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Review article

Exhaustion of T lymphocytes in the tumor microenvironment: Significance and effective mechanisms

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ABSTRACT

T lymphocytes play crucial roles in adaptive immune responses to tumors. However, due to different tolerance mechanisms and inhibitory effects of the tumor microenvironment (TME) on T cells, responses to tumors are insufficient. In fact, cellular and molecular suppressive mechanisms repress T cell responses in the TME, resulting in senescent, anergic and exhausted lymphocytes. Exhaustion is a poor responsive status of T cells, with up-regulated expression of inhibitory receptors, decreased production of effective cytokines, and reduced cytotoxic activity. Low immunogenicity of tumor antigens and inadequate presentation of tumor-specific antigens results in inappropriate activation of naive T lymphocytes against tumor antigens. Moreover, when effector cytotoxic T cells enter TME, they encounter a complicated network of cells and cytokines that suppress their effectiveness and turn them into exhausted T cells. Thus, the mechanism of T cell exhaustion in cancer is different from that in chronic infections. In this review we will discuss the main components such as inhibitory receptors, inflammatory cells, stromal cells, cytokine milieu as well as environmental and metabolic conditions in TME which play role in development of exhaustion. Furthermore, recent therapeutic methods available to overcome exhaustion will be discussed.

1. Introduction

Tumorigenesis is usually initiated following stepwise accumulation of genetic and epigenetic alterations that control cellular apoptosis and mortality [1]. Mutations may evoke neoplastic phenotypes in normal cells. These accumulated mutations alone generally do not result in cancer formation. Hence, the interaction and crosstalk between cancer cells and the supporting cell types that form the tumor microenvironment (TME) indicate that this environment plays a critical role in cancer development [2]. The TME is a key factor in the escape of tumor cells from the immune system. This region contains mainly angiogenic vascular endothelial cells, infiltrating immune cells, and stromal cells [3].

Pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) are upregulated in most tumors and can result in the development of immature vessels with abnormal structures in the TME. Vascular hyper-permeability is a hallmark of tumor pathophysiology, and one of its consequence is diminished tumor perfusion. Reduced perfusion leads to hypoxia and acidification of the TME and results in decreased leukocyte infiltration [4].

The altered metabolism of cancer cells influences the nutritional status in the TME and influences the metabolic fitness of leukocytes leading to low glucose and acidification of the TME [5–7]. Hypoxia promotes tumorigenesis by enhancing cancer cell proliferation, and the low pH increases the suppressive activity of tumor-infiltrating myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs), and reduces the cytotoxicity and proliferation of T lymphocytes in the TME [4].

Cytotoxic $CD8^+$ T cells (CTLs) are considered as one of main effector cell types of the adaptive immune system responsible for combating cancer cells. Despite the presence and activation of several immunologic components, especially CTLs in the TME, tumor cells are not easily eradicated [8]. Mechanisms involved in this impaired

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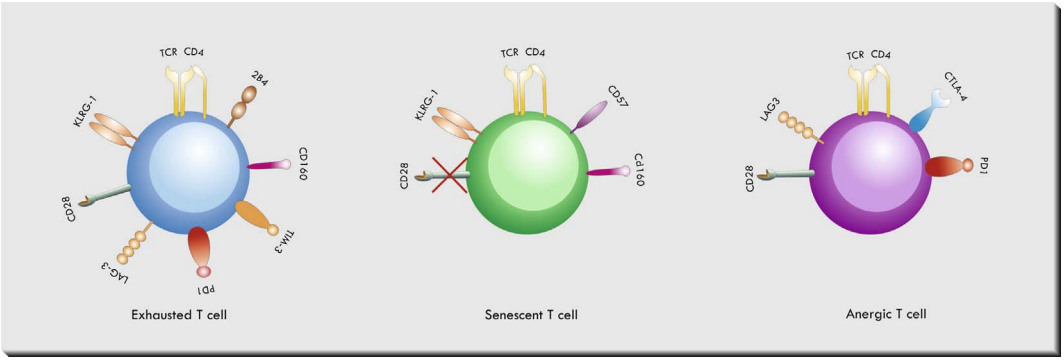

Fig. 1. Dysfunctional subsets of T lymphocytes in the tumor microenvironment and their features.

responsiveness include depletion of naive anti-tumor T cells during thymic lymphocyte development, unresponsiveness of CTLs due to expression of reduced amounts of the co-stimulatory molecules CD80 (B7-1) and CD86 (B7-2), and prolonged presence of immunomodulator cells and secretion of soluble factors from those cells, which can directly inhibit the defensive mechanisms of leukocytes or indirectly disrupt their activation process and undermine the CTL response in the TME [9]. The presence of immune suppressive agents in the TME leads to T cell dysfunction in the TME in the context of anergy, senescence, and exhaustion (Fig. 1). Consequently, although immune cells are found in the TME, they are not fully effective.

The concept of T cell exhaustion was first described in CTLs in chronic lymphocytic choriomeningitis virus (LCMV) infection in mice [10,11], and was subsequently reported in human chronic viral infections and cancers as well. T cell exhaustion is a state of T cell dysfunctionality and the severity of T cell exhaustion appears to increase according to antigen concentration and decreased $CD4^+$ T cell numbers [12]. Exhausted T cells progressively lose their proliferation, cytokine production, and cytotoxic capabilities. Several studies confirm that exhausted T cells express increased levels of inhibitory receptors, including programmed cell death protein 1 (PD-1), cytotoxic T lymphocyte antigen-4 (CTLA-4), lymphocyte activation gene 3 (LAG-3), T cell immunoglobulin and mucin domain containing-3 (TIM-3), B and T lymphocyte attenuator (BTLA), and T cell immunoreceptor with Ig and ITIM domains (TIGIT) [13–19]. Interestingly, it was reported that exhausted CTLs co-express inhibitory receptors and the pattern and number of inhibitory receptors correlate with T cell exhaustion levels [20]. Blocking inhibitory receptors with specific antibodies may reverse the functionality and anti-tumor responses of exhausted T cells. However, application of blocking antibodies against inhibitory receptors in combination therapy has shown promising results in the treatment of advanced stages of cancer. In this paper we discuss mechanisms that may limit CTL effectiveness and promote the development of inefficient T cell subsets, especially those exhausted status in the TME that cannot efficiently combat tumor cells, allowing them to survive and grow.

2. Exhausted T lymphocytes differ from other subsets in the TME

As previously mentioned, evidence indicates that effector T cell phenotypes and capabilities are dramatically impaired in the TME, resulting in T cell exhaustion. Various molecular and cellular mechanisms contribute to this phenomenon. In this section, we discuss lymphocyte dysfunctional phenotypes including anergic, senescent, and exhausted T cells, and also their underlying developmental mechanisms.

2.1. Anergic T lymphocytes

T cell anergy is generally described as an induced state of hyporesponsiveness with impaired proliferation and IL-2 secretion (Table 1). It has been proposed that T cell anergy induces peripheral tolerance and protection from autoimmune disease development [21–25]. Although several studies support T cell anergy in cancer, the exact reasons for anergic T cell development in the TME are unclear.

T cell anergy generally happens following their incomplete activation in response to suboptimal amounts of IL-2 or absence of co-stimulatory signals [21,23]. Obviously, tolerance mechanisms inhibit the immune response against cancer; however, the exact mechanisms of their induction in the TME is not known. Effective mechanisms responsible for T cell anergy in the TME depend mostly on molecular properties of these cells, such as expression of surface molecules. In the TME, B7 family stimulatory and inhibitory receptors do not have the arbitrary distribution as in immunological response to tumor cells. In fact, in this microenvironment, tumor cells and the antigen-presenting cells (APCs) overexpress programmed death-ligand 1 (PD-L1), while expression of the stimulatory receptors CD80 and CD86 are diminished [10,26,27]. Studies in mouse cancer models demonstrated that inducing B7-1 expression on tumor cells or stopping the expression of inhibitory receptor expression on these cells can reduce tumor growth [26–32]. In addition, appropriate stimulation of anergic T cells in the mouse cancer model expands the specific anti-tumor T cell population and returns them from the anergic to the effective mode and helps them to overcome and eradicate the tumor cells [33–35]. Overall T cell anergy is a reversible dysfunctional state of a subset of T cells and tumor-induced T cell anergy in the TME may be one of the immune evasion mechanisms in cancer.

2.2. senescent T lymphocytes

Senescent T cells are characterizes by telomere shortening, arrested cell cycle, and phenotypic changes including downregulated CD28 receptor expression (Table 1) [24,25,36,37]. Telomere shortening is an inherent consequence of cell division, which affects cell function and leads to cellular senescence [38]. Cell cycle controlling proteins including p16, p21, and p53 normally inhibit cell cycle progression and accumulate in senescent cells [39–41]. In addition to phenotypic changes, senescent T cells manifest defective killing abilities and develop negative regulatory functions [42,43].

Aging is a normal physiological process of cells; however, the percentage of senescent T cells in young individuals with autoimmune diseases and chronic infections is higher than in normal individuals [44], indicating that repeated activation and proliferation may promote aging in these cells [45]. Moreover, co-culture of cancer cells with T cells induced senescence in the T cells [46]. Studies of patients with lung or head and neck cancers suggests that following T cell senescence, $CD28^{-/dim}$ CTLs will predominate [47,48]. In addition, it has been shown that reduction in CD28 expression and overexpression of TIM-3, CD57, and killer cell lectin-like receptor subfamily G member 1 (KLRG1) is associated with T cell senescence [49–54]. Some researchers believe that DNA damage, which occurs in thymic lymphocyte progenitors, may exit these cells from the normal cell cycle and induce

Table 1
Comparison of dysfunctional T cell.

Features	Exhausted T cells	Anergic T cells	Senescent T cells	Refs.
Phenotype	Reduced proliferation Increased experssion of inhibitory receptor Decreased cytokine production Reduced cytotoxicity	Reduced proliferation Reduced IL-2 secretion	Telomere shorting Cell-cycle arrest Phenotypic changes	[12-19,21-25,36,37]
Surface marker profile	$CD45RO^+$ $CD57^+$ $CD95^+$ $PD\text{-}1^+$ $CTLA\text{-}4^+$ $TIM\text{-}3^+$ $LAG\text{-}3^+$ $BTLA^+$	$LAG\text{-}3^+$ $PD\text{-}1^+$	$KLRG1^+$ $CD28^-$ $CD57^+$	[12-19,21-25,36,37]
Transcription profile	NFAT, $T\text{-}bet^{low}/Eomes^{high}$, Blimp-1, BATF, FoxP3	NFAT, NF-κB/RelA, Ikaros, Egr1/Egr2	FoxP3	[12-19,21-25,36,37]
Main causes	Excessive or continuous stimulation	Suboptimal stimulation	Repetitive stimulation	[19,24,25,36]
Detailed reason of development	Persistent overstimulation of signal 1 and signal 3 leads to progressive loss of effector and memory function	Absent or weak signal 2 and/or signal 3 during priming of T cells results dysfunctionality	Intermittent and/or multiple stimulations after the primary response leads to limited cell replication	[19,24,25,37,68]
Time frame of occurrence	Typically within a few weeks	Typically within a few days	Typically within months to years	[19,24,25,36,68]
Proliferative capacity	Low	Low	Low	[19,24,25,36]
Effector function	Low to moderate	None or low	High	[19,24,25,36]
Proper priming	Yes	No	Yes	[19,24,25]

Signal 1: Interaction of MHC-peptide complex with specific T cell receptor.
Signal 2: Involvement of co-stimulatory or co-inhibitory molecules.
Signal 3: Signals from soluble cytokines.

their senescence; however, this mechanism in the TME has not been confirmed [55]. Senescent T cells are dysfunctional and incapable of killing target cells, and may in fact inhibit normal T cell activity in the TME [42,43].

2.3. Exhausted T lymphocytes

Exhaustion is hypo-responsiveness of T cells, along with reduced proliferation, increased expression of the inhibitory receptors PD-1, LAG-3, TIM-3, CTLA-4, BTLA, and TIGIT, and decreased production of IL2, IFNγ, TNFα, and granzyme B. The impaired cytotoxicity of these cells is shown in Table 1 [12-19,24,25]. Severity of T cell exhaustion appears to rise with increased antigen load and decreased specific T $CD4^+$ cells [12]. The typical sign of T cell exhaustion is expression of the inhibitory receptor, PD-1 [13]. Interestingly, results of studies in mice and humans have shown that exhausted CTLs co-express inhibitory receptors and the pattern and number of these receptors correlate with the levels of T cell exhaustion [20]. Approximately one-third to one-half of $CD8^+$ tumor-infiltrating lymphocytes (TILs) co-express PD-1 and CTLA-4. $PD\text{-}1^+$ $CTLA4^+$ $CD8^+$ TILs are more severely exhausted and show lower proliferation and cytokine production capability than normal CTLs [43]. In addition, other inhibitory receptors including TIM-3, LAG-3, BTLA, and TIGIT have been demonstrated to regulate T cell exhaustion in cancer. Most researchers believe that T cell exhaustion occurs due to continuous stimulation of these cells, resulting in their loss of effectiveness and eventual apoptosis. However, some researchers believe that the developmental disorders of memory T cells are mainly due to exhaustion [20]. Although the steps that direct T cell exhaustion in chronic infections are clear, the process in the TME is not yet known. Albeit, since chronic conditions occur in both of those conditions, similar characteristics can be considered for exhausted T cells in either case. Obviously, the TME has a great impact on the phenotype, metabolism, functionality, and maintenance of these cells [56].

3. $CD8^+$ T lymphocyte exhaustion in the TME

In chronic infections, the characteristics of exhausted T cells are the following: 1) progressive impairment in effectiveness or functionality, 2) upregulated co-expression of inhibitory receptors, 3) diminished production of effective cytokines such as IL-2, IFNγ, and TNFα, 4) impaired in vitro cytotoxic activity, 5) poor responsiveness to survival factors such as IL-7 and IL-15, and 6) alteration in cellular metabolism, DNA transcription level, and functionality of transcription factors including *T*-bet and Eomes [53,57,58]. While some of the mentioned criteria such as alterations of the expression level of some of receptors, cytokines, and transcription factors are observed in all types of exhausted cells, certain criteria are merely restricted to exhausted T cells in chronic infections. Due to similarities of tumor antigens with self-antigens and also the existence of central and peripheral tolerance mechanisms, effectiveness of CTL responses in cancer is mediocre. In addition, presentation of cancer antigens in the absence of inflammatory cytokines and the presence of inhibitory mechanisms such as self-antigen-specific regulatory T cells ameliorate the condition [59]. Therefore, mechanisms of T cell exhaustion in cancer differ from those of chronic infections.

In chronic infections, the naive T cells naturally encounter presented antigens and differentiate into effective CTLs, but following high resistance of the pathogen and failure in antigen removal, effective CTLs become exhausted. However, in the TME, due to low expression and immunogenicity of tumor antigens, low affinity of specific T cell receptors (TCRs) to tumor antigens [60], insufficient presentation of the tumor antigens to T cells [56], absence of inflammatory cytokines, and reduced expression of co-stimulatory molecules, the process of differentiation of naive CTLs to effective cells is disrupted and naive T cells are not fully activated. On the other hand, following entry of effective CTLs to the TME, they encounter a complicated regulatory network of various cells including cancer, inflammatory, and stromal cells, and secreted cytokines, which could suppress and convert them to exhausted phenotype [9]. Changes in the metabolic state and nutrient availability of cancer cells may alter the functional fate of T cells in the TME. Some researchers believe that exhaustion of CTLs may be triggered by metabolic stresses within the TME [61]. Cancer cells strongly consume glucose through glycolysis as a main metabolic program, thus they capture a high percentage of environmental glucose for energy production. Consequently, T cells will encounter a lack of glucose (hypoglycemia) in the TME due to competition with cancer cells. Hypoglycemia may prevent full activation of CTLs and also decrease the expansion, differentiation, and effector functions of these cells in the TME [62]. In addition, these metabolic conditions increase PD-1

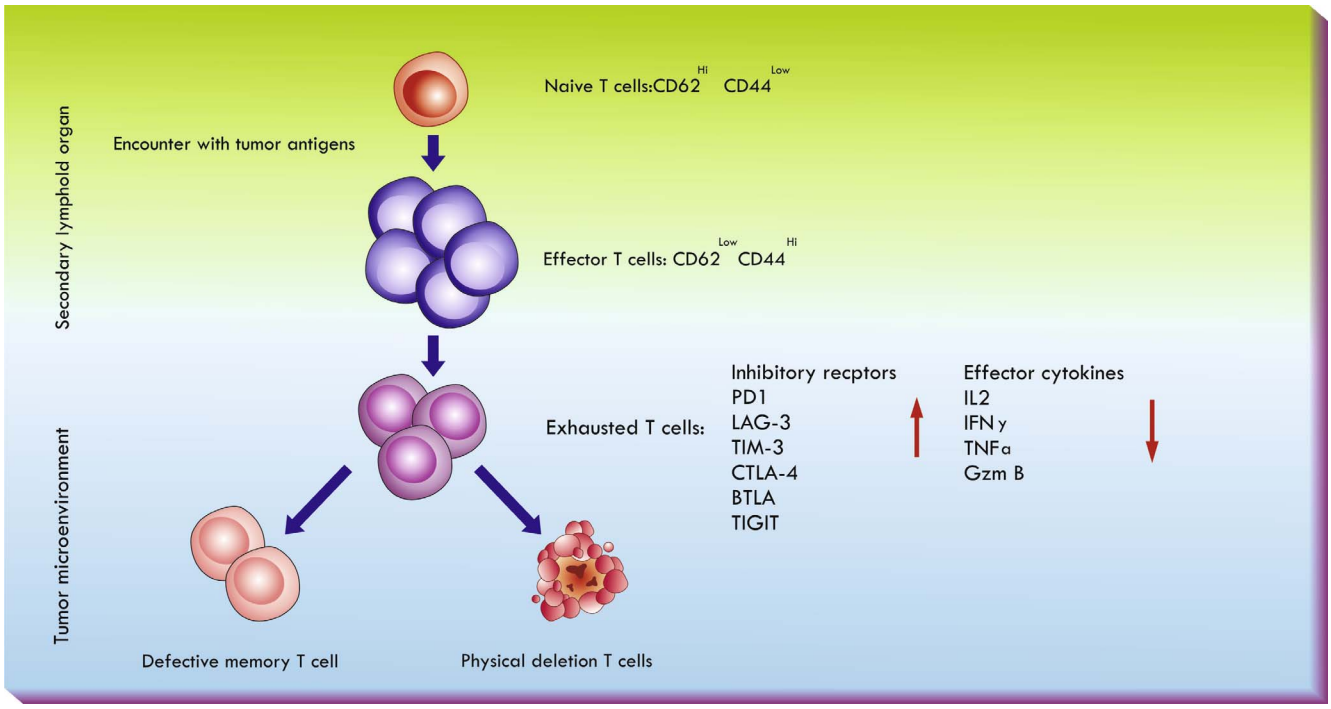

Fig. 2. Differentiation of T CD8$^+$ T lymphocyte to exhausted cells in the tumor microenvironment. In the secondary lymphoid organs, the encounter of naive T cells with tumor antigens results in proliferation and differentiation of effector T cells. In the tumor microenvironment, effector T cells are polarized into exhausted T cells, with decreased effector cytokine production and increased inhibitory receptor expression. Subsequently, exhausted T cells may differentiate to defective memory T cells or be deleted physically.

expression on activated CTLs and promote differentiation of T cells into regulatory T lymphocytes (Tregs) [63]. On the other hand, excessive consumption of glucose through glycolysis in cancer cells produces large amounts of lactic acid, which can suppress proliferation, production of effective cytokines, and cytotoxic activity of CTLs in the TME [64]. Also excessive metabolism of the amino acids tryptophan, arginine, and glutamine by cancer cells causes an amino acid deficiency that can suppress CTL anti-tumor responses in the TME [65,66]. Accumulation of adipocytes and adipocyte-like fibroblasts, and production of large amounts of fatty acids by cancer cells lead to lipid-enrichment in the TME. These metabolic conditions may promote development of Tregs and suppress effector CTL functions in the TME [67].

4. Differentiation of CD8$^+$ T lymphocytes to exhausted cells in the TME

After differentiation of CD44^{low}, CD62L^{high} naive CTLs to CD44^{high}, CD62L^{low} effective cells in secondary lymph tissues, these cells may enter the TME and become exhausted cells, which eventually will be removed or differentiate to defective memory cells (Fig. 2). So, it seems that in the TME, the exhausted CTLs originate from the effective CTLs that leave the environment and become dysfunctional by the mentioned mechanisms. Some researchers believe that exhaustion of CTLs is due to impaired formation of memory T cells [20]; however, Pauken et al. illustrated that exhausted T cells are a distinct lineage from effector or memory CTLs [68].

5. Correlation of the exhaustion of CTLs with deletion of CTLs in the TME

PD-L1, one of the major factors in the TME because of its high expression in cancer tissues and also its capability to induce apoptosis in CTLs, plays a crucial role in the viability of these cells. It was shown that increasing the expression of this molecule on the surface of cancer cells results in impaired CTL survival and elimination of their population in the TME [69]. Immunohistochemical staining showed that upregulated expression of PD-L1 in hepatocellular carcinoma is directly associated with increased apoptosis of CTLs in the TME. On the other hand, CTLs produce IFNγ, which induces the expression of PD-L1 on hepatocytes and causes apoptosis in these cells. These observations suggest that the local reduction of CTLs occurs in advanced stages of exhaustion [70].

6. Effective Internal signals in exhaustion of CTLs in the TME

Involvement of the inhibitory receptors PD-1, LAG-3, and TIM-3, as well as alteration of transcription factors, are effective mechanisms for the induction of exhaustion in CTLs and is discussed in detail below (Fig. 3).

6.1. Inhibitory receptors

T cell activation requires three signals; these include: 1) recognition of MHC-peptide complexes by the TCR, 2) pairing of co-stimulatory or co-inhibitory molecules, and 3) alarms from proper soluble cytokines [71].

Of these signals, the second plays a crucial role in production of the cytokine profile responsible for differentiation of lymphocytes into activator or inhibitor phenotypes. In normal conditions, molecules that generate co-inhibitory messages, such as PD-1 and CTLA-4, inhibit inflammation and prevent the inappropriate escalation of immune system responses and tissue damage. However, excessive increases of co-inhibitory signals weaken immune responses and result in exhaustion of CTLs in the early stages, and finally enhance the expression of the inhibitory receptors PD-1, LAG-3, TIM-3, CTLA-4, BTLA, and TIGIT, as well as reduced production of granzyme B and the stimulatory cytokines IL-2, IFNγ, and TNFα [56,72]. During this process, exhausted CTLs gradually lose their ability to produce cytokines. For example, in the early stages of exhaustion, IL-2 production decreases significantly [19]; in the middle stages, TNFα production is decreased, and in the advanced stages, T cells cannot produce IFNγ or granzyme B, thus the performance of CTLs as tumor cell eradicators is limited [12].

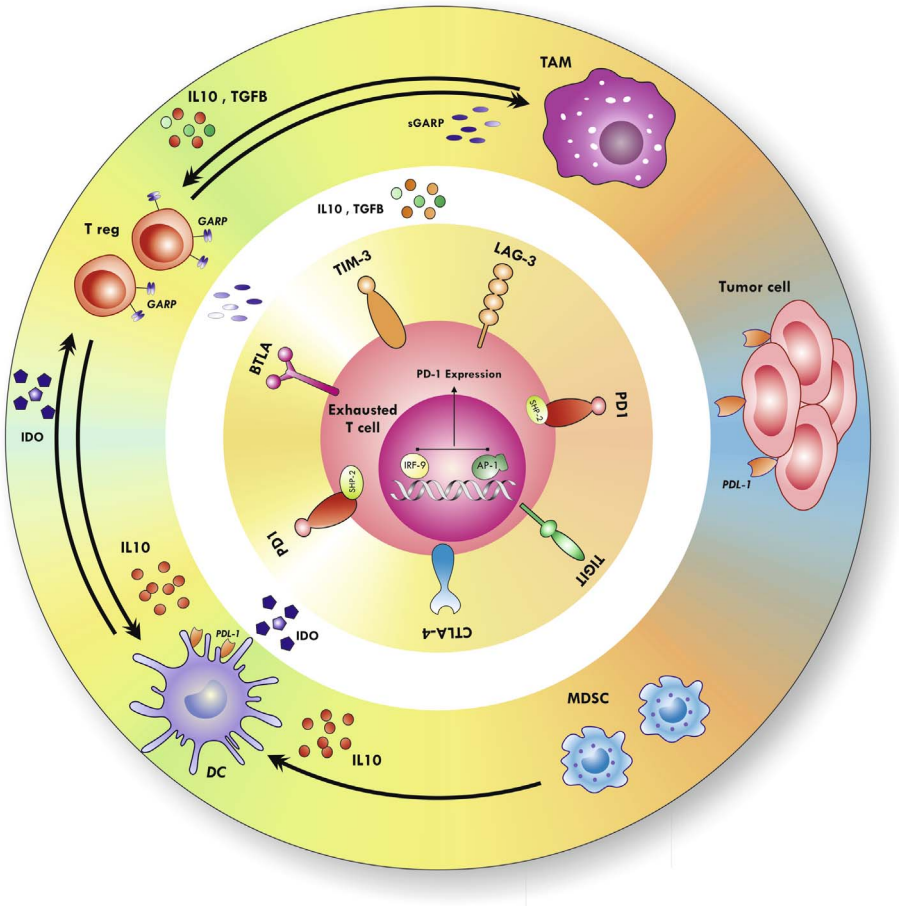

Fig. 3. Regulatory mechanisms of T cell exhaustion in the tumor microenvironment. Cancer cells, regulatory T lymphocytes (Tregs), tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs) and plasmacytoid dendritic cells are major extrinsic cells that promote T cell exhaustion by different mechanisms. Cytokines, including IL-10 and TGF-β, are both extrinsic factors involved in T cell exhaustion in the TME. GARP is shed from the surface of activated Tregs, which in its soluble form (sGARP) induces peripheral Tregs and TAMs with the M2 phenotype. Notably, proliferation of cytotoxic T cells and their effector functions are inhibited by sGARP. Increased expression of inhibitory receptors, including PD-1, CTLA-4, LAG-3, TIM-3, BTLA, and TIGIT, on $CD8^+$ T cells is the major intrinsic factor for T cell exhaustion. SHP-2, IRF-9, and AP-1 may affect PD-1 expression in the $CD8^+$ T cells.

6.1.1. PD-1

PD-1 was first established as a negative regulator of T cell activation. Based on possession of a cytoplasmic immunoreceptor tyrosine-based inhibition motif (ITIM), this molecule is regarded as an inhibitory receptor of the immunoglobulin superfamily of receptors [73]. This inhibitory receptor is expressed on T and B cells, and some myeloid cells following their activation [74]. Following TCR engagement, PD-1 expresses and this expression eventually declines after antigen resolving, while during chronic antigen exposure, PD-1 expression is sustained. It has been shown that after co-culture of Jurkat cells with cancer cells, PD-1 expression is upregulated on the Jurkat cell surfaces. Although it is not well understood how PD-1 suppresses T cell functions in vivo, five different mechanisms have been proposed (Fig. 4). PD-1 may: 1) antagonize TCR signaling by recruiting phosphatases [11,75–78] 2) modulate the PI3K / AKT / mTOR pathway, implementing PD-1 in nutrient sensing, metabolism, survival, and cell growth [79–81], 3) modulate the Ras pathway, linking PD-1 to the cell cycle [81], 4) induce expression of basic leucine zipper transcription factor (BATF), which can repress expression of effector genes [82], and, 5) influence T cell motility [83–85]. A significant increase in PD-1 expression on the surface of CTLs in the TME and its correlation with reduction of effective cytokines has been documented in patients with lymphoma, Hodgkin disease, and liver and stomach cancers [73,86,87].

6.1.2. CTLA-4

CTLA-4, an inhibitory receptor belonging to the CD28 protein family, is expressed on the T cell surface. Following connection to its ligands, CD80 and CD86, CTLA-4 exerts inhibitory messages that diminish IL-2 production, thus reducing T cell functionality [88,89]. In effector T cells, CTLA-4 competes strongly with CD28 for effective co-stimulation by CD80/86, leading to functional inactivation of the specific lymphocytes. CTLA-4 promotes the “stop signals” initiated by T cells upon encounter of the TCR with peptide-loaded MHC molecules [90]. Moreover, CTLA-4 induces *trans*-endocytosis of costimulatory ligands including CD80 and CD86, thus restricting opportunities for further T cell activation [91]. In the TME, 30–50% of CTLs simultaneously express CTLA-4 and PD-1. CTLs that express these two receptors are less capable of proliferation and cytokine production than those that do not, and are prone to exhaustion [92]. Stimulation of these inhibitory receptors can inhibit the AKT signaling pathway. The AKT pathway plays an important role in the regulation of glucose metabolism in T cells and increases glucose transporter 1 expression, resulting in increased glycolysis in these cells. Thus, activation of CTLA-4 and/or PD-1 leads to impaired glucose metabolism and reduced lymphocyte functionality, and eventually, exhaustion [80].

6.1.3. TIM-3

Like PD-1, TIM-3 is transiently upregulated on virus-specific CTLs during acute infections, remains elevated on activated T cells, and may help to drive exhaustion under prolonged antigen exposure, which occurs during chronic infections and cancer [93–95]. In melanoma, co-expression of PD-1 and TIM-3 on exhausted CTL surfaces has been observed in the TME. Interestingly, cells with the $TIM\text{-}3^+$ $PD\text{-}1^+$ phenotype fail more in proliferation and production of typical cytokines such as IL-2, IFNγ, and TNFα than $TIM\text{-}3^-$ $PD\text{-}1^+$ and $TIM\text{-}3^-$ $PD\text{-}1^-$ cells; they also show more severe exhaustion [96].

6.1.4. LAG-3

LAG-3, or CD223, is an MHC class-II ligand that is structurally similar to CD4 and expressed on activated and exhausted T cells. LAG-3 suppresses the expansion of $CD4^+$ T cells in response to antigen recognition [97] and was found to be synergistic with CTLA-4 and PD-1 in

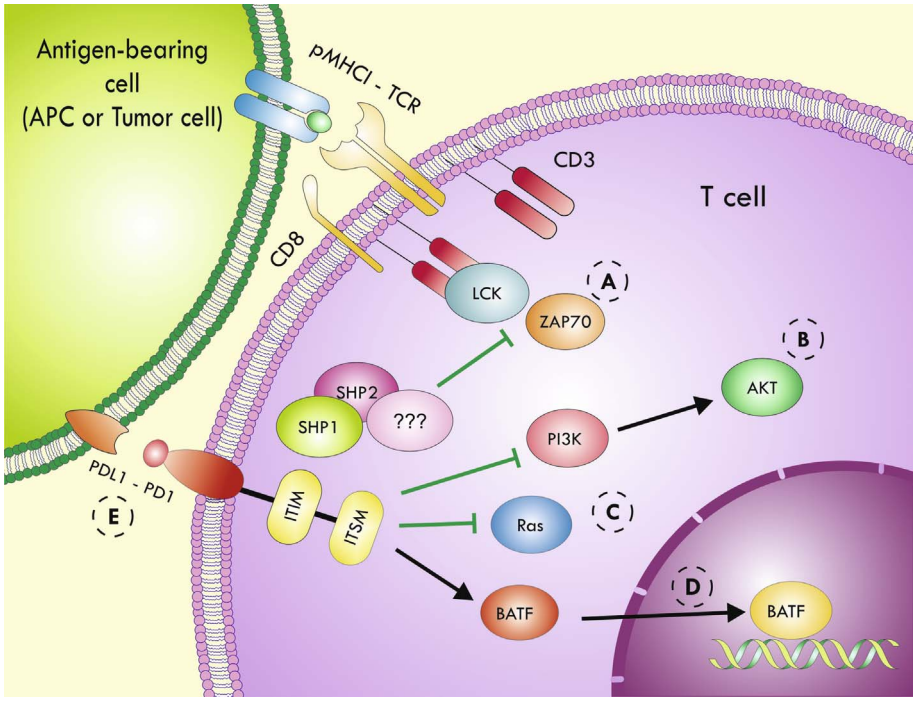

Fig. 4. Inhibitory mechanisms of Programmed Cell Death-1 on T lymphocytes. PD-1 affects T cell function by various mechanisms. A) PD-1 directly antagonizes T cell receptor (TCR) signaling by preventing the phosphorylation of zeta-chain-associated protein kinase 70 (ZAP70); (B) PD-1 hinders the CD28-induced activation of phosphatidyl inositide 3-kinase (PI3K), leading to diminished AKT activation; (C) PD-1 inhibits the Ras pathway; (D) PD-1 upregulates the expression of suppressive transcription factors including basic leucine zipper transcription factor ATF-like (BATF); and (E) PD-1 may affect the contact duration between T cells and antigen-presenting cells (APCs).

mediating T cell suppression during chronic antigenic stimulation [98,99]. Additionally, LAG-3 is important in promoting the functionality of regulatory T cells [100]. LAG-3 has been shown to inhibit calcium fluxes associated with TCR signaling, and dampen cytokine production and lymphocyte proliferation [97]. However, it is believed that upregulated expression of LAG-3 on T cells alone may not be sufficient to drive them toward exhaustion, but it may cooperate with other inhibitory receptors to influence the extent of T cell exhaustion [101]. In the ovarian TME, it was shown that PD-1 and LAG-3 are simultaneously expressed on exhausted CTL surfaces, and lymphocytes with the LAG-3^+ PD-1^+ phenotype fail more in TNFα and IFNγ production than LAG-3^- PD-1^+ or LAG-3^-,PD-1^- cells [58,102].

6.1.5. BTLA

BTLA, an inhibitory receptor on T cell surfaces, through binding to herpes virus molecules, causes induction of inhibitory messages in CTLs. In advanced skin cancers, CTLs with BTLA$^+$ PD-1^+ TIM-3^+ phenotypes were the most dysfunctional cells. So it seems that in skin cancers, BTLA plays an important role in CTL exhaustion [17,103].

6.1.6. TIGIT

Recently, the role of the co-inhibitory receptor TIGIT was demonstrated in T cell exhaustion. TIGIT and CD226 molecules compete for binding to their CD112 and CD155 ligands, which is very similar to the binding of CTLA-4 to CD28. Notably, binding of CD226 to its ligands transfers positive activation signals while TIGIT binding leads to negative signals in T lymphocytes and their impairment [18,104]. It was shown that melanoma-specific CTLs highly express TIGIT. Furthermore, simultaneous blocking of PD-1 and TIGIT increases T cell proliferation and cytokine production, and enhances CTL performance, leading to the eventual elimination of tumor cells [105,106]. Overall, these findings demonstrate that the inhibitory molecules, including PD-1, have crucial roles in regulation of CTL exhaustion and PD-1 expression, and along with other inhibitory receptors, may determine the severity of CTL exhaustion.

6.2. Transcription factors

Transcription factors including B lymphocyte–induced maturation protein 1 (Blimp-1), *T*-bet, nuclear factor of activated T cells, cytoplasmic 1 (NFATc1), and BATF play substantial roles in CTL exhaustion in chronic infections [82,107,108]. However, the signaling pathways and transcription factors that affect CTL exhaustion in cancer are not yet clear. Downstream signaling pathways should also be considered. It has been demonstrated that Src homology 2 domain-containing phosphatase-2 (SHP2) plays an important role in the downstream signaling pathway of PD-1 [109]. On the other hand, PD-1 expression is upregulated on the surface of CTLs in Hodgkin's lymphoma. Blocking the PD-1 pathway affects the phosphorylation of tyrosine phosphatases such as SHP2, which consequently results in reduced IFNγ production, and hence, impaired lymphocyte function. Alpha interferon via IFN-responsive factor 9 (IRF9) signaling causes stable expression of PD-1 on T cell surfaces, indicating that IRF9 may induce T cell exhaustion [110]. Studies have also demonstrated that aberrant expression of the C-FOS subunit of the AP1 transcription factor in CTLs increases PD-1 expression through binding to the PD-1 promoter, which indicates the crucial role of C-FOS in the regulation of CTL exhaustion in the TME [111].

7. Effective external signals in exhaustion of CTLs in the TME

The TME consists of stromal and inflammatory cells that express inhibitory ligands such as PD-L1 and PD-L2 enclosed with tumor cells and a network of regulatory cells and cytokines. This alters the metabolic status, lowers nutrient accessibility, and provides a complex arrangement of immunosuppressors, which limits cytotoxic activity CTLs and causes their dysfunction. In addition, the abundance of tumor antigens in this microenvironment leads to chronic T cell activation and probably CTL exhaustion [20]. Effective external signals in exhaustion of CTLs in the TME include the inhibitory ligands of PD-L1 and PD-L2, immunosuppressive cells, soluble factors, and environmental and metabolic conditions (Fig. 3).

7.1. The inhibitory ligands PD-L1 and PD-L2

The main inhibitory ligands of PD-1 are PD-L1 and PD-L2. PD-L1 is highly expressed on stromal and cancer cell surfaces, and the PD-1/PD-L1 signaling pathway is considered as one of substantial regulatory routes of CTL exhaustion in the TME. Therefore, blocking this signaling

pathway may augment the antitumor activity of CTLs [112]. PD-L2 is intermediately expressed on macrophages in response to cytokine activation, as well as dendritic cells (DCs) and mast cells. It was shown that coupling of these ligands to PD-1 drives T cells toward regulatory types [113].

7.2. Immunosuppressive cells

A complex of immunosuppressor cells in the TME includes regulatory $CD4^+$ T cells, plasmacytoid DCs, macrophages, and myeloid-derived suppressor cells that directly or indirectly suppress CTL responses.

7.2.1. Regulatory T $CD4^+$ lymphocytes

Regulatory $CD4^+$ T cells (Tregs) are defined as subsets of inhibitory T cells that in normal conditions maintain peripheral tolerance and prevent autoimmune diseases. These lymphocytes are abundant in peripheral blood and tumor tissues and may aid tumor cells to escape from immune responses [114,115]. These cells, through their functional ectoenzymes including CD39 and CD73, mediate production of extracellular adenosine, which may couple with adenosine A2A receptors on the cell membrane of effective CTLs and exert inhibitory effects [116,117]. By production of inhibitory cytokines such as IL-10 and TGFβ, and overexpression of CD25 (IL-2R), Tregs suppress CTL functions [118–120]. Furthermore, the increased IL-10 secretion from regulatory lymphocytes results in upregulated expression of PD-L1 on DC surfaces.

Glycoprotein A repetitions predominant (GARP), also known as LLRC32, which induces peripheral tolerance via TGF-β, is an activation marker for Tregs. GARP is shed from activated Treg surfaces, and in its soluble form (sGARP), induces peripheral Tregs and M2 phenotype TAMs. Notably, cytotoxic T cell proliferation and their effector function is inhibited by sGARP [121].

7.2.2. Plasmacytoid dendritic cells

Dendritic cells are distinct subsets of professional APCs. Plasmacytoid DCs, by production of indolamine 2, 3-dioxygenase (IDO), induce Tregs and eventually suppress immune responses in the TME [122]. It was shown that a population of plasmacytoid DCs in the TME of mice with prostate cancer expressed lower levels of the co-stimulatory ligands CD80, CD86, and CD40 and greater levels of the inhibitory ligands PD-L1 and IDO than non-cancerous mice. These findings reflect the crucial role of plasmacytoid DCs in the induction of CTL exhaustion [123].

7.2.3. Macrophages

Macrophages play a vital role in innate immunity against foreign pathogens. M1 phenotypes are capable of producing a significant amount of pro-inflammatory cytokines, while M2 phenotypes, by producing several growth factors, are involved in tissue remodeling and control of innate immune responses. Macrophages that accumulate in the TME, known as TAMs, mainly demonstrate M2 phenotype and favor tumor establishment [124]. An increased number of these cells in the TME may worsen the prognosis. Tumor cells, by production of VEGF, CCL2, M-CSF, and angiopoietin 2, promote migration of bloodstream monocytes to the TME, which then differentiate to TAMs [125,126]. CCL2 overexpression in mice fibrocarcinoma cells results in recruitment of TAMs and assists tumorigenesis [62]. Tumor-associated macrophages, by production of the inhibitory cytokines IL-10 and TGFβ, suppress lymphocyte proliferation in the TME and also lead the immune responses toward conversion to Tregs [127].

7.2.4. Myeloid-derived suppressor cells

Accumulation of MDSCs is a substantial mechanism of tumor promotion. These suppressor cells are progenitors of DCs, monocytes/macrophages, and granulocytes [128]. In the case of human cancers they exhibit $CD11b^+$ $CD33^+$ $CD34^+$ $CD14^-$ $HLA\text{-}DR^-$ phenotype, while in mice tumors they exhibit $CD11b^+$ $Gr\text{-}1^+$ characteristics. MDSCs are commonly divided into the monocytic subgroup, with typical characteristics of $CD11b^+$ $Ly6G^{low}$ $Ly6C^{high}$, and the granulocytic subgroup, with cellular characteristics of $CD11b^+$ $Ly6G^{high}$ $Ly6C^{low}$ [129]. By several mechanisms, MDSCs may inhibit the functionality of activated lymphocytes and induce CTL exhaustion. For example, in a mouse model of ovarian cancer, MDSCs highly express PD-L1 and CD80, which significantly inhibits the immune response against tumor antigens [130,131]. IL-10-producing cells, such as some DCs, may stimulate MDSCs to increase PD-L1 expression on their surfaces, which ultimately leads to CTL dysfunction through the PD-1/PD-L1 pathway, emphasizing the role of MDSCs in CTL exhaustion [132].

7.3. Soluble factors (IL-10 and TGF-β as inhibitory cytokines)

Several inhibitory cytokines contribute to CTL exhaustion. Among them IL-10 and TGFβ could be released from tumor cells, TAMs, and Tregs in the TME and contribute to reduced effectiveness of local lymphocytes [127,133]. These cytokines can impair the cytotoxic effects of natural killer cells, which play a major role in innate immunity against tumors. They also may suppress macrophage and DC functions in the TME [134]. These cytokines may also upregulate PD-L1 expression on DCs [132], which represents a crucial step in CTL exhaustion [135]. Cancer cells, local fibroblasts, and several leukocytes are the main sources of TGFβ [136]. TGFβ shows various roles in tumor immunobiology, depending on cancer cell type and disease stage. In the early stages of cancer, TGFβ hinders tumor cell growth and induces their apoptosis, however in the later stages TGFβ may inhibit immune responses, upregulate cancer cell invasiveness, and increase the possibility of metastasis [137]. It was recently shown that TGFβ-mediated transcription inhibition of lymphocyte effector molecules including perforin, granzyme, and cytokines, directly inhibits cytotoxic properties of CTLs [120,138]. Moreover, tumor cell-derived TGFβ may increase miR-23a expression and reduce Blimp-1 expression and consequently inhibit CTL toxicity [139]. In addition, TGFβ-treated naive T cells differentiate to Tregs, which have a major role in CTL exhaustion [140].

7.4. Environmental and metabolic conditions

Researchers believe that differentiation status and metabolic programing determine the functional fate of T cells in the TME. As previously mentioned, changes in metabolic state of and nutrient availability to cancer cells may alter T cell function in the TME. Under physiological conditions, most nonmalignant cells rely on mitochondrial respiration, in which oxidative phosphorylation (OXPHOS) occurs as a primary metabolic pathway to generate energy in the form of adenosine triphosphate (ATP). But cancer cells, due to oncogenic mutations and dysfunction of the tumor suppressor genes, including p53, switch their metabolism to glycolysis, a biochemical process that occurs in the cytoplasm without the requirement for oxygen. This phenomenon, called the “Warburg effect,” is a hallmark for cancer cell metabolism [141].

In cancer cells, glycolysis occurs in parallel with the tricarboxylic acid (TCA) cycle, which is linked to OXPHOS and oxidizes acetyl-CoA, and also other metabolic pathways to enhance the biosynthetic processes and thus support cancer cell proliferation and growth. Similar metabolic features were discovered in T lymphocytes during activation, even though this metabolic transition in T cells is part of a physiological process. Naive CTLs primarily use OXPHOS to produce ATP, but activated CTLs switch their metabolic program to glycolysis [142]. Signal transduction via the TCR and CD28 results in activation of phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), and mammalian target of rapamycin (mTOR) pathways, which consequently may increase the activity of hypoxia-inducible factor (HIF)-1α and Myc [143]. Upregulation of HIF-1α results in increased expression of glucose

transporter protein (Glut1), which augments glucose uptake. PI3K activation decreases the expression of enzymes involved in the TCA cycle as well as OXPHOS, whereas Akt and Myc increase the activity of several glycolytic enzymes [144–146]. All these events induce CTLs to increase glycolysis after activation steps [147]. Glycolysis is less efficient than OXPHOS due to lower ATP production per glucose molecule, but generates ATP molecules faster than OXPHOS and supports differentiation and functionality of effector T cells [61]. Hypoxia, excessive consumption of essential metabolites such as glucose and essential amino acids, production of large amounts of lactic acid, fatty acids, reactive oxygen species (ROS) by cancer cells may lead to decreased functionality of effector TCD8$^+$ lymphocytes and suppression of anti-tumor immune responses in TME [142].

7.4.1. Hypoxia

Hypoxia is low oxygen concentration status that commonly occurs following reduction of blood perfusion within tumoral tissue due to structural and functional alterations in the vessels, and also increased oxygen consumption, which refers to the uncontrolled proliferation of cancer cells. This results in insufficient delivery of oxygen and nutrients into the TME [148,149]. Different opinions were proposed about the effects of hypoxia on metabolism and CTL functions in the TME. Some researchers believe that HIF-1α, the main transcription factor that senses and responds to hypoxia, regulates CTL metabolism and suppresses their responses in the TME. Upon entry of CTLs to the TME and their confrontation with hypoxic conditions, HIF-1α becomes activated. In hypoxia, HIF-1α, by increasing pyruvate dehydrogenase kinase 1 (PDK1) expression, inhibits mitochondrial respiration, prevents the oxidation of pyruvate to acetyl-CoA, and by promoting the activity of lactate dehydrogenase A (LDHa), enhances glycolysis for energy generation [150,151]. Furthermore, increased HIF-1α activity inhibits the sustained Ca^{2+}-NFAT pathway, which controls production of effector molecules in activated T cells [152]. Several in vitro and in vivo studies have shown that hypoxia and increased HIF-1α activity suppress T cell activation, reducing their proliferation and decreasing their ability to produce necessary cytokines and lytic enzymes [153–157]. Additionally, hypoxia, due to increasing ROS accumulation, may induce apoptosis in activated CTLs [158,159]. Interestingly, some studies reported that cytokine production was increased in activated CTLs with a partial deficiency in HIF-1α [160,161]. Doedens et al. demonstrated that hypoxia or increased HIF-1α activity in CTLs may increase expression of co-inhibitors such as CTLA-4, LAG-3, and TIM-3, and decrease expression of *T*-bet, a key transcription factor that controls of T cell function. Moreover, these studies showed that hypoxia and increased HIF-1α activity may promote T cell effector functions, especially production of the proteolytic enzymes granzyme B and perforin. Of note, in these studies after 48 h of activation, CTLs were rested for several days in the IL2-supplemented medium before being subjected to hypoxia. Thus, maintenance of T cells in the IL-2-supplemented medium, due to metabolic reprograming and their decreased energy demand, may allow them to improve some functions in hypoxia [162]. Some other researchers believe that the effect of hypoxia or HIF-1α is limited to CTLs. They confirmed that deletion of VHL, a protein involved in the ubiquitination and degradation of HIF, may lead to HIF upregulation, resulting in differentiation of cytotoxic CD8$^+$ cells (CTLs), while depletion of HIF-1α reduces CTL function [61]. Hypoxia, in addition to its direct effect on CTL responses in the TME through increased surface expression of PD-L1 on cancer cells and enhanced the suppressive activity of tumor-infiltrating myeloid suppressor cells and tumor-associated macrophages, impairs PD-1$^+$ CTL survival and function [163–165].

7.4.2. Hypoglycemia and lactic acid

Glucose is critical to the survival, growth, and differentiation of T lymphocytes. Several studies in vitro and in vivo have demonstrated that hypoglycemia suppresses the effector functions of CTLs. High competition for glucose intake by cancer cells and activated T lymphocytes leads to hypoglycemia in the TME. Activated CTLs express increased levels of Glut1 to increase glucose uptake, but this effect may be neutralized by tumor cells. Consequently, impaired naive CTL activation may dampen their effectiveness and decrease generation of effector cytokines, even though some levels of proliferation may be preserved through OXPHOS [62,166–171].

The mTOR pathway and AMP-activated protein kinase (AMPK) are central energy monitoring systems of cells. Disorders of mTOR and AMPK activity are key signals that merge metabolic activity with cell activation and differentiation in T cells [146,151].

Hypoglycemia exerts its immunosuppressive effects via increasing PD-1 expression and decreasing mTOR signaling in activated CTLs, which lead to reduced glycolysis, enhanced fatty acid metabolism, and diminished IFN-γ and IL-2 production [63], as well as blockade of PD-1, resulting in augmentation of the glycolytic capacity through increased mTOR signaling and improvement of CTL function in the TME [62].

Glucose deprivation, with enhanced AMP and alteration of the AMP/ATP cellular ratio, leads to activation of AMP-activated protein kinase (AMPK) as an energy sensor in activated CTLs. AMPK, through inhibition of the mTOR pathway, decreases glycolysis and anabolic processes, while enhancement of OXPHOS by fatty acids and glutamine, as well as AMPK via blocking cytokine production, decreases T cell energy expenditure [172,173]. Furthermore, AMPK activation promotes Treg formation in both in vitro and in vivo conditions [174,175].

The glycolytic metabolite phosphoenolpyruvate (PEP) promotes T cell activation by sustaining TCR-mediated Ca^{2+}-NFAT signaling and suppressing sarco/ER Ca^{2+}-ATPase (SERCA) activity. SERCA is a Ca^{2+} ATPase that transfers Ca^{2+} from the cell cytosol to the sarcoplasmic reticulum (SR) lumen, increasing T cell effector functions [170].

Hypoglycemia due to high glucose flux in cancer cells results in limited glucose availability for T cell utilization. As a consequence, T cells not only are unable to develop tumoricidal effects, but also alter their differentiation program resulting in the generation of cell types that develop due to limited glucose supplies, such as Tregs and exhausted T lymphocytes [174,176].

Although increased glycolysis in activated CTLs causes lactic acid production and release, PD-1 signaling in these cells reduces glycolysis and lactic acid production; therefore, tumor cells are most likely the main source of lactic acid in the TME [176]. Increasing glycolysis in cancer cells produces excess lactic acid resulting in microenvironmental acidification. Microenvironmental acidification can: (1) suppress proliferation, cytokine production, and cytotoxic activity of CTLs [65,177], (2) alter macrophage polarization and shift them to the M2 suppressive phenotype [178], and (3) induce arginase 1 (Arg1), which promotes the depletion of extracellular arginine levels, resulting in inhibition of efficient T cell proliferation and activation [178,179]. Increasing lactic acid concentration in CTLs causes local acidification, which inhibits the activity of phosphofructokinase, a key glycolytic enzyme [177].

7.4.3. Fatty acids and cholesterol metabolism

Accumulation of adipocytes and adipocyte-like fibroblasts and production of large amounts of fatty acids by tumor cells indicate that the TME could be lipid-enriched [67,180,181]. Excessive amounts of fatty acids lead to leukocyte dysfunction in the TME. Many studies have shown that tumor-infiltrating MDSCs and DCs with abnormally high lipid content are respectively associated with strong immunosuppressive activity and weakened antigen presentation [182–185]. The fatty acid-enriched TME may affect effector T cell function, promote Treg development, and shift macrophages to the M2 suppressive phenotype [5,186].

Cholesterol is an important component of the plasma membrane in mammalian cells that often clusters in lipid rafts. Lipid rafts cluster at TCR-rich regions of T cells, thus cholesterol is regarded as a critical factor for TCR signaling [187]. Free cholesterol can be esterified by the cholesteryl esters ACAT1 and ACAT2, which can help their intracellular

storage [188]. Yang et al. proposed a mechanism by which the anti-tumor response of mouse CTLs can be potentiated via modulating cholesterol metabolism. Inhibiting cholesterol esterification in T cells by genetic ablation or pharmacological inhibition of ACAT1 leads to enhanced functionality and proliferation of $CD8^+$, but not $CD4^+$, T cells. This is probably due to selective increment of cholesterol in CTL plasma membranes, which causes enhanced TCR clustering and signaling, as well as increased immunological synapse formation. CTLs controlled melanoma growth and metastasis in ACAT1-deficient mice better than wild-type cells. Yang et al. indicated that avasimibe, an ACAT inhibitor that was previously applied in clinical trials to treat atherosclerosis, and also to treat melanoma in mice, has proved safe in humans, thus it may be an effective human antitumoric. Recently, a combined therapy of avasimibe plus an anti-PD-1 antibody showed greater efficacy than monotherapies in control of tumor progression [189].

7.4.4. Amino acid depletion

Tryptophan, an essential amino acid, has a crucial role in cancer cell survival. Overexpression of IDO, a metabolic enzyme that degrades tryptophan to kynurenine via reduced infiltration of tryptophan to *T*-lymphocytes, may suppress lymphocyte proliferation. Furthermore, kynurenine production promotes Tregs in the TME [65,190]. Cancer cells, MDSCs, and M2 type macrophages, through increased expression of Arg1, which degrades arginine, may have reduced arginine levels in the TME [178,191]. Glutamine is also required for T cell differentiation and function. This amino acid is also utilized by lymphocytes as an essential nutrient and helps in effector CTL development [172,192]. In several cancer types, enhanced glutamine metabolism, due to mutations and altered signaling pathways, may result in its decreased availability. Therefore, T cells encounter glutamine-deprived conditions in the TME [66]. Overall, excessive amino acid metabolism by cancer cells causes amino acid paucity in the TME, which can suppress CTL anti-tumor responses in this region.

8. Therapeutic interventions to restore exhausted CTL function in the TME

Recently, cancer immunotherapy via inhibitory receptor targeting by specific antibodies has been a major breakthrough. Blocking inhibitory receptors with specific antibodies may reverse exhausted T cell functions and anti-tumor responses. Currently-available antibodies that block T cell inhibitory receptors target CTLA-4, PD-1, and PD-L1 (Table 2). Among CTLA-4 blocking monoclonal antibodies, Tremelimumab and Ipilimumab are the most studied. Early-phase clinical trials demonstrated that Tremelimumab treatment resulted in sustained anti-tumor responses [193]. Ipilimumab was approved in 2011 by the American Food and Drug Administration (FDA) for the treatment of patients with advanced skin cancer [194,195], and is currently under study for treatment of patients with metastatic prostate cancer. As previously mentioned, blocking the PD-1-PD-L1 pathway could restore exhausted CTL functions and increase their anti-tumor responses. For this purpose, various antibodies have been produced and applied. Nivolumab was the first monoclonal anti-PD-1 antibody used to treat patients with advanced skin, kidney, and lung cancers; unfortunately, this antibody was only effective for up to one year and could not be applied for prolonged treatments [112, 196]. Pembrolizumab, another anti-PD-1 monoclonal antibody, in the first phase of clinical trials, caused satisfactory anti-tumor responses and no toxic side effects on T lymphocytes were seen [197]. Similarly, Pidilizumab, another anti-PD-1 monoclonal antibody, was initially used to treat hematologic malignancies and generated some steady-state responses [198]. In other studies, humanized monoclonal antibodies, including BMS-936559 and MPDL3280A, were used to block PD-L1. In clinical trials BMS-936559 led to long-term tumor regression in patients with kidney, lung, ovarian and skin cancers, caused limited side effects, and was well tolerated by patients [199]. Similarly, MPDL3280A was antitumoric in the treatment of prostate cancer and other advanced and metastatic solid tumors [200]. MEDI4736, another PD-L1 blocking antibody showed acceptable results in early stage clinical trials for treatment of advanced solid tumors; moreover, molecular engineering of the Fc region of this antibody improved results [201]. Currently, combinations of blocking antibodies against inhibitory receptors have shown promising results in advance stage cancer treatments. For example, a combination of Pidilizumab and Rituximab is, chimeric monoclonal antibody against CD20, recommended for the treatment of follicular lymphoma [113], a combination of Nivolumab and Ipilimumab has been used to treat advanced skin cancers [202], and a combination of Pembrolizumab and Ipilmumab has been approved for the treatment of malignant skin cancers [203]. Overall, combination therapies have been shown to be more effective against tumors than monoclonal monotherapies.

Table 2
Therapeutic interventions for blocking immune checkpoints.

Antibody	Target	Status of clinical trial	Cancer type	Refs.
Tremelimumab	CTLA-4	Phase II	Mesothelioma	[188]
Ipilimumab	CTLA-4	FDA approved	Melanoma	[189,190]
		Phase II and III	Solid tumors	
Nivolumab	PD-1	Phases I and II	Solid tumors, melanoma, NSCLC, RCC, ovarian cancer	[65,191]
Pembrolizumab	PD-1	Phase I	Melanoma, NSCLC, head and neck cancer	[192]
Pidilizumab	PD-1	Phases I and II	Hematologic malignancies	[66]
BMS-936559	PD-L1	Phase I	Solid tumors	[193]
MPDL3280A	PD-L1	Phase I	Solid tumors, melanoma, NSCLC, bladder cancer	[194]
MEDI4736	PD-L1	Phase I	Solid tumors, melanoma, head and neck cancer, gastric cancer	[195]

Abbreviations: CTLA-4, cytotoxic T lymphocyte antigen-4; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1; PD-L1, programmed cell death1 ligand 1; RCC, renal cell carcinoma.

Another cancer immunotherapy approach is application of CAR T cells. In this method T cells from peripheral blood are modified to express chimeric antigen receptors (CARs), which recognize cell surface-expressed tumor-associated antigens independent from the major histocompatibility complex and also co-stimulatory molecules, enhancing T cell anti-tumor responses [204]. The therapeutic outcomes of CAR T cells on acute lymphatic leukemia and B cell lymphoma were acceptable; however, in patients with solid tumors the results were disappointing. It is believed the TME can reduce CAR T cell-induced anti-tumor immunity [205–209]. Reducing the number of Tregs, MDSCs, or TAMs, or suppressing their functions as well as blocking the immune checkpoints, could improve the efficacy of CAR T cells in cancer immunotherapy [210,211]. Interestingly, CAR T cells, combined with PD-1 blockade, strongly augmented anti-tumor responses [212], Laboratory results confirmed that inhibitory checkpoint pathways such as PD-1 and CTLA-4 signaling can modify the metabolic programs of T cells [77,176]. PD-1-dependent signaling may inhibit glucose and glutamine transport and hinder hexokinase 2, which catalyzes the first step of glycolysis, leading to reduced glycolysis and amino acid metabolism in T cells. However, this signals through inhibiting the lipid oxidation PI3K pathway induce lipolysis and decrease lipid biosynthesis that increase the rate of fatty acid oxidation (FAO) in T lymphocytes

[176,213]. Increasing FAO after receiving PD-1 signals in T cells causes longevity of these cells in patients with chronic infections and cancer, thus PD-1 ligation alters the metabolic programming of T lymphocytes by inhibiting glycolysis and promoting FAO. In contrast, CTLA-4 signals without FAO augmentation may decrease glycolysis in T cells only through inhibiting expression of the glucose and glutamine transporters. It seems that CTLA-4 ligation maintains immune tolerance by preserving the metabolic profile of T lymphocytes [214]. As previously mentioned, the metabolic state of the tumor cells has a significant influence on longevity and functional fate of CTLs by altering nutrient availability and modifying their metabolic profile before their infiltration to the TME. Some studies revealed that pharmaceutical targeting of the glycolytic pathway in tumor cells by inhibitors of GLUTs, HK, PKM2, or LDHA may lead to tumor regression and increased glucose sources within the TME [215,216]. Chang et al. indicated that blocking PD-L1 reduces glucose consumption through suppressed glycolysis in tumor cells and increases glycolysis and effective function of CTLs in the TME [62]. Ho et al. showed that a glucose-deficient TME leads to reduction of phosphoenolpyruvate (PEP), a glycolytic metabolite essential for Ca^{2+}-NFAT signaling in CTLs, and increases the expression of phosphoenolpyruvate carboxykinase 1 (PCK1), which converts the TCA cycle intermediate oxaloacetate to PEP, augments NFAT signaling, and improves CTLs function within the TME [170]. On the other hand, some studies indicated that inhibition of glycolysis in CTLs increases their efficacy. During their activation naive CTLs switch their metabolic program to glycolysis, which may lead to terminal differentiation and shorten their survival. Thus, reducing glycolysis in these cells may improve therapeutic effects [217]. For example, inhibition of glycolysis in CTLs by 2-deoxyglucose enhances their anti-tumor function in a mouse melanoma model [218]. Furthermore, a series of studies demonstrated that CTLs, when treated with IL-7 or IL-15 in vitro, differentiate toward memory and enhancement of anti-tumor function in vivo [219–221]. Also in adoptive transfer condition, central memory CTLs have stronger anti-tumor responses than effector $CD8^+$ T lymphocytes [222,223]. We think that use of FAO and OXPHOS as the primary metabolic pathway in memory CTLs causes their superior performance within the TME.

9. Conclusion

Tumor cells utilize various mechanisms to overcome patients' immune systems. The presence and functions of specific T cells in the TME are directly related to the fate of tumor cells and subsequent patient prognosis. Numerous mechanisms limit T cell activity and tumor cell survival. Lack of nutrients, increased metabolic waste, and expression of inhibitory ligands by tumors lead to reduced metabolic fitness and decrease the ability of T cells to import nutrients. Moreover, immune suppressive agents in the TME lead to T cell dysfunction characterized by exhaustion and Treg phenotypes. In the TME, exhaustion is an important mechanism that reduces the cytotoxic function of CTLs and induces secretion of regulatory cytokines. Currently, restoring the function of these cells and increasing the anti-tumor response is regarded as a novel strategy in cancer treatment. Blocking inhibitory receptors, especially PD-1 and PD-L1, with monoclonal antibodies, has opened a new perspective in cancer treatment. Application of combination therapy has improved results in this area. In the case of using a singular blocking antibody, some types of exhausted CTLs change their phenotypes to effective ones, while simultaneous blocking of multiple inhibitory receptors resulted in improved outcomes. However, limitations exist in this therapeutic approach: first, the various functions of inhibitory receptors are not yet well known, for example, PD-1 and TIM-3 may regulate as yet undefined pathways in CTLs, second, restoring CTL function may intensify their cytotoxic activity, and third, blocking each inhibitory receptor alone had little effect, while application of combination therapies was more effective.

On the other hand, drugs that directly target key metabolic enzymes or their upstream regulators will likely effect metabolism of both cancer and T cells. Thus, understanding the similar and different metabolic processes of the two cell types may help to develop therapies that simultaneously improve anti-tumor responses while eradicating tumor cells. We have only begun to understand how the TME effects cancer and its treatment options, but we are confident that restoring the function of exhausted CTLs can be considered as a promising therapeutic approach for cancer treatment.

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