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Review article

Regulation of T cells in the tumor microenvironment by histone methylation: LSD1 inhibition—a new direction for enhancing immunotherapy

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ABSTRACT

Although immune checkpoint blockade (ICB) has been shown to achieve durable therapeutic responses in various types of tumors, only 20–40 % of patients benefit from this therapy. A growing body of research suggests that epigenetic modulation of the tumor microenvironment may be a promising direction for enhancing the efficacy of immunotherapy, for example, histone methylation plays an important role in the regulation of T cells in the tumor microenvironment (TME). In particular, histone lysine-specific demethylase 1 (LSD1/KDM1A), as an important histone-modifying enzyme in epigenetics, was found to be an important factor in the regulation of T cells. Therefore, this paper will summarize the effects of histone methylation, especially LSD1, on T cells in the TME to enhance the efficacy of *anti*-PD-1 immunotherapy. To provide a strong theoretical basis for the strategy of combining LSD1 inhibitors with *anti*-PD-1/PD-L1 immunotherapy, thus adding new possibilities to improve the survival of tumor patients.

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1. Introduction

Tumor microenvironment (TME) refers to the non-cancerous cells and components present in a tumor, including the molecules they produce and release. The ongoing interactions between tumor cells and the tumor microenvironment play a decisive role in tumorigenesis, progression, metastasis, and response to therapy. Functioning as a therapeutic target for cancer, TME has attracted great research and clinical interest [1]. Immune cells in the TME are known to have anti-tumor or pro-tumor functions, according to which immunotherapy for tumors was created [2]. Although advances in immunotherapy are very exciting, most patients do not respond to immunotherapy alone, except in melanoma [3,4]. In recent years, immune checkpoint modulators, represented by blocking antibodies

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against programmed cell death receptor 1 (PD-1: receptor on T cells) or programmed cell death 1 ligand 1 (PD-L1: an inhibitory ligand for PD-1, which is expressed at different levels by cancer cells), have shown unexpected anti-tumor effects in a variety of tumors, bringing a new era in tumor therapy [5]. However, most cancer patients are resistant to *anti*-PD-1 therapy due to multiple immunosuppressive mechanisms in the tumor microenvironment, including dysfunctional T cells and lack of T cell infiltration or recognition [6,7]. This raises the pressing question of whether the combination of *anti*-PD-1 therapy with other agents can robustly scale up clinical response and efficacy across a wider range of cancer subtypes.

To investigate this question, the method of epigenetic alterations is introduced. Epigenetic alterations can promote carcinogenesis by affecting the expression of a wide range of proto-oncogenes and oncogenes [8,9], and even by influencing the activation, differentiation, and immune function of immune cells (e.g., T-cells and natural killer cells [NK], which constitute immune surveillance mechanism) [10–14]. Epigenetic-based therapies aim to modulate the transcriptional programming of various signaling pathways affecting immune cells, other normal cells, and/or cancer cells, thereby altering the state of cell populations to achieve improved efficacy of antitumor treatment regimens [15–17]. Thus, epigenetic drugs are chemicals that act on the cellular epigenome to perform their functions. These drugs include DNA methyltransferases (DNMTs), DNA demethylases, histone deacetylases (HDACs), histone acetyltransferases (HATs), histone methyltransferases (HMTs), histone demethylases (HDMs/KDMs), and other inhibitors of related enzymes [18].

Histone lysine-specific demethylase 1 (LSD1/KDM1A), specifically removes one or two methyl groups located at the position 4 or 9 of histone 3 (Histone H3 Lysine 4, H3K4/Histone H3 Lysine 9, H3K9) with the help of Flavin adenine dinucleotide (FAD), thus leading to different transcriptional regulation modes. As high expression of LSD1 has been found in a variety of solid tumors and the phenomenon is closely associated with malignant transformation, epithelial-mesenchymal transition (EMT), stem cell biology, cell proliferation and differentiation, targeted inhibition of LSD1 is suggested to be a new direction for cancer therapy [19]. In addition, LSD1 can promote the process of tumor development by changing TME through regulating NK cells and macrophage polarization [20,21]. From this, we can see that LSD1 also has a regulatory role in the tumor microenvironment that is difficult to ignore. Specifically, LSD1 was found to have the function of regulating T cells in the TME and affecting the anti-tumor immunity of the body.

In summary, it is very feasible and extremely important to start from an epigenetic perspective to explore new strategies for combining immunotherapy to improve anticancer efficacy. Due to space limitations, this paper will only discuss in detail the regulatory role of histone methylation on T cells in TME. More importantly, the pharmacological or genetic inhibition of LSD1, which enhances the anti-tumor immunity of T cells and its molecular mechanism, will be the focus of this paper, with which we hopes to provide a theoretical basis for the combination of targeted LSD1 inhibition and *anti*-PD-1 mAb, and thus bring new hope for the treatment of "cold" tumor patients who have little response to immunotherapy.

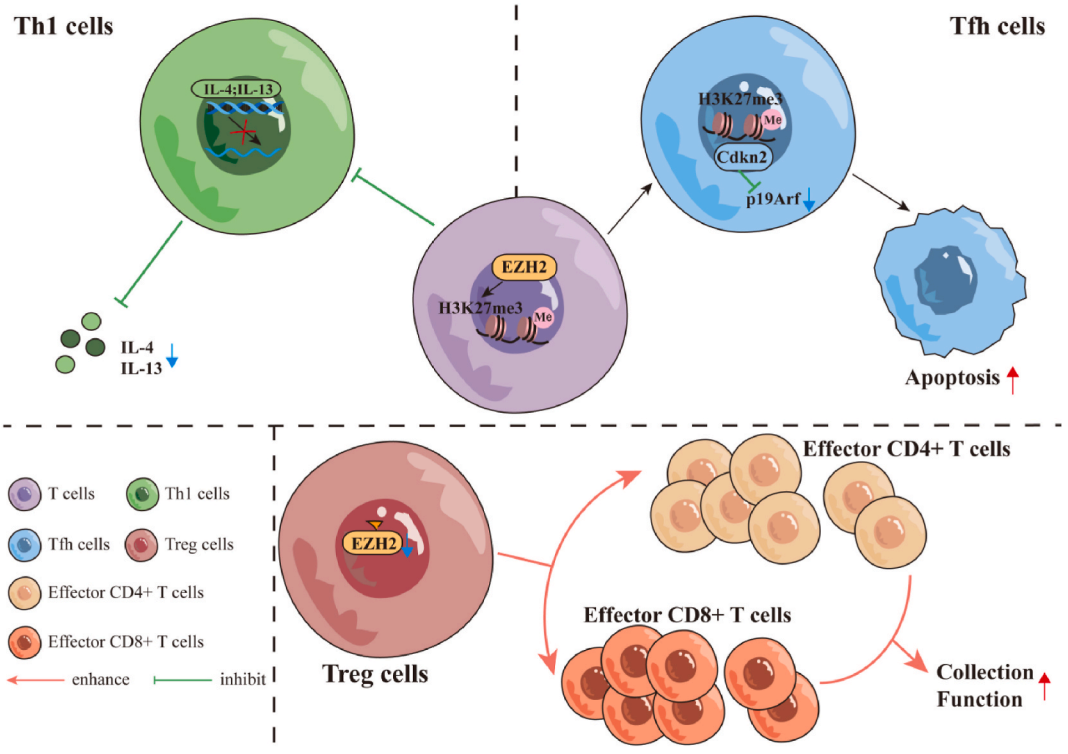

Fig. 1. Regulation of $CD4^+$ T cells in the TME by EZH2.

2. Histone methylation and T cells in TME

As the third major epigenetic modification, histone methylation is a reversible process, in which two major classes of enzymes (i.e., HMTs) that catalyze methyl addition are involved [22]. HMTs that methylate arginine residues are called protein arginine methyltransferases (PRMTs) [23], while those that methylate lysine residues are called histone lysine methyltransferases (HKMTs) [24]. In contrast, enzymes called HDMs/KDMs play the opposite role [25]. In general, methylation of histone H3 lysines 4, 36, and 79 (H3K4, H3K36, and H3K79) is associated with nonchromatin, whereas methylation of H3K9, H3K27, and H4K20 is associated with heterochromatin and gene silencing [26,27]. There are three forms of methylation of histone tail residues corresponding to the addition of mono-, dimethyl-, and trimethyl groups, each of which may have different effects on the formation of chromatin states [26,28].

It is found that certain HMTs and KDMs play important roles in the activation, differentiation, and functional stability of immune cells [18]. To further detail this view, here the author will describe the role of histone methylation or demethylation in the regulation of T cells in the TME.

2.1. $CD4^+$ T cells

$CD4^+$ T cells perform a variety of functions in the adaptive immune system, the best known of which is their role as T helper (Th) cells, including subsets such as Th1, Th2, Th17, and regulatory T cells (Tregs) [29]. Various Th cells can produce cytokines that influence the processes of $CD4^+$ effector T cells [30]. Specifically, Th1 cells secrete interferon-gamma (IFN-γ) and provide protection to the organism against intracellular pathogens and cancer; Th2 cells produce the cytokines interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13), which stimulate the production of antibodies by B-cells and are also involved in host defense against parasites [31]; Th17 cells involve in neutrophil-mediated protection against bacteria and link innate and adaptive immunity by producing the cytokines interleukin-17 (IL-17), interleukin-21 (IL-21), and interleukin-22 (IL-22) [32].

It has been well demonstrated that histone methylation plays a key role in the cellular differentiation and function of Th1 and Th2 cells [30] (Fig. 1). For $CD4^+$ T cells, histone methylation is involved in the regulation of the expression or silencing of key genes and key cytokines. For example, enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) and histone 3 trimethyl lysine 27 (H3K27me3) are associated with the gene silencing of IL-4 and IL-13 in Th1 cells [33]. EZH2 is an HMT that catalyzes H3K27me3 and affects Th1, Th2, and Tregs mainly by influencing HMT activity [18]. In addition, EZH2 causes apoptosis of T follicular helper (Tfh) cells and impairs the activation of Tfh transcriptional programming. Mechanistically, EZH2 deploys H3K27me3 to the promoter region of the cyclin dependent kinase inhibitor 2 (Cdkn2/p19Arf) gene and inhibits its expression in Tfh cells, resulting in abnormal downregulation of p19Arf and triggering apoptosis of Tfh cells [34]. Meanwhile, EZH2 is also critical to the regulation of Tregs. Wang D et al. found that the destruction of EZH2 activity in Tregs by genetic or pharmacological means resulted in the acquisition of proinflammatory functions by tumor-infiltrating Tregs and enhanced recruitment and function of $CD8^+$ and $CD4^+$ effector T cells [35]. In another study, genetic deletion of EZH2 in Tregs resulted in robust anti-tumor immunity in a mouse model [36].

EZH2-catalyzed H3K27me3 leads to: a. Silencing of IL-4 and IL-13 genes in Th1 cells. b. Apoptosis of follicular Tfh and impaired activation of Tfh transcriptional programming. In addition disruption of EZH2 activity resulted in tumor-infiltrating Tregs acquiring pro-inflammatory functions and even generating potent anti-tumor immunity and enhancing the recruitment and function of $CD8^+$ and $CD4^+$ effector T cells.

2.2. $CD8^+$ T cells

$CD8^+$ T cells contribute to the homeostasis of the organism. Cytotoxic $CD8^+$ T cells (CTLs) help destroy pathogens by secreting cytokines and directly killing infected cells, whereas long-lived memory $CD8^+$ T cells provide enhanced protection against reinfection. Memory $CD8^+$ T cells are differentiated from naive $CD8^+$ T cells through the phases including initial activation, proliferation, and antigen presentation after they are activated by antigens [37]. The whole process is tightly regulated by cell surface receptors, soluble factors, and transcriptional programs [37]. CTLs are the main immune cell population for the control and clearance of tumor cells. To generate an effective immune response, CTLs must be induced and activated before they are recruited to the tumor site [38].

Recent studies have shown that histone methylation has a non-negligible regulatory effect on $CD8^+$ T cells. Bian Y et al. found that tumor cells disrupted methionine metabolism in $CD8^+$ T cells, which reduced intracellular methionine levels and the methyl donor *S*-adenosylmethionine (SAM), thus leading to the loss of H3K79me2 [39]. Chromatin immunoprecipitation assay (ChIP) showed that H3K79me2 was directly involved in the regulation of the transcription of signal transducer and activator of transcription 5 (STAT5) in $CD8^+$ T cells through high binding to the promoter region of STAT5 [39]. Therefore, H3K79me2 loss leads to low STAT5 expression and impaired T cell immunity (mainly manifested as induction of $CD8^+$ T cell apoptosis and inhibition of $CD8^+$ T cell cytokines TNF-α and IFN-γ production in a dose-dependent manner) [39]. Existing studies have found a general relationship between gene expression levels and histone methylation distribution in $CD8^+$ T cell subsets, i.e., gene expression is positively correlated with H3K4me3 levels and negatively correlated with H3K27me3 levels [40]. H3K27me3 is predominantly catalyzed by the Polycomb Repressive Complex 2 (PRC2)and is associated with gene silencing [41,42]. The function of the genes in H3K27me3-rich genomic regions in naive $CD8^+$ T cells was found to be associated with cell signaling, transcription, metabolism, cell junctions, and cell adhesion. Meanwhile, memory $CD8^+$ T cells also are found to involve H3K27me3-rich genomic regions, in which exist relevant genes of type I interferons, apoptosis, cell adhesion, signaling, transporter, and cytoskeleton [40].

3. LSD1 and CD8$^+$ T cells in TME

LSD1 is epigenetically involved in the control of many cellular processes, such as autophagy, stemness, differentiation, senescence, cell proliferation and motility, and organogenesis [43–47]. Meanwhile, LSD1 regulates gene expression in cancer cells and immune cells to adapt tumor cells to TME and thus escape the body's immune surveillance [48]. Therefore, whether targeted inhibition of LSD1 can improve the body's anti-tumor immunity and prolong the survival of tumor patients with low response to immunotherapy has become a hot topic in recent years. Fortunately, the current study shows that pharmacological or genetic inhibition of LSD1 expression positively regulates the proliferation, activation, chemotaxis, and cytotoxicity of CD8$^+$ T cells in the TME (Fig. 2). In addition, such inhibition also can inhibit or even reverse the exhaustion of CD8$^+$ T cells (Fig. 2). In summary, targeting LSD1 is expected to be an important strategy to improve immunotherapy and provide a strong theoretical basis for opening a new chapter in anti-tumor immunity.

3.1. LSD1 regulates T cell proliferation and activation

In recent years, it has been found that during the co-culture of tumor cells or tumor-like organs in gastric cancer (GC), breast cancer (BC), especially triple-negative breast cancer (TNBC) and T cells, the inhibition of LSD1 expression can significantly elevate the secretion of three major reactive cytokines IL-2, IFN-γand TNF-α secreted during T cell proliferation and activation, and improve the expression of Ki-67 and CD69, one of the earliest cell surface antigens, in CD8$^+$ T cells [49–51]. In addition, Liu Y et al. found that compared to wild-type CD8$^+$, LSD1-deficient CD8$^+$ tumor-infiltrating leukocytes (TILs) still showed a significant increase in the number of cells after the use of the T-cell recruitment blocker FTY720 [52]. By molecular mechanism, the anti-tumor immunity of CD8$^+$ T cells is strengthened by the enhancement of the TCF1-controlled transcriptional network involved in CD8$^+$ T cell survival and self-renewal which is led by LSD1 exhaustion. These results suggest that while sustained CD8$^+$ T cell recruitment is a key basis for antitumor immunity, knockdown of LSD1 acts primarily on recruited CD8$^+$ T cells to enhance their intratumor proliferation [52].

In summary, these studies have shown that inhibiting the expression of LSD1 in tumor cells, such as gastric cancer and breast cancer, can effectively enhance the proliferation and activation of T cells, thereby improving the anti-tumor immunity of the body, regardless of in vivo and in vitro. However, most of the current studies only found that LSD1 can regulate the phenotype of T cell proliferation and activation and that, in terms of the specific mechanism, this process is related to the transcriptional network controlled by TCF1. Next, we can focus on exploring whether LSD1 affects T cell activation by altering the histone methylation level of the cytokines promoter region of T cell proliferation and activation and regulating its transcription, and whether the alteration of LSD1 expression regulates T cell proliferation and activation by affecting the TCR signaling pathway.

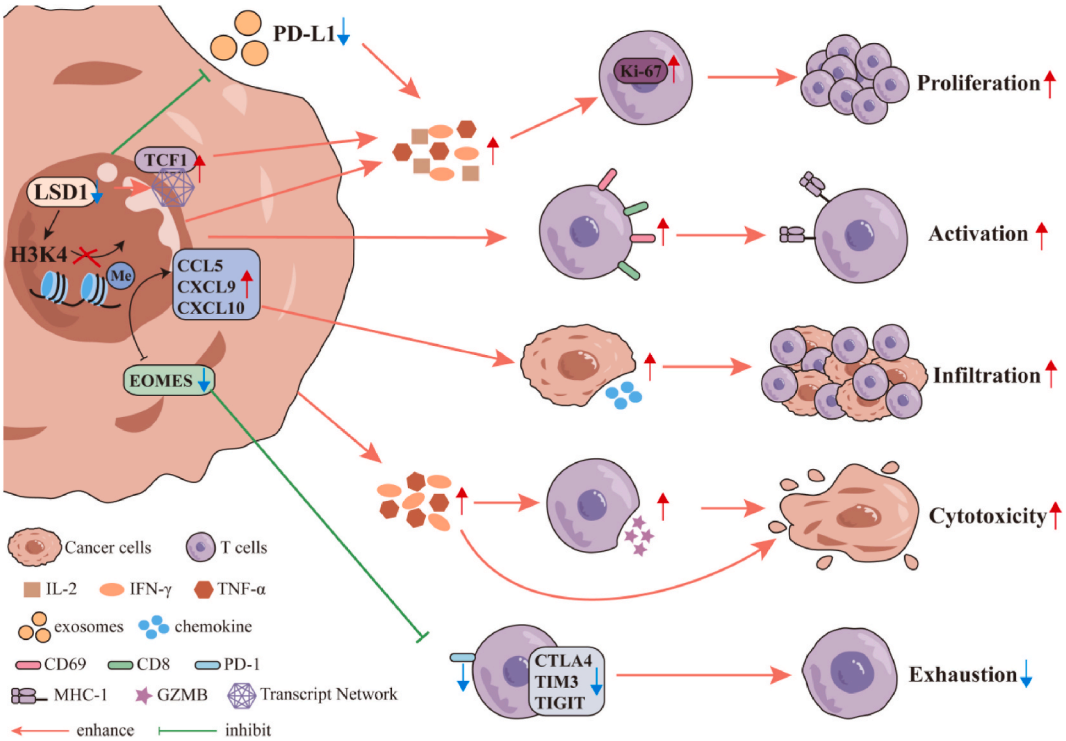

Fig. 2. Inhibition of LSD1 remodels the tumor microenvironment and enhances the anti-tumor immunity of T cells.

3.2. LSD1 regulates CD8$^+$ T cell infiltration into tumors

Anti-tumor responses mediated by immune checkpoint inhibitors (ICIs) depend on the infiltration of T cells capable of recognizing and killing tumor cells. ICIs are ineffective against "cold tumors" due to the lack of T cell infiltration. In order to realize the full potential of immunotherapy and address this obstacle, it is critical to understand the drivers of T cell infiltration into tumors [53].

It was found that knockdown of LSD1 in cancer cells of melanoma could upregulate the expression of endogenous retroviral element (ERV), activate dsRNA production thus leading to the activation of IFN pathway, and significantly increase the number of CD4$^+$ and CD8$^+$ T cells in mouse tumors [54]. More importantly, by knocking down LSD1 and blocking PD-1 and TGF-β, the T cell infiltration in B16 tumor bodies of melanoma in mice could be increased [55]. In addition, after inhibiting LSD1 expression in the tumor cells of gastric cancer, breast cancer, small cell carcinoma of the ovary-hypercalcemic type (SCCOHT), and head and neck squamous cell carcinoma (HNSCC) with pharmacological or genetic method, CD8$^+$ T cells were found to have higher tumor infiltrability [50–52,56,57]. Even when LSD1 of T cells was knocked down, there was a dramatic enhancement of infiltration of CD8+TILs in the late growth phase (day 18) of the tumor cells in mice [52]. Regarding the mechanism, Qin et al. found that the treatment of breast cancer with the LSD1 inhibitor HCI-2509 enhanced H3K4me2 enrichment in the remote region upstream of the Transcriptional Start Site (TSS) of the chemokine *C*–C motif chemokine ligand 5 (CCL5) promoter [58]. Meanwhile, Han Y et al. also found that in HNSCC, a negative correlation existed between LSD1 (KDM1A) and the expression of chemokines (CCL5, *C*-X-C motif chemokine ligand 9, chemokine ligand 10, *C*-X-C motif chemokine receptor 3, *C*-X-C motif chemokine receptor 4, *C*-X-C motif chemokine receptor 6, and *C*-X-C motif chemokine receptor 8 [CXCL9, CXCL10, CXCR3, CXCR4, CXCR6, and CXCR8]) which attracting CD8$^+$ T cells [57].

From the above, it is clear that inhibition or knockdown of LSD1 results in enhanced CD8$^+$ T cell infiltration in a variety of solid tumors. This phenomenon is mostly associated with increased expression of chemokines CCL5, CXCL9, CXCL10, and others. However, the currently clear mechanism by which LSD1 regulates chemokines is that the inhibition of LSD1 in breast cancer enhances H3K4me2 enrichment in the remote region upstream of the TSS of the chemokine CCL5 promoter, thereby promoting the transcription of CCL5. However, in other tumors, what was found is only a negative correlation between LSD1 and the expression of some chemokines, while the specific regulatory mechanisms for chemokines have not been clarified. Moreover, whether the effector chemokines regulated by LSD1 in multiple tumors are consistent remains to be explored.

3.3. LSD1 regulates the cytotoxicity of CD8$^+$ T cells

In addition to Granzymes (Gzms), cytotoxic granules secreted by CD8$^+$ T cells contain perforins, which are pore-forming proteins that disrupt the cell membranes of target cells and mediate the entry of Gzms into the cytoplasmic lysosomes of target cells, thereby leading to the death of tumor cells [59]. Furthermore, in TME, IFN-γconsistently coordinates pro-tumorigenic and anti-tumor immunity, acting as a cytotoxic cytokine along with Granzymes B (GzmB) and perforin to initiate apoptosis in tumor cells [60].

Shen et al. found that LSD1 KO cell-derived exosomes increased IFN-γexpression in mouse forestomach carcinoma (MFC) cells tumors of 615 mouse with gastric cancer [49]. In addition, Zhou Z et al. also found that inhibition of LSD1 in breast cancer significantly upregulated the expression levels of GzmB, IFN-γ, and TNF-α in CD8$^+$ T cells. Importantly, tumor patient-derived organoids (PDOs) treated with GSK-LSD1 exhibited higher T cell-mediated cytotoxicity i.e. higher mortality of tumor cells after co-culture of PDOs with CD8$^+$ T cells [51]. Surprisingly, different results were found in melanoma: in LSD1 KO tumors of mice, GzmB protein levels of CD8$^+$ GzmB$^+$ TIL were suppressed, while such levels were restored when TGF-β was also knocked down from tumor cells [55].

Therefore, it is concluded that in terms of the cytotoxicity of T cells, LSD1 also has a regulatory role. However, the regulatory role of LSD1 seems to be dual. Inhibition or knockdown of LSD1 in gastric and breast cancers resulted in a significant increase in cytotoxicity of CD8$^+$ T cells, but knockdown of LSD1 in melanoma instead suppressed the expression of GzmB proteins, which was restored when TGF-β was also knocked down in tumor cells. Besides, the mechanism by which LSD1 regulates cytotoxicity in T cells is ambiguous, and the intermediate genes or proteins that mediate this process are unknown. Meanwhile, how to find a way to positively enhance anti-tumor immunity from the duality of LSD1's regulatory role for T cell-mediated cytotoxicity remains to be addressed. Finally, whether the enhanced cytotoxicity will cause side effects to normal cells in the body needs to be further explored with experiments.

3.4. LSD1 regulates CD8$^+$ T cell exhaustion

In cancer, CD8$^+$ T cells are continuously exposed to antigens and inflammations, which compromises the effectiveness of CD8$^+$ T cells, and this state is called "exhaustion". Exhausted T cells are characterized by progressive loss of effector function (cytokines production and killing), expression of multiple inhibitory receptors (e.g., PD-1 and Lymphocyte activating 3 [LAG3]), metabolic dysregulation, poor immune memory responses, and homeostatic proliferation. These altered functions are closely related to altered transcriptional programs and epigenetics [61].

Researchers found that in breast cancer and melanoma, suppressing LSD1 in tumor cells could significantly inhibit the expression of depletion markers (PD-1, CTLA4, TIM3, and TIGIT, etc.) in CD8$^+$ T cells [50,58]. The regulatory mechanism of this process is that Eomesodermin (EOMES), a transcription factor for T cell depletion and effector, co-exists with Nuclear LSD1 phosphorylated at serine 111 (nLSD1p) in PD-1$^+$ CD8$^+$ T cells of drug-resistant patients, and nLSD1p regulates the nuclear dynamics of EOMES through a demethylation/acetylation switch of key EOMES residues, such that targeted inhibition of nLSD1p can reverse T cell depletion [50].

In conclusion, as an epigenetically important demethylase, LSD1 plays a regulatory role in T cell exhaustion. Compared with the mechanisms by which LSD1 regulates other biology phenotypes of T cells, the LSD1 regulation mechanism behind T cell exhaustion is more clear, namely, nuclear LSD1 regulates T cell exhaustion by modifying key residues of EOMES. However, current studies on this

mechanism are relatively few, which results in that the universality of this mechanism cannot be clear. In the future, a large number of molecular mechanism studies on a variety of tumors are still needed to explore the regulatory mechanism of LSD1 on T cell exhaustion, during which process, it is likely to find more molecular mechanisms in other cancer directions.

After inhibiting LSD1 in tumors, the secretion of IL-2, IFN-γ, and TNF-α is increased, and the proliferation and activation of T cells are therefore promoted. Meanwhile, the expression of chemokines CCL5, CXCL9, and CXCL10 are increased, thus enhancing the infiltration of T cells into the tumor. In addition, the high expression of IFN-γ, TNF-α, and GzmB results in a significant enhancement of the T cell-mediated cytotoxicity. Finally, the expression of T cell exhaustion markers PD-1, CTLA4, TIM3, and TIGIT is reduced, suggesting that inhibition of LSD1 could also inhibit T cell exhaustion.

4. Inhibition of LSD1 enhances immunotherapeutic efficacy of *anti*-PD-1 mAb

Genetic or pharmacological inhibition of LSD1 in combination with *anti*-PD-1 mAb significantly controls tumor growth in most solid tumors. In addition, in some tumors the phenomenon was associated with an increase in $CD8^+$ T-cell infiltration.

During the processes of tumorigenesis and tumor progression, PD-1 and PD-L1 synergistically inhibit anti-tumor immunity of the tumor host by the following routes: a. Inhibiting the activation of TILs and inducing its apoptosis, b. Inhibiting CTL granzymes, such that leading to tumor immune escape and perforin production, c. Decreasing the secretion of inflammatory cytokines, such as IFN-γ, IL-2, and TNF-α, and promoting the secretion of the immune suppressive cytokine IL-10, d. Stopping the T cell cycle, therefore leading to G0/G1 phase cell aggregation, e. Promoting tumor cell epithelialization, tumor metastasis, and tumor infiltration [62]. Based on the molecular mechanisms of the PD-1/PD-L1 pathway, various types of *anti*-PD-1/PD-L1 antibodies have been applied to treat tumors. However, the success of this therapy is only limited to a small percentage of patients [62].

A growing number of studies have found that LSD1 inhibitors, when combined with PD-1 blocking antibodies, have a strong effect in suppressing the growth of a wide range of solid tumors (Table 1). This combination therapy helps most patients overcome their resistance to PD-1 blocking antibodies, and more notably, improves TME, especially enhancing T cell infiltration and cytotoxicity (Table 1). Mechanistically, inhibition of LSD1 results in aberrant PD-L1 expression in tumor cells. However, the regulatory effects can be opposite in different types of tumors. Inhibition of LSD1 in melanoma, TNBC, SCCOHT, OCCC, and HNSCC leads to upregulation of PD-L1 expression, which is attributed to the fact that inhibition of LSD1 leads to increased H3K4me2 enrichment in the proximal element or core region of the transcription start site of the PD-L1 promoter, and thus activating PD-L1 transcription [54–58]. In contrast, LSD1 in GC is positively correlated with PD-L1 expression [49]. High expression of PD-L1 may inhibit the functional activity of CD8+TIL and induce tumor immune escape [63]. This shortcoming is eliminated when LSD1 inhibitors are combined with PD-1 blocking antibodies, which should be thanks to the fact that inhibition of LSD1 in GC downregulates PD-L1 expression, thereby inhibiting immune escape. In addition, in depleted $CD8^+$ T cells, a subpopulation of $TCF1^+$ PD-1^{int} progenitor cells within the tumor is an important determinant of effective response to PD-1 blockade. Knockdown of LSD1 in colon cancer cells preserves the subset of the TCF1+PD-1^{int} progenitor cell of depleted CD8+T cells in the TME, therefore enhancing the effective response of the organism to PD-1 blockade [52].

In conclusion, LSD1 is found to function as a regulator of PD-L1 expression in tumors but has different regulatory trends in different tumors. What is consistent is that using a blocking antibody to PD-1 while inhibiting LSD1 can greatly inhibit tumor growth or even eradicate the tumor. Therefore, the combination therapy of LSD1 inhibitors and PD-1 blocking antibodies may be one of the important strategies for combining epigenetics with immunotherapy to improve cancer treatment in the future.

5. Conclusion and future directions

Since LSD1 is an epigenetic regulator involved in a variety of physiological processes, it has been implicated in a variety of diseases,

Table 1
Inhibition of LSD1 in combination with *anti*-PD-1 therapy.

Cancers	LSD1 suppression	Immunotherapy (PD-[L]1)	Effects
Melanoma (B16)	LSD1 Knockout (LSD1 KO)	*anti*-PD-1 mAb	Inhibits tumor growth
	LSD1 Knockout (LSD1 KO) + TGF-β Knockout		Cooperatively potentiate both T cell infiltration and cytotoxicity that enables the eradication tumors
Breast cancer (BC [EMT6])	LSD1 inhibitor HCI-2509		a. Inhibits tumor growth b. Reduces the size of metastatic lesions in the lungs c. The ratio of $CD4^+$ to $CD8^+$ T cells in lymph nodes was significantly reduced
Colon adenocarcinoma (MC38)	LSD1 inhibitor GSK2879552		a. A cooperative effect on controlling tumor growth b. LSD1 inhibition preserves the progenitor exhausted $CD8^+$ TILs and sustains intratumoral T cell expansion, resulting in long-lasting responses of tumors to *anti*-PD-1 treatment
Head and neck squamous cell carcinoma (HNSCC [SCC7])	LSD1 inhibitor SP2509		a. Tumor growth inhibition b. Reduced Ki-67 levels c. Enhanced $CD8^+$ T cell infiltration

of which cancer is the most represented disease. LSD1 promotes cancer cell survival and makes the microenvironment cancer-friendly. Therefore, inhibiting LSD1 function is a powerful strategy to inhibit cancer and improve the body's anti-tumor immunity [48].

In summary, inhibition or knockdown of LSD1 regulates multiple biological phenotypes of T cells in a variety of cancers, such as gastric cancer, breast cancer, and melanoma. The effects of such inhibition or knockdown are detailed as follows: (a) the expression and secretion of IL2 increases; expression of Ki - 67 is significantly higher; the number of $CD8^+$ T cells increases significantly, which indicates that the T cell proliferation ability has improved. (b) In terms of activation, CD69 expression on $CD8^+$ T cells is significantly enhanced; secretion of three main reactive cytokines IL - 2, IFN-γ, and TNF-α generated during the process of T cells activation increases; the proportion of total $CD8^+$ IFN-γ^+ T cells is also upregulated. (3) The expression and secretion of CCL5, CXCL9 and CXCL10 are significantly increased after inhibition of LSD1 or co-inhibition LSD1 with TGF-β. Notably, H3K4me2 enrichment in the CCL5 promoter region is enhanced; subcutaneous transplantation tumor shows high $CD8^+$ T cell infiltration. (4) The secretion of IFN-γ and GzmB reflecting the cytotoxicity of $CD8^+$ T cells is significantly increased; when $CD8^+$ T cells are co-cultured with LSD1-inhibiting tumor cells, the proportion of remaining tumor cells also reflects the enhanced cytotoxicity of $CD8^+$ T cells. (5) expression of $CD8^+$ T cell exhaustion markers (PD-1, CTLA4, TIM3, and TIGIT) significantly increases; demethylation/acetylation of key residues of the exhaustion regulatory element EOMES is also switched by nLSD1p, thereby reversing T cell exhaustion.

Nevertheless, there are still many problems that need to be further explored. First, although a lot of research results show that inhibiting LSD1 can enhance the anti-tumor immunity of T cells, most current experiments only focus on its effects on the biology phenotypes of T cells, while the molecular mechanisms causing the phenotypes are mostly unknown. So far, only the regulatory mechanism of LSD1 on chemokine CCL5 in T cells is clearly understood, i.e. it is induced by the methylation of the promoter region at the transcriptome level, and the molecular mechanism of high expression of other chemokines has not been studied. In terms of exhaustion, what has been stated is that its molecular mechanism is associated with EOMES and dynamics. For the molecular mechanisms of proliferation, activation, and cell toxicity, current studies only focus on the high expression of the related proteins and the increase of the secretion of marked cytokines. Secondly, in terms of T cell infiltration capacity, although the fact that the inhibition of LSD1 results in the increasing of a variety of chemokines has been found, it is necessary to provide a lot of in-depth fundamental research in different solid tumors to prove the consistency of such conclusion.

It is worth noting that inhibition of LSD1 enhances the immunity of T cells, which brings new hope for the fight against tumors. However, whether the enhanced T cells will attack the normal cells of the body and produce other side effects needs to be further explored by enough basic and clinical experiments, thus to confirm the safety of this strategy before it can be considered a new idea for enhancing tumor immunotherapy.

Ethics statement

Review and/or approval by an ethics committee was not needed for this study because this is a review article and all experimental data were taken exclusively from the references.

Informed consent was not required for this study because this is a review article and all case data were taken from references.

Data availability statement

All data included in this article as a review are data from publicly available references. They are all available through Pubmed access.

CRediT authorship contribution statement

Xie Xueqing: W. **Peng Yongcan:** Writing – review & editing, Formal analysis, Data curation. **Lu Wei:** Methodology, Investigation. **Yin Qingling:** Resources. **Ding Jie:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.Ding Jie reports financial support was provided by the Department of Science and Technology of Guizhou Province. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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