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Review

The functions of polycomb group proteins in T cells

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ABSTRACT

T cells are involved in many aspects of adaptive immunity, including autoimmunity, anti-tumor activity, and responses to allergenic substances and pathogens. T cells undergo comprehensive epigenome remodeling in response to signals. Polycomb group (PcG) proteins are a well-studied complex of chromatin regulators, conserved in animals, and function in various biological processes. PcG proteins are divided into two distinct complexes: PRC1 (Polycomb repressive complex 1) and PRC2. PcG is correlated with the regulation of T cell development, phenotypic transformation, and function. In contrast, PcG dysregulation is correlated with pathogenesis of immune-mediated diseases and compromised anti-tumor responses. This review discusses recent findings on the involvement of PcG proteins in T cell maturation, differentiation, and activation. In addition, we explore implications in the development of the immune system diseases and cancer immunity, which offers promising targets for various treatment protocols.

1. Introduction

Epigenetics is defined as heritable changes in genomic function without altering the DNA sequence. DNA methylation and histone modification are two extensively studied epigenetic mechanisms involved in T cell development and immune responses. Epigenetics controls the whole lifespan of T cells. In the process of T cell lineage commitment, development, activation, or responding to external stimulus, the up-regulation of lineage specific genes and down-regulation of genes associated with other lineages, are controlled by dynamic epigenetic changes.

In mammals, DNA methylation is associated with gene silencing, and is catalyzed by DNA methyltransferases, which include DNMT1 (DNA methyltransferase 1) (maintenance of DNA methylation), DNMT3a and DNMT3b (*de novo* DNA methyltransferases). DNA demethylation is mediated by the ten-eleven translocations (TETs) family, which is composed of three members, TET1, TET2, and TET3. A genome-wide DNA methylation map for MPPs (multipotent progenitors), CLPs (common lymphoid progenitors), CMPs (common myeloid progenitors), GMPs (granulocyte/macrophage progenitors), DN1 (double negative cells), DN2, and DN3 demonstrates that lineage-related genes are dynamically regulated by differential DNA methylation (Ji et al., 2010). These results indicate an essential role of DNA methylation in T cells commitment.

Histones serve as the organizers of DNA, and they also undergo post-translational modifications that affect gene regulation. Although histone modifications may do not determine the transcriptional state of target genes directly, transcriptionally active genes tend to have an open chromatin structure that is marked by active histone modifications; repressive epigenetic markers are associated with closed chromatin structures that block promoter and gene access. H3K27ac (histone H3 lysine 27 acetylation) and H3K4me3 (histone H3 lysine 4 trimethylation) are associated with active promoters, H3K27ac and H3K4me1 (histone H3 lysine 4 monomethylation) are found at active genetic enhancers, while H3K27me3 (histone H3 lysine 27 trimethylation) and H2AK119ub1 (histone H2A lysine 119 monoubiquitination) are involved in promoter regions of transcriptionally silenced genes (Onodera and Nakayama, 2015; Tumes et al., 2017). The PcG proteins mediate histone modifications that affect cell development, differentiation, proliferation, activation, apoptosis, and fate transition via regulation of gene expression and chromatin structure (Gaballa et al., 2018). This phenomenon was originally identified in *Drosophila* and has been found in countless other species. The PcG machinery can be divided into two main complexes, namely PRC1 and PRC2 (Wu et al., 2013). PRC2 methylates histone H3 on lysine 27, and PRC1 catalyzes monoubiquitylation of histone H2A on lysine 119.

The purpose of this review is to summarize recently published data on the involvement of PcG in modulating T cell fate. This review will focus on four main topics: PcG in T cell development; PcG in T cell differentiation and activation; PcG regulation of T cell responses in immune system diseases; and PcG in tumor infiltrated T cells.

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2. Main body

2.1. Composition of polycomb complexes

PcG complexes form large multi-subunit complexes that maintain transcriptional repression by histone modification and chromatin compaction (Wu et al., 2013). The catalytic subunit of PRC2 is EZH1 or EZH2, which also contains RBAP46/48, SUZ12 and EED. The composition of PRC1 is more complicated compared with PRC2. The catalytic subunit of PRC1 is E3-ligases RING1A/B, which forms a heterodimer with one of the six PCGFs (PcG RING finger domain paralogs 1–6) (Chan and Morey, 2019). In mammals, PRC1 is categorized into canonical PRC1 (cPRC1) and noncanonical PRC1 (ncPRC1), depending on the presence or absence of a CBX (biological functions of chromobox) subunit. cPRC1 contains a PHC subunit (polyhomeotic-like protein 1/2/3), a CBX subunit (CBX2/4/6/7/8), and PCGF2 (MEL-18) or its homologue PCGF4 (BMI-1) (Chan and Morey, 2019; Wu et al., 2013). In contrast, ncPRC1 consists of RYBP (RING1A/B and YY1-binding protein) or its paralog, YAF2 (YY1 associated factor 2), which can associate with any of the six PCGF proteins (PCGF1/2/3/4/5/6) to assemble the PRC1.1-PRC1.6 complexes (Chan and Morey, 2019; Wu et al., 2013). ncPRC1 lacks the CBX subunit, instead, KDM2B, E2F6, and other components are associated (Fig. 1) (Table 1).

2.2. The mechanism of PcG transcription inhibition

PcG is one of the most studied epigenetic regulators generally associated with gene silencing (Nutt et al., 2020). Mechanisms include chromatin compaction, inhibition of transcription activation-associated histone modification, and maintenance of chromatin interaction to form a transcription inhibition-associated structure.

1) Chromatin compaction

Occupancy of nucleosomes within promoters and enhancers, and chromatin compaction, are essential mechanisms that maintain transcriptional silencing. Preincubation of PRC1 with nucleosomal arrays blocks chromatin remodeling complex SWI/SNF, and inhibits the recruitment of transcriptional machinery (Shao et al., 1999). In addition, PRC1 induces compaction of nucleosomal arrays, each Polycomb complex compacts three nucleosomes (Francis et al., 2004). And, cPRC1 compacts adjacent nucleosomes into globular structures (Lau et al., 2017). PRC2 also participates in the chromatin compaction. Chromatin compaction could occur before the establishment of H3K27me3. The high density of nucleosome arrays contributes to enhanced PRC2 activity (Yuan et al., 2012). Subsequently, H3K27me3 catalyzed by PRC2 is recognized by PRC1 protein CBX, and further recruits PRC1 to enhance the efficiency of chromatin compaction.

2) Inhibition of transcription activation-associated histone modification

PcG not only maintains transcriptional inactivity, but also inhibits the transcriptional activation-associated histone modifications to modulate the expression of some active PcG targeting genes. For example, CBX interacts directly with the histone acetyltransferase CBP (CREB-binding protein) to inhibit acetylation of histone H3, to exerts a repressive effect on transcription (Tie et al., 2016). Histone H2A ubiquitylation (ubH2A) catalyzed by PRC1 represses di- and trimethylation of H3K4, resulting in regulation of transcriptional initiation (Nakagawa et al., 2008). Similarly, all H3K36-specific histone methyltransferases show compromised enzymatic activities on nucleosome substrates containing ubH2A (Yuan et al., 2013).

3) Maintenance of chromatin interaction to form a transcription inhibition-associated structure

PRC1 clusters into speckles known as "PcG bodies'' in *Drosophila* and mammals. PRC1 and PRC2 recognize and bind to H3K27me3, forming a chromatin loop to keep target genes silenced. In this process, the SAM domain of PHC2 functions as an essential module to mediate PRC1 clustering (Isono et al., 2013). From studies using 5C and super-resolution microscopy, gene loci repressed by PRC1 forms isolated self-interacting domains mediated by PRC1 in pluripotent ESCs (Kundu et al., 2017).

2.3. Involvement of PcG in gene transcription activation

Interestingly, genome-wide profiles in both flies and mammals show PRC1 and PRC2 complexes are also recruited to active genes and active enhancer regions, and even act as transcriptional activators (Brand et al., 2019; Chan and Morey, 2019; Cohen et al., 2018; Cohen et al., 2019; Kuroda et al., 2020; Morey et al., 2015; van den Boom et al., 2016; Zhao et al., 2017). But the molecular mechanisms remain largely unknown. One possible reason is that PcG mediates the interaction between promoter and positive regulatory elements (Kondo et al., 2014). ncPRC1 complexes directly affect activation of gene expression through the recruitment of co-activators such as p300 (Brand et al., 2019; Zhao et al., 2017). Proteomic approaches and promoter occupancy analyses show Pcgf3/5 interacts with Tex10 (testis-expressed 10), which may directly contribute to transcriptional activation via the acetyltransferase p300. Coincident with this, Pcgf3/5 deletion in mouse ES cells significantly reduced the occupancy of Tex10 and p300 at target genes, and reduced expression of target genes (Zhao et al., 2017). ncPRC1.5 consists of at least five components, including RING1A/B, PCGF5, RYBP/YAF2, AUTS2 (autism susceptibility candidate 2), and CK2 (casein kinase 2). CK2 thwarts PRC1 repressive activity through phosphorylation of RING1B at S168, while AUTS2 mediates the recruitment of p300 to induce gene activation (Chan and Morey, 2019; Gao et al., 2014). PcG also regulates the topological transition of cis-regulatory elements. *Meis2* is a solitary PcG target gene with an essential developmental role in mammals. In early development, PcG is involved in both activation and repression of *Meis2* in a tissue-specific manner. Interaction between promoter, RBS (3' region downstream of the poly-A site, a Ring1B binding site), and MBE (midbrain specific enhancer), which is dependent

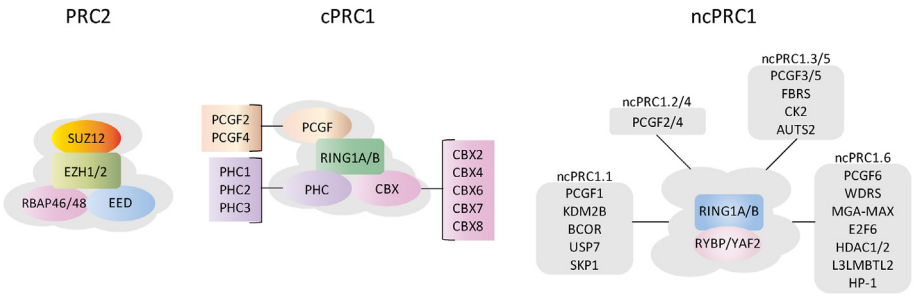

Fig. 1. Polycomb complexes in mammals. Schematic representation of PRC2, cPRC1 and ncPRC1 complexes in mammals. PRC2 is comprised of EZH1 or EZH2 with EED, SUZ12 and RBAP46 or 48. RING1B or its homologue RING1A comprises the catalytic subunit of PRC1. PRC1 is divided into cPRC1 and ncPRC1, which respectively include a CBX protein, or RING1 and YY1 binding protein (RYBP) or its homologue YY1- associated factor 2 (YAF2). They associate with either PCGF2/PCGF4 or PCGF1-6. The presence of several other interacting proteins give rise to PRC1 with different functionalities.

Table 1
PRC1 and PRC2 subunits and their roles.

Complex	Subunit	Important domain	Molecular function
PRC2	EZH1/2	SET	Histone methyltransferase (H3K27)
	SUZ12	VEFS-Box	Mediate the interaction between PRC2 and proteins
	EED	WD40	Required for allosteric activation
	RBAP46/48	WD40	Mediate the interaction between PRC2 and proteins
	JARID2	JmjC, zinc finger	Regulate PRC2 recruitment
	AEBP2	zinc finger	
	PHF19/PCL3	PHD, Tudor	
	PHF1/PCL1	PHD, Tudor	
	MTF2/PCL2	PHD, Tudor	
Canonical PRC1	CBX2/4/6/7/8	Chromodomain	Binding to chromatin (H3K27me3 - dependent)
	PHC1/2/3	SAM	Help form high- order structures, chromatin compaction
	RING1A/B	RING-finger	E3 ubiquitin ligase, catalyze H2AK119ub1
	PCGF2/4	RING-finger	Regulate enzyme activity and PRC1 recruitment
	SCMH1/2	SAM	Protein-protein interaction
Non- canonical PRC1	RYBP/YAF2	Zinc finger	Mediate the interaction between PRC1 and proteins, reader of H2AK119ub1
	RING1A/B	RING-finger	E3 ubiquitin ligase, catalyze H2AK119ub1
	PCGF1-6	RING-finger	Regulate enzyme activity and PRC1 recruitment
Accessory subunits of non-canonical PRC1	KDM2B	Jmjc, CxxC	Binding to unmethylated CGIs, histone demethylase (H3K36me2)
	CK2		Thwarts PRC1 repressive activity through phosphorylation of RING1B at S168
	AUTS2		Positive activator of transcription
	L3MBTL2	Zinc finger, MBT domain	Repressor of transcription, mediates chromatin compaction
	E2F6	E2F_DD, E2F_TDP	Regulate PRC1 recruitment

PRC, Polycomb repressive complex; EZH1/2, enhancer of zeste homologue 2; SUZ12, suppressor of zeste 12; EED, embryonic ectoderm development; RBAP46/48, retinoblastoma protein associated protein 46/48; JARID2, Jumonji and AT-rich interaction domain containing 2; AEBP2, AE binding protein 2; PHF, PHD finger protein; MTF2, metal response element binding transcription factor 2; CBX, chromobox; PHC, polyhomeotic-like protein; RING1A/B, really interesting new gene 1A/B; PCGF, Polycomb group ring finger; SCMH1/2, Scm polycomb group protein homologue 1/2; RYBP, RING1 and YY1 binding protein; KDM2B, lysine-specific demethylase 2B; CK2, casein kinase 2; AUTS2, autism susceptibility candidate 2; L3MBTL2, L3MBTL histone methyl-lysine binding protein 2; E2F6, E2F transcription factor 6; H3K27me3, trimethylated histone H3 Lys 27; H2AK119ub1, monoubiquitylated histone H2A Lys 119.

on Ring 1, is critical for the transcriptional status of the *Meis2* promoter. The promoter-RBS interaction is correlated with *Meis2* silencing. However, the interaction between promoter and MBE is correlated with activation of the *Meis2* promoter. The key step for transcriptional activation of *Meis2* is evicting Ring1B-bound RBS from the transient promoter-MBE-RBS tripartite (Kondo et al., 2014). PcG also participates in the regulation of active promoters in quiescent B cells and T cells. Ring1B binds to a range of active genes in resting B cells and G1-activated B cells, but the composition of PRC1 enriched at active genes changes during activation. Knockout of *Ring1B* in murine resting B cells and T cells shows a dramatic reduction in transcription of a group of active genes, compared with WT (wild type) cells. And, there is reduced binding of unphosphorylated RNA polymerase II (RNAPII) and RNAPII-S5ph at active gene promoters, and PNAPII-S2ph within transcription units (Frangini et al., 2013). PcG proteins are also positive regulators of signature cytokine genes in Th cells (Hod-Dvorai et al., 2011; Jacob et al., 2011). Overall, PcG complexes play dual roles in both gene repression and activation.

2.4. PcG in T cell development

T cells are derived from HSCs (hematopoietic stem cells) in BM (bone marrow), and undergo a series of maturation steps in the thymus, the primary site of T cell lymphopoiesis. The earliest precursor and the most immature double-negative (DN) subpopulation in the thymus is ETP (early thymic progenitor). T cell commitment occurs at DN2 stage, with the loss of ability to differentiate into other hematopoietic lineages. Transcription factor Bcl11b is expressed starting from DN2 stage, and is critical for normal T cell development. T cells undergo a series of well-documented differentiation steps, in which cells with a functional TCR mature into single-positive (SP) $CD4^+$ or $CD8^+$ T cells. These cell types leave the thymus and travel to the periphery (Fig. 2). In the process of cell fate determination or lineage commitment, there is a general trend that stemness related genes, and genes associated with other lineages, are deposited with repressive epigenetic marks; but genes specific for one lineage become epigenetically more “open” (Kitagawa et al., 2015; Naito and Taniuchi, 2013). Polycomb proteins are differentially expressed in distinct thymocytes and T cells, suggesting they are involved in the regulation of hematopoiesis (Raaphorst et al., 2001).

Polycomb repressive mechanisms, H3K27me3 and H2AK119ub1, restrict the expression of lineage specific genes in progenitors, delay their activation and ensuing lineage commitment (Pease et al., 2021). In early T cell progenitors, Notch signals induce the expression of Tcf 7 and Gata3, which activates *Bcl11b*. Although the upstream regulators become active rapidly after pro-thymocytes enter the thymus gland, *Bcl11b* activation and T cell commitment occur 5–10 days later. This time-delay in gene activation is partly caused by an epigenetic switch. Both *Bcl11b* alleles are inactive in bone marrow progenitors, with broad H3K27me3 marks across the 5’ end. These abundant H3K27me3 marks are reduced by half in monoallelic *Bcl11b* expressing DN2 progenitors, and are almost completely lost in biallelic *Bcl11b* expressing DN2 progenitors, indicating H3K27me3 is gradually removed from the *Bcl11b* locus upon activation (Pease et al., 2021). When treated with small molecule inhibitors of Ezh2, or knockdown of *Eed*, the activation rate of the silent allele is significantly increased in the monoallelic *Bcl11b* expressing DN2 progenitors. In contrast, the activation rate is decreased when progenitors are treated by the inhibitor of H3K27 demethylases Kdm6A/B, GSK-J4. These results indicate H3K27me3 levels at the *Bcl11b* locus control the timing of *Bcl11b* activation (Pease et al., 2021).

The importance of PRC1 in T cell development has also been evaluated. *Mel-18* mutation in mice decreases absolute number of lymphoid cells in bone marrow, and T and B cells in spleen, suggesting it plays an essential role in lymphocytes development. *Mel-18*$^{-/-}$ causes defects in the early thymocyte development as early as c-Kit^{hi} DN1 stage (Akasaka et al., 1997; Miyazaki et al., 2005). YY1 (Yin Yang 1) is a ubiquitous transcription factor as well as a Polycomb protein, which contains a YY1 PcG function/REPO domain. In a context dependent manner, YY1 acts as a transcriptional activator or repressor. YY1 is a crucial factor regulating cell viability, proliferation, apoptosis, cellular metabolism, and inflammatory response (Atchison, 2014; Meliala et al., 2020; Verheul et al., 2020). Xuan Pan et al. measured the effect of *Yy1* conditional knockout on early T cell development, and demonstrated that YY1 PcG function/REPO domain is required for promoting early T cell survival and DN1 to DN2 transition. YY1 binds to the enhancer of *Notch1* locus, to positively regulate the expression of *Notch1* and target genes (Assumpcao et al., 2021). Bmi-1 is involved in the survival of activated DN3 cells and

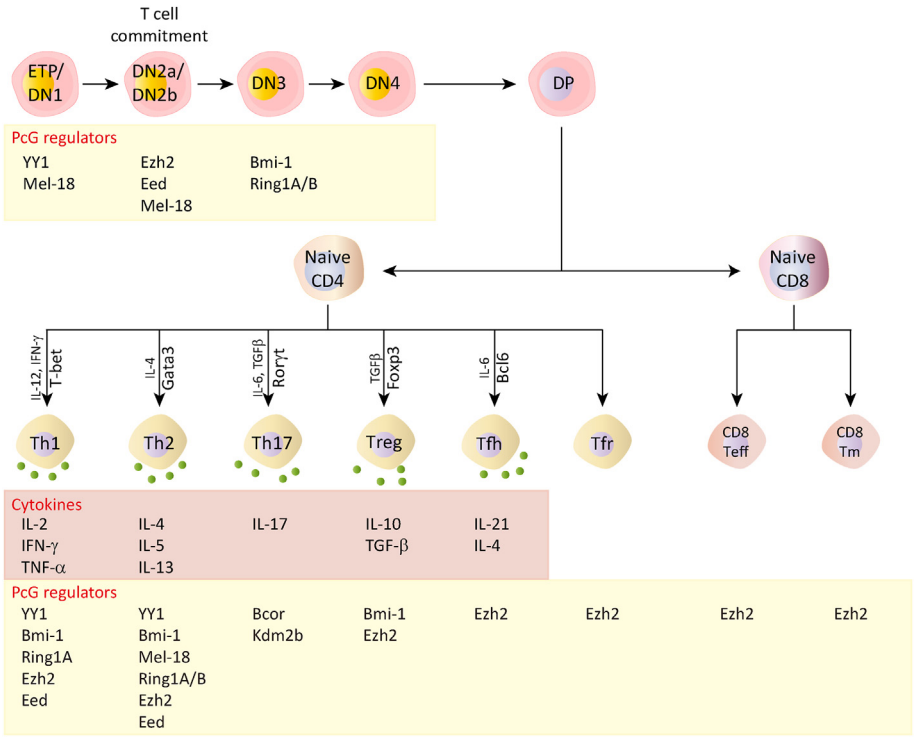

Fig. 2. PcG proteins in T cell development and differentiation. The figure shows T cell development and differentiation, the key transcription factors for each subtype, signals that induce differentiation, cytokines secreted by Th cells, and Polycomb proteins involved at each stage or cell type. Signals that induce differentiation are shown by the left of arrows, key transcription factors are shown on the right, PcG proteins are shown in yellow boxes, specific cytokine profiles produced by Th cells are shown in the orange box. Immature T cells migrate from bone marrow to the thymus for differentiation. Early T cell precursors (ETP) differentiate from double negative (DN) to double positive (DP) to single positive (SP) stages. The ability to differentiate into other hematopoietic lineages is lost, with irreversible commitment to the T cell lineage occurring at the DN2 stage, which requires the transcription factor Bcl11b. Upon stimulation, naive $CD4^+$ T cells differentiate into effector Th cells (Th1, Th2, Th17, Treg, Tfh and Tfr). Naive $CD8^+$ T cells differentiate into effector T cells ($CD8^+$ Teff) and memory T cells ($CD8^+$ Tm) after encountering antigen and in response to signals in their microenvironment.

thymocytes development. Bmi-1, as well as CBX2, bind to the *Cdkn2a* gene locus in immature DN cells to maintain the repressive H3K27me3 modification. Therefore, *Bmi-1* ablation induces p19Arf (encoded by *Cdkn2a*) overexpression in DN cells, and activation of the p53 pathway that leads to a differentiation arrest at DN3 stage and a blockade of DN-DP transition in mice (Miyazaki et al., 2008).

Epigenetic suppression of B-lineage gene program is critical for the maintenance of T cell fate. Ring1B binds to several B-lineage-associated genes, as well as H3K27me3 modification in thymocytes, to inhibit the expression of these genes during early T cell development. Using a T cell specific *Ring* knockout mouse model ($Lck^{Cre}Ring1A^{-/-}Ring1B^{fl/fl}$), *Ring1B* expression is deleted at the DN2–3 stage in the thymus. In this double knockout model, $TCR\beta^+$ cells are nearly absent, and T cell development is arrested at the DN3 stage, but B cell lineage-specific genes are up-regulated. These cells fail to generate T cells, instead, they give rise to $CD19^+IgM^+$ B cells when transferred to irradiated immunodeficient mice. By contrast, in $CD4^{Cre}Ring1A^{-/-}Ring1B^{fl/fl}$ mice, frequency of T cells is unchanged, implying Ring1A/B is dispensable at later stages of T cell development (Ikawa et al., 2016).

2.5. PcG in $CD8^+$ T cell differentiation and activation

During antigenic stimulation, naive $CD8^+$ T cells differentiate into heterogeneous effector T cells (or cytotoxic T lymphocytes [CTLs]) and long-lived memory T cells (Fig. 2). Upon activation, Ezh2 is induced in $CD8^+$ T cells (Gray et al., 2017). This epigenetic regulator is required for the expansion, terminal differentiation, and anti-viral capacity of effector $CD8^+$ T cells. Deficiency of *Ezh2* in $CD8^+$ T cells impairs cell cycle progression from G0/G1 to S phase, leading to defection in activation induced proliferation. In *Ezh2* knockout studies, the H3K27me3 marks at *Cdkn1c* and *Cdkn2a* (cyclin-dependent kinase inhibitors) are down-regulated, resulting in increased expression of *Cdkn1c* and *Cdkn2a*, which may contribute to the interrupted cell cycle (Chen et al., 2018). The involvement of PRC2 in proliferation of $CD8^+$ T cells has been confirmed by other groups. *Ezh2* deficiency in activated $CD8^+$ T cells ($Ezh2^{fl/fl}GzmB^{Cre}$ mice) impairs the generation of LCMV-specific anti-viral $CD8^+$ T cells, and has delayed viral clearance compared with control littermates (Gray et al., 2017). PRC2 is required to maintain epigenetic silencing of pro-memory genes in effector $CD8^+$ T cells. During viral infection, most $CD8^+$ T cells differentiate into terminal effector (TE) cells, lose memory potential, and die. However, a subset $CD8^+$ T cells, named memory precursor (MP) effector cells, are multipotent and more fit to persist (Gray et al., 2017). Genome-wide analysis of H3K27ac and H3K27me3 in MP and TE effector $CD8^+$ T cells show that in TE cells many pro-memory genes are modified by high levels of repressive H3K27me3. But in MP cells, both the MP- and TE-associated genes are maintained at permissive or active chromatin states. That is say, while TE cells lose memory potential, MP cells still possess multipotency for both memory and effector fates. When *Ezh2* or *Eed* is knocked out in T cells or activated $CD8^+$ T cells, the differentiation of TE $CD8^+$ T cells is significantly reduced. As the generation of TE $CD8^+$ T cells decrease, more MP-like cells are acquired. Unlike TE cells, PRC2 has little effect on MP T cell generation, but is required for their protective immunity (Gray et al., 2017).

2.6. PcG in $CD4^+$ T cell differentiation and activation

Naive CD4 T cells, on activation, differentiate into specific subtypes, including Th1 (T-helper 1), Th2 (T-helper 2), Th17 (T-helper 17) cells, Treg (regulatory T cell), Tfh (follicular helper T cell), and Tfr (follicular regulatory T cell); all induce different types of immune responses (Onodera and Nakayama, 2015). The differentiation of Th cells mainly depends on cytokines they received. IL-12 and IFN-γ contribute to Th1 differentiation, IL-4 is involved in Th2 cell differentiation, IL-6 and TGF-β are involved in the differentiation program of Th17 cells, IL-6 strongly potentiates Tfh differentiation, and Treg differentiation requires TGF-β. Lineage-specific master transcription factors that impart specific effector cytokine profiles in different Th subsets have been identified. T-bet (Szabo et al., 2000), GATA3 (Zheng and Flavell, 1997) and RORγt (Ivanov et al., 2006) are master regulators of differentiation of Th1, Th2 and Th17 cells, respectively. Bcl6 is the master transcription factor of Tfh cells (Chtanova et al., 2004), and Foxp3 controls the differentiation of Treg (Fontenot et al., 2003; Hori et al., 2003; Khattri et al., 2003). Different Th cells produce distinct cytokines that define their subsets.

Th1 cells produce IL-2, IFN-γ and TNF-α; Th2 cells produce IL-4, IL-5 and IL-13; Th17 cells secrete IL-17; Tfh cells secrete IL-21 and IL-4; and the signature cytokines of Treg cells are IL-10 and TGF-β (Fig. 2).

The binding activity of the Polycomb proteins to cytokine genes in Th cells is associated with cytokine transcription. YY1 binds to the promoter and CNS1 (conserved non-coding sequence) of *Ifng* in Th1, but not Th2 cells. In contrast, the promoter and other transcription regulation elements of *Il4* are more strongly bound by YY1 in Th2 cells. The binding is enhanced in response to stimulation *in vitro*. Bmi-1 and Ring1A also bind to *Ifng* and *Il4* genes, in a similar way to YY1. Generally, binding activity is positively associated with expression of cytokine genes. Ezh2 and Eed selectively bind to the promoter and CNS1 of *Ifng* in Th1 cells, but bind to the promoter of *Il4* in Th2 cells. However, the binding activity to other regulatory elements of *Il4* is less selective. PcG proteins binding to the active genes implies they are involved in gene activation (Jacob et al., 2008).

The importance of PcG proteins in regulating $CD4^+$ T cell differentiation has been shown by generating effector Th subsets *in vitro*. Under the Th2-polarizing condition, *Bmi-1* overexpression in fresh mice $CD4^+$ T cells enhances the development of Th2 cells and the production of IL-4, IL-5, and IL-13; in contrast, under the Th1-polarizing condition, no difference was observed in Th1 generation. *Bmi-1* deficiency has no effect on the formation of Th1 and Th2 cells under Th1/Th2- skewed conditions, but there is a significant reduction of GATA3 expression and Th2 cell generation under neutralizing conditions. In mouse T cell line TG40, Bmi-1 overexpression inhibits the degradation of GATA3. Therefore, Bmi-1 controls the stability of GATA3 proteins to facilitate Th2 cell differentiation (Hosokawa et al., 2006). Similarly, Mel-18 also contributes to Th2 cell differentiation. Formation of Th2 cells is compromised in the absence of *Mel-18 in vitro*, with an impaired production of IL-4 and decreased expression of GATA3 under the Th2-polarizing condition, which can be rescued by introduction of *Gata3*. Th2 differentiation is accompanied by demethylation at the *Il4* gene. Interestingly, reduced demethylation at *Il4* locus is correlated with *Mel-18* deficiency, which may contribute to the defect of Th2 differentiation (Kimura et al., 2001). Ezh2 directly binds and facilitates correct expression of *Tbx21*, *Gata3* and other lineage-specific genes in differentiating Th1 and Th2 cells via H3K27me3 modification. During Th cell differentiation, the occupancy of Ezh2 at *Tbx21 and Gata3* loci show the most dramatic changes. Ezh2 binds at the *Gata3* locus and catalyzes H3K27me3 in naive and Th1 cells, but is impaired in Th2 cells. In contrast, Ezh2 is recruited to the *Tbx21* loci to catalyze H3K27me3 in Th2, but is absent in Th1 cells. Inhibition of Ezh2 activity in naive $CD4^+$ T cells induces depleted H3K27me3 marks at *Tbx21* and *Gata3* loci, coincident with strongly enhanced *Tbx21* and *Gata3* expression, as well as Th1 and Th2 cells differentiation under the Th1 or Th2 cell-inducing conditions (Tumes et al., 2013). Even under the nonpolarizing Th0 conditions, the production of Th1 cytokine IFN-γ and the Th2 cytokines IL-4, IL-5, and IL-13 are elevated in *Ezh2* knockout $CD4^+$ T cells (Yang et al., 2015; Zhang et al., 2014). Furthermore, both *Ezh2*-deficient Th1 and Th2 appear to be more plastic. However, Ezh2 occupancy at the *Rorc* locus in Th cells activated without polarizing cytokines and Th17 cell-skewed conditions are very low (Tumes et al., 2013; Zhang et al., 2014). Although *Ezh2* deficient Th cells produce more effector cytokines under Th1- or Th2- polarizing conditions, they are functionally inadequate, as they can't prevent the *T. gondii* infection effectively *in vivo* (Yang et al., 2015).

Polycomb dictates naive $CD4^+$ T cells dividing into distinct effector T subsets during acute viral infection (Cho et al., 2013; Kotov et al., 2019; Li et al., 2020). *Ezh2*-deficiency in T cells impairs the competitiveness compared with WT T cells when they are mixed at equal ratios and co-transferred into congenic WT recipients that are subsequently infected with acute lymphocytic choriomeningitis virus (LCMV) Armstrong strain virus. After infection, the ratio of *Ezh2*-deficient $CD4^+$ T cells is decreased. Moreover, upon acute viral infection, virus-specific $CD4^+$ T cells differentiate into either Tfh cells or Th1 cells. *Ezh2* deficiency blunts the mTOR signaling pathway, leading to impaired expansion of both Tfh and Th1 cells. These results reveal the Ezh2 protein enhances $CD4^+$ T cell expansion in response to acute viral infection by fine-tuning the target of rapamycin (mTOR) signaling (Li et al., 2020). Th17 is a more phenotypically flexible Th lineage, its differentiation is regulated by PRC1.1 components BCOR and KDM2B. They are required for maximal differentiation of Th17 cells in response to Th17-inducing pathogen-*Streptococcus pyogenes* infection by repressing Th17 fate suppressors (Kotov et al., 2019).

A specialized subset of suppressor $CD4^+$ T cells, termed Treg cells, play a particularly important role in maintaining immune homeostasis, prevention of autoimmunity, and represents a major barrier to the induction of robust anti-tumor immune responses (Savage et al., 2020). FOXP3, encoded by the *Foxp3* gene on the X chromosome, is the master transcriptional factor of Treg cells. This transcription factor enforces lineage development and immune suppressive function, and is specifically expressed in Treg. Dysfunction of *Foxp3* in mice and humans causes a complex autoimmune disorder known as IPEX (immune dysregulation polyendocrinopathy, enteropathy, X-linked syndrome) (Bennett et al., 2001; Brunkow et al., 2001; Wildin et al., 2001). The roles of Ezh2 in various subsets of $CD4^+$ T cells, including Treg, are not clear. For example, in one report, *Foxp3* promoter is epigenetically regulated by Ezh2. *Foxp3*-negative T cells (T cells that do not express Foxp3) have an enrichment of H3K27me3 modification on its promoter. Overexpressing Ezh2 in primary naive murine $CD4^+$ T cells blocks *Foxp3* induction upon stimulation, indicating Ezh2 binds to the core promoter of *Foxp3* to maintain repressed state until the moment of its induction. PCAF (p300/CBP-associated factor), the GCN5 family member of HATs, functions to antagonize the silencing effects of PRC2 on *Foxp3* (Xiong et al., 2012). But in a different study, *Ezh2* knockout in T cells ($Ezh2^{fl/fl}CD4^{Cre}$) results in reduction of both the percentage and number of $Foxp3^+$ cells in the secondary lymphoid organs. In addition, T cells fail to differentiate into Treg cells in the absence of Ezh2. The lower Foxp3 expression is attributed to aberrant expression of effector cytokines in $Ezh2^{fl/fl}CD4^{Cre}$ T cells. Elevated IL-4 and IFN-γ activate downstream STAT6 and STAT1, respectively, to inhibit the expression of Foxp3. Therefore, IL-4 and/or IFN-γ neutralizing antibodies can rescue the expression of Foxp3 effectively. Furthermore, *Ezh2*-deficient Treg cells fail to protect against experimental colitis, suggesting they are functionally defective (Yang et al., 2015). Consistent with Ezh2 positively regulating the immunosuppressive function of Treg, Bluestone et al. showed that anti-CD3 and anti-CD28 antibody co-stimulation enhanced Ezh2 expression in murine Treg cells. There are more H3K27me3 marks in aTreg (activated Treg) cells than rTreg (resting Treg) cells *in vivo* (DuPage et al., 2015). Ezh2 is required to maintain the identity of Tregs during Treg cell responses, as a result, the deficiency of *Ezh2* in Treg causes cells to fail immune homeostasis, resulting in lethal multiorgan autoimmunity (Sarmento et al., 2017). By RNA-seq analysis of aTreg or rTreg cells from WT, $Ezh2^{fl/+}Foxp3^{cre}$ and $Ezh2^{fl/fl}Foxp3^{cre}$ mice, the authors revealed that Ezh2, via H3K27me3 deposition, is required for the repressive gene program in Treg cells during cellular activation. More interesting, 58.8% of all DEGs (differentially expressed genes) in *Ezh2*-deficient Treg cells overlap with DEGs from *Foxp3* knockout Treg cells. Foxp3 acts predominantly as a repressor of gene transcription in Treg cells. Foxp3-bound sites are less accessible, with a deposition of H3K27me3 marks after Treg activation. This indicates Foxp3 cooperates with PRC2 to promote the Treg cell program in response to TCR signaling and inflammatory cues (Arvey et al., 2014; DuPage et al., 2015). Results indicate Foxp3 forms a complex with Ezh2 in aTreg cells but not in rTreg cells. Following activation, Ezh2 is recruited to the Foxp3-bound site and deposits H3K27me3, which is associated with diminished accessibility of chromatin and compromised expression of nearby genes (Arvey et al., 2014).

After antigen activation, rapidly proliferating B cells expand and undergo somatic hypermutation and selection, and further differentiate into memory B cells, or long-lived antibody producing plasma cells in the germinal centers (GCs) (Ding et al., 2021; Mintz and Cyster, 2020). In this

process, the balance of Tfh/Tfr cells is critically important for the maintenance of immune homeostasis. $CD4^+$ GC-Tfh cells are a subset of specialized $CD4^+$ T cells that provide costimulatory molecules and cytokines to help in the differentiation of B cells into memory cells or plasma cells. The transcriptional repressor Bcl6 functions as a "master regulator" to govern early Tfh cell formation by inhibiting the expression of genes that promote differentiation into other lineages. According to recently published data, Ezh2 is required for Tfh differentiation. Tfh cell differentiation occurs within 48 hours after acute viral infections. Generally speaking, during the early response to acute viral infection, the expression level of Ezh2 and H3K27me3 marks in activated virus-specific $CD4^+$ T cells are positively associated with Tfh formation. In contrast, cells with low Ezh2 and H3K27me3 tend to differentiate into Th1 cells. Loss of Ezh2 (by conditional knockout or by Ezh2 inhibitor treatment) leads to compromised Tfh differentiation and defects in the activation of a broad Tfh transcriptional program, including *Bcl6* (Chen et al., 2020; Li et al., 2018). Ezh2 is an important regulator of early Tfh fate commitment, but is not required for the late differentiation and the maintenance of virus-specific Tfh cells in response to an acute viral infection (Chen et al., 2020). In Tfh, most of the Ezh2 peaks are correlated with H3K27Ac, not H3K27me3, so the predominant role of Ezh2 appears to be related with gene activation. In one hand, Ezh2 is recruited to selective Tfh gene loci in a Tcf 1 (a critical transcription factor for *Bcl6* induction in Tfh cells) dependent way to exert activating function, and this function seems dependent on Ezh2 Ser 21 phosphorylation. On the other hand, while p19Arf physically interacts with Bcl6 to perturb Bcl6-mediated gene repression, Ezh2 can repress p19Arf to promote Tfh cell survival and differentiation (Li et al., 2018). Although *Ezh2* deletion in Treg ($Ezh2^{fl/fl}Foxp3^{cre}$) increases the percentages of total Treg cells and Tfr cells, *Ezh2* deficiency impairs the ability of Tfr cells to fully suppress B cell responses, indicating Ezh2 is required for the suppressive function of Tfr cells (Hou et al., 2019).

PcG complexes also participate in the regulation of higher order chromatin structures in T cells. When naive T cells differentiate into effector lineage, the cells enlarge, differentiate, proliferate, and are accompanied by major changes in the chromatin structure. TCR stimulation for 24 hours in either Th1 or Th2 cell-inducing conditions induces nuclear F-actin (filamentous actin) in the Th1 and Th2 cells. This protein is required for proper regulation of DNA replication, gene expression, chromatin organization, and nuclear expansion during mitotic exit, and plays an important role in chromatin spreading and nuclear expansion in differentiating Th cells. Nuclear Ezh2 is colocalized with F-actin, as well as actin regulator Vav1 and chromatin fibers, and is required for chromatin spreading. Inhibition of Ezh2 by small-molecule inhibitor UNC1999 diminishes the presence of nuclear F-actin, reduces the expression of β-actin, γ-actin and Vav1, leading to a more condensed organization of chromatin fibers. In summary, Ezh2 is required for the assembly of intranuclear F-actin to re-organize chromatin in differentiating Th cells (Titelbaum et al., 2021).

2.7. PcG regulation of T cell responses in immune system diseases

Recent studies demonstrate the involvement of Polycomb in allergies and autoimmune diseases. SLE (systemic lupus erythematosus) is a chronic autoimmune disorder with a variety of clinical manifestations. Results show there is an expansion of $CD8^+CD38^{high}$ T cells in the peripheral blood from SLE patients. This population displays less cytolytic activity, and increased risk for infections. $CD8^+CD38^{high}$ T cells show decreased expression of cytotoxic-related molecules, including IFN-γ, perforin, and granzyme A/B compared with control subjects. The elevated level of CD38 leads to decreased cytotoxicity of $CD8^+$ T cells through the NAD/Sirtuin1/EZH2 axis. Briefly, high expression of CD38 limits the activation of NAD+/SIRT1, which deacetylases Ezh2 in T cells. Therefore, acetylated EZH2 with methyltransferase activity increases. Consequently, the acetylated protein encourages methyltransferase activity to repress the expression of cytotoxic molecules. Therefore, inhibition of the Sirtuin1/EZH2 Axis by Ezh2 inhibitor restores $CD8^+$ T cytotoxicity. Thus, this pathway might offer therapeutic opportunities to restore cell cytotoxicity in SLE (Katsuyama et al., 2020).

Th2 cells play central roles in allergic asthma. Airway and blood eosinophilia, and unwarranted Th2 mediated immune response, are the hallmarks of airway inflammation in type-2-high asthma (Hammad and Lambrecht, 2021; Lambrecht et al., 2019). In an OVA-induced airway inflammation model, T cells with *Ring1B* knockout significantly decrease the total infiltrating leukocytes, eosinophils, and the percentage of $CD4^+$ cells in the bronchoalveolar lavage (BAL) fluid. The mRNA expression of Th2 cytokines *Il-4, Il-5*, and *Il-13*, and Th2 master gene *Gata3* in the BAL fluid cells are dramatically reduced. Furthermore, *Ring1B* knockout Th2 cells are more sensitive to apoptosis. Therefore, Ring1B has an essential role in Th2-driven allergic airway responses (Suzuki et al., 2010).

ITP (immune thrombocytopenia) is an autoimmune bleeding disorder characterized by isolated thrombocytopenia (Cooper and Ghanima, 2019). Patients with ITP have platelet-reactive autoreactive T lymphocytes that cause B cells to produce antiplatelet autoantibodies (Cooper and Ghanima, 2019; Ware and Howard, 1993). $CD4^+$ T cells from ITP patients express higher BMI-1 than that of healthy donors. Knocking down *BMI-1* in $CD4^+$ T cells from active ITP patients reduces cell proliferation, increases apoptosis, and impairs the production of IL-4. In contrast, overexpression of *BMI-1* in sorted $CD4^+$ T cells from healthy donors enhances cell proliferation and the production of IL-4, and decreases apoptosis compared with controls. These findings suggest that BMI-1 promotes proliferation and inhibits apoptosis of autoreactive $CD4^+$ T cell from ITP patients (Ma et al., 2012).

Chronic inflammatory bowel disease (IBD) is an autoimmune disorder that includes ulcerative colitis (UC) and Crohn's disease (CD) (Seyedian et al., 2019). Evidence suggests that the abnormal adaptive immune response is an important reason to induce gut inflammation in IBD patients (Gonzalez et al., 2021). Although Treg cells are expanded within the inflamed intestine of human CD, there is a defect in FOXP3-mediated gene repression within these cells. Several laboratories have shown that Polycomb proteins have important Treg-specific roles in preventing multiorgan inflammation and colitis. Ezh2 is the most well investigated PcG protein in Treg cells. Treg specific *Ezh2* knockout mice display various symptoms of autoimmunity. In a minimal chemical colitis experiment in WT mice induced by oral dextran sodium sulfate (DSS) to stimulate the mucosal immune response, the Ezh2 inhibitor DZNep causes heightened intestinal immune reactivity (DuPage et al., 2015; Gonzalez et al., 2021; Sarmento et al., 2017). The Interaction between PRC2 and Foxp3 is important to maintain immunosuppressive function of Treg cells. FOXP3 constitutively forms a complex with the core subunits of PRC2 - Ezh2, SUZ12, and EED - on their target loci to regulate gene expression in murine and human Treg cells (Bamidele et al., 2019). In addition, gene expression profiles in $CD4^+$ T cells isolated from the ileum of CD patients and healthy donors shows that FOXP3 is among the top three up-regulated transcription factors, and nearly half of EZH2-associated gene targets are differentially expressed. Furthermore, 187 FOXP3-EZH2 common target genes are differentially up-regulated, and 88 are down-regulated. Therefore, dysregulation of common FOXP3 and EZH2 targeting genes is a hallmark of human CD (Sarmento et al., 2017). While FOXP3-EZH2 interaction in Treg is required for the FOXP3 associated repressive transcription program and properly immunosuppressive function, IBD-associated FOXP3–C232G mutation disrupts FOXP3 and EZH2 interaction, leading to impaired Treg function. Investigating the interaction between FOXP3 and EZH2 proteins via PLA using freshly isolated $CD4^+$ T cells from the intestinal lesions of human CD shows a significantly reduced FOXP3 and EZH2 protein interaction. Results of ChIP-qPCR demonstrate that this mutation diminishes H3K27me3 modification at the promoter of the IBD-relevant gene *IFNG*, and impairs the repression of *IFNG* promoter (Bamidele et al., 2019). PRC1 is also involved in the regulation of CD-associated gene networks in Treg cells. ATAC-seq data in human induced Treg cells (iTregs) shows that

ATAC regions are significantly more open in iTreg cells compared with naive T cells at the DEGs loci in CD-associated $CD4^+$ lymphocytes. There is correlation between open chromatin areas with the PRC2 and PRC1 proteins, including EED, SUZ12, EZH2, RNF2, BCOR, and BMI-1. PRC1 target genes are enriched within the upregulated CD-DEGs. Treg cells uniquely display RING1B-BMI-1 protein interaction in contrast to naive cells. Knockout of *Bmi-1* in Treg cells *in vivo* causes a spontaneous inflammatory phenotype with increased production of Th1/Th17-associated proinflammatory cytokines TNFα, IFN-γ, and IL-17, and shows a failure to suppress colitis in mice. The refractory CD associated DEGs (from a scRNA-seq data set derived from CD associated ileum) are up-regulated in *Bmi-1* knockout Treg. Therefore, BMI-1 plays a role in maintain Treg identity and the immunosuppressive function in murine and human (Gonzalez et al., 2021).

Polycomb proteins have also been shown to play a role in graft-versus-host disease (GVHD) pathology. GVHD is caused by donor T cells that destroy recipient cells. In a mouse GVHD model, *Ezh2* ablation in donor T cells inhibits lethal GVHD. Loss of *Ezh2* does not impair activation or proliferation of donor T cells during the GVHD priming phase. But at later stages, the proliferation and expansion of knockout $CD4^+$ T cells are significantly reduced compared with WT $CD4^+$ T cells. *Ezh2* knockout reduces IFN-γ^+ effector cells frequency and impairs Th1 cells development. Although less potent than WT T cells, *Ezh2* deficiency preserves anti-leukemia activity in donor T cells, leading to improved overall survival of the recipients (He et al., 2013). However, inhibiting H3K27me3 by Ezh2 selective inhibitors GSK126, UNC 1999, or EPZ6438 that specifically reducing H3K27me3 without altering Ezh2 protein fails to control GVHD. Ezh2 interacts with molecular chaperone Hsp90, which is required for the stability and function of number of key signaling proteins (Huang et al., 2017; Neckers, 2007). Hsp90 inhibitor AUY922 treatment induces a dose dependent decrease of Ezh2 protein in TCR-activated $CD8^+$ T cells without changing the levels of H3K27me3, H3K4me3, H3K36me3, and H3K9me3. As a result, destabilizing Ezh2 by inhibiting Hsp90 in T cell reduces GVHD in mice, causes a decrease of alloantigen-activated cells, downregulates IFN-γ production by donor T cells, but preserves the anti-leukemia activity of donor T cells. These results are important for future designing of Ezh2 inhibiting-based GVHD therapy (Huang et al., 2017).

2.8. PcG in tumor infiltrated T cells

Growing evidence shows epigenetic alterations contribute to altered gene expression in cancer. However, how the tumor infiltrated immune cells are manipulated by epigenetic mechanisms are still not understood. Multiple studies show Ezh2 modifies the anti-tumor efficiency of $CD8^+$ T cells and promotes memory precursor formation. Furthermore, higher proportions of $EZH2^+CD8^+$ T cells are associated with improved cancer patient survival (He et al., 2017; Zhao et al., 2016). In an adoptive cell therapy model, transfer of WT but not *Ezh2*$^{-/-}$ Pmel-1 naive $CD8^+$ T cells (Ezh2 is deleted in melanoma-associated antigen gp100-specific $CD8^+$ T-cell receptor-transgenic Pmel-1 cells) represses the growth of pre-established B16 melanoma in lymphodepleted mice. Further research reveals that the persistence of tumor-reactive T cells is impaired upon *Ezh2* deficiency. It is known that the level of tumor infiltrated memory T cells is associated with improved cancer patient survival (Zhao et al., 2016). *Ezh2* ablation leads to an extended terminal differentiation and compromised memory potential of $CD8^+$ T cells (He et al., 2017). There is an up-regulation of effector differentiation associated genes *Id2*, *Prdm1*, and *Eomes*, and down-regulation of *Id3*, which regulates memory formation upon *Ezh2* deficiency in the *in vitro* activated Pmel-1 cells (He et al., 2017). $Ezh2^+$ T cells represent a polyfunctional T cell subtype (T cells that produce multiple effector cytokines). In the peripheral blood mononuclear cells from ovarian cancer, $EZH2^+CD4^+$ and $EZH2^+CD8^+$ T cells are different from anergic and senescent memory T cells. Instead, they are enriched with polyfunctional T cells, and resistant to cisplatin-induced apoptosis. Inhibition of the methyltransferase function of Ezh2 causes reduced H3K27me3 modification, decreased polyfunctional $CD8^+$ T cells, and impaired polyfunctional T cell-mediated anti-tumor immunity. The absolute number and proportion of $EZH2^+CD8^+$ T cells are correlated with improved overall survival and disease-free interval of ovarian cancer patients. However, tumors can impair EZH2 expression in both $CD4^+$ and $CD8^+$ T cells, and reduce polyfunctional T cells via glucose restriction (Zhao et al., 2016). Infiltrating $CD4^+$ and $CD8^+$ T cells from B16F10 melanoma demonstrate to be exhausted and display a reduced H3K27me3 peak compared with T cells from tumor-draining lymph nodes and *in vitro* activated T cells. *In vitro* treatment with highly specific and effective small-molecule inhibitors of Ezh2 reveals an exhausted phenotype in primary $CD8^+$ T cells, implying a relationship between *Ezh2* down-regulation and T cell exhaustion. Consistent with the role of Ezh2 in controlling of T cell effector function and memory precursor formation, exogenous expression of gain-of-function $EZH2^{Y641F}$ mutant improves tumor control (Koss et al., 2020). The role of Ezh2 in T cells can be regulated by phosphorylation. Although $CD8^+$ T cells express elevated level of Ezh2 in response to antigen activation, Ezh2 is phosphorylated at serine 21 ($pEzh2_{S21}$) by Akt upon TCR activation, leading to suppressed Ezh2 enzymatic activity, correlated with decreased H3K27me3. Phosphorylation of Ezh2 impairs memory precursors formation; but, overexpressing Akt phosphorylation-resistant mutant Ezh2, $Ezh2_{S21A}$, dramatically improves their memory differentiation and anti-tumor efficiency (He et al., 2017). To be noticed, the tumor microenvironment is complicated. Although Ezh2 positively regulates the anti-tumor efficiency of $CD8^+$ T cells, the Ezh2 inhibitor CPI-1205 leads to enhanced proportions of $CD8^+$ T cells, dramatic inhibition of tumor progression (MB49 and B16–F10 and MC38), and increases the efficiency of anti–CTLA-4 therapy in tumor-bearing immunocompetent mice (Goswami et al., 2018; Wang et al., 2018).

Treg cells in tumor tissues suppress effective tumor immunity and often portends poor outcomes in cancer patients. Tumor infiltrated Treg cells have enhanced EZH2 expression and activity compared to effector $CD4^+Foxp3^-$ T cells in tumors (Wang et al., 2018). Disruption of EZH2 activity in Treg cells, either pharmacologically or genetically, reprograms their phenotype to effector-like T cells, impairs Treg suppressive capacity, remodels the tumor microenvironment, and leads to robust antitumor immunity (Goswami et al., 2018; Wang et al., 2018).

3. Concluding remarks and future perspectives

PcG proteins are essential for cell fate maintenance and transition. They are required for the proper function of T cells, mediating their development, differentiation, activation, and responses in inflammatory and cancer. Recently, we studied how Kdm2b, a subunit of ncPRC1, regulates the activation and immunosuppressive function of Treg cells. We found that Kdm2b is indispensable for Treg cells to maintain immune homeostasis. Kdm2b not only has a role in regulating repressive program in Treg cells, but is also positively associated with gene transcription (unpublished data). However, as we have mentioned, the roles of Polycomb proteins in T cells are controversial and their mechanisms of actions are unclear. The exact mechanisms by which PcG orchestrates genetic factors to regulate the gene expression profile of T cells in response to stimulation through histone modification are not understood. To solve these questions, there are several important and interesting lines of investigation to be addressed. First, although there is a rapid development of genome-wide mapping technology, the datasets of the epigenetic status and Polycomb proteins genome-wide binding in each population of T cells (activated, exhausted, tumor infiltrated T cells) are far from entirely complete. Establishment of histone modification profiles and the binding landscapes of PcG in T cells will help scientists better understand the regulating mechanism of PcG on T cells. Second, the control of PcG induction and expression in T cells remains relatively unclear. $CD8^+$ T cells with higher levels of Ezh2 is associated with

enhanced memory fate and polyfunction. Therefore, understanding the PcG network will help us to fine-tune T cell responses in immune system diseases and cancers. Third, Polycomb proteins are mutated (including loss-of-function and gain-of-function), or highly expressed in several types of cancers. Consequently, targeting Polycomb proteins for cancer therapy is a hot research topic now.

Considering Polycomb proteins have activity against cancer cells, effector T cells and regulatory T cells, systemic application of small molecular inhibitors may cause complicated physiologic changes, and the prognosis is hard to predict. Thus, novel therapeutic strategies targeting PcG are challenging and need to be evaluated comprehensively. Fortunately, advances in transgenic mice, single cell RNA sequencing, and microcellular multi-omics association analysis have significantly enhanced our capacity to establish the chromatin landscape in T cells. We feel the knowledge acquired from PcG studies in T cells will assist with targeted pharmacological or genetic interventions for the diagnosis and treatment of allergies, autoimmune diseases, and cancer.

Declaration of competing interest

The author declares no competing financial interests.

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