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SETDB1: a perspective into immune cell function and cancer immunotherapy

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Abstract

Oncogene SETDB1/ ESET, an H3K9 methyltransferase, was originally discovered over two decades ago, however its function in the immune response was not first reported until 2011. SETDB1 immune functions include B cell maturation, T cell activity regulation, and immune escape in cancer cells. In B lymphocytes, SETDB1 mediates the transition from pro-B to pre-B cells and represses endogenous retroviruses (ERV) to encourage B cell lineage differentiation and maturation. SETDB1 alters T cell function by methylating IL-2 and IL-17 promoters and mediating T cell lineage commitment and development. Additionally, SETDB1 plays a critical role in ERV silencing within a variety of immune cells, which can indirectly weaken the immune response. Although SETDB1 is critical for normal immune cell function, overexpression in cancer cells negatively impacts immune cell fights against cancer through decreased tumor immunogenicity. Within cancer cells, SETDB1 overexpression represses production and infiltration of antitumor immune cells, mediates immune escape through TE and ERV silencing, represses the type I interferon pathway, and interferes in immune checkpoint blockade (ICB) outcomes by regulation of PD-L1 expression and IFN signaling. In this review, we further discuss the immunological mechanisms of SETDB1 in normal and cancerous cells and its implications in cancer immunotherapy.

Graphical Abstract

SETDB1, an H3K9 methyltransferase, plays a critical role in the regular function of the immune system; however, overexpression of SETDB1 is associated with poor outcome in many cancer types. This review serves to detail the abundance of recent findings regarding SETDB1 in its role in the normal immune system and its detrimental effect in relation to anti-tumor mechanisms.

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Author Contributions

Conceptualization of the study: Shujie Yang. Acquisition of data: Eleanor Johnson and Kiarash Salari. Writing and editing of the original draft: Eleanor Johnson and Kiarash Salari. Writing and editing of the reviewed manuscript: Eleanor Johnson and Shujie Yang. Figures: Eleanor Johnson. Supervision and editorial assistance: Shujie Yang. All authors were involved in final revisions and discussion of the manuscript.

Disclosure of Conflict of Interest

The authors report no conflicts of interest.

Keywords

Cancer; Tumor/Immunology; Regulation/Suppression; Cytokines; Cell Differentiation

Introduction

SET Domain Bifurcated 1 (SETDB1) is a histone H3K9 methyltransferase and has been reported to be responsible for the silencing of many different genes and pathways important in the cell cycle, apoptosis, differentiation, Epithelial-Mesenchymal Transition (EMT), cell growth, and metabolism [1–6]. Formation of heterochromatin through histone methylation by SETDB1 is often associated with recruitment of several different co-repressors and repressor complexes. These complexes aid SETDB1 in binding proximal to gene promoters and stimulate their transcriptional repression. Among these complexes are the Human Silencing Hub (HUSH) complex, KRAB-Zinc finger proteins (KRAB-ZFP) and KAP1/TRIM28 co-repressors [7–9]. In our previous review [10], we addressed the role of SETDB1 in promoting cancer progression, survival, growth, proliferation, and development. Over the past decade, SETDB1 has emerged as a key chromatin modifier regulating important immune cell processes and functions (Figure 1). These functions are mainly attributed to immune cell development and cell fate as well as immune evasion in cancer. Throughout these findings, H3K9 trimethylation by SETDB1 appears to play a pivotal role in regulating differentiation and phenotypic switches of many immune cells such as that of B and T lymphocytes [11, 12] as well as its role in production of different cytokines [13] and activation of innate immune related genes [14]. This histone mediated silencing of gene expression by SETDB1 has been described to take place either directly on the gene promoters responsible for the observed phenotypes or act on endogenous retroviral (ERV) elements maintaining their suppression [15], which could otherwise act as gene enhancers and promote their activation. In this review, we first focus on the regular function of SETDB1 in different immune cells to better understand the influence of the methyltransferase in an immunological capacity (Figure 2). We then turn to an oncological standpoint and examine the role of SETDB1 in recruitment of immune cells to the tumor site in cancer (Figure 3) as well as its prevalence in innate immune responses.

SETDB1 is Crucial for Normal Immune Cell Function

SETDB1 Mediation of B Cell Development

Research by Pasquarella and colleagues showed that, in SETDB1 knockout mice, B cells are halted in the Pro B cell stage and unable to develop into mature B cells [16]. RNA-seq further revealed an increase in specific transcriptions regularly suppressed by SETDB1 that restricts the organization of the B cell program. VDJ recombination, a process responsible for creating diversity of antibodies for B and T cells, is also weakened in SETDB1 knockout mice. These findings were supported later in 2015 by Collins and colleagues who demonstrated the influence of SETDB1 in the immune system as an epigenetic regulator affecting B cell development [17]. They revealed that deletion of SETDB1 using an Mb1-CRE transgene targeting floxed SETDB1 in pre-B cells specifically leads to eradication of B cell population in bone marrow and spleen. This defect in maturation of pro-B cells into pre-

B cells can be traced back to de-repression of endogenous retroviruses (ERVs), which act on other proximal gene promoters as enhancers of gene expression. Upregulation of ERVs happens due to decreased histone H3K9 methylation at specific ERV loci. SETDB1 deletion correlates with induced expression of genes related to innate immunity, non-hematopoietic lineages, and even T cell specific genes. As a result, B cell lineage specific differentiation and activation genes were largely hindered. In 2016, Pasquarella and colleagues revisited the role of SETDB1 in the development of pro-B cells as a retrotransposons silencer [11]. A mouse strain was generated to execute deletion of SETDB1 in the transition of pro-B cells from pre-cells to committed cells. Cells lacking SETDB1 showed MLV as the most upregulated retrotransposon. Upregulation of MLV alters chromatin structure and triggers the unfolded protein response (UPR), inducing apoptosis. These studies strongly exhibit the role of SETDB1 in the maturation of B cells by ensuring B cell program establishment, repressing target ERVs to promote lineage differentiation, and silencing target retrotransposons to protect cell vitality (Figure 2).

SETDB1 Role in T Cell Function and Lineage Commitment

Wakabayashi and colleagues introduced the importance of two TGF-β-downstream transcription factors, Smad2 and Smad3, in suppressing the production of IL-2 within CD4+ T cells in collaboration with H3K9 methyltransferases [18]. They proposed that Smad2/3 recruit H3K9 methyltransferases SETDB1 and SUV39H1 to the IL-2 promoter to perform methylation and thus hinder transcription of T cell receptor-dependent IL-2. Smad3 expressed in 68–41 T cells had moderate effect on IL-2 promoter activity. When SETDB1 and SUV39H1 were introduced, the interaction with Smad3 suppressed IL-2 activation. Furthermore, introducing expression of either Smad3 or SUV39H1 greatly decreased IL-2 mRNA production whereas expression of SETDB1 had a lesser but still suppressive effect on IL-2 transcription within T cells. Xiao and colleagues later studied the effect of SETDB1 on another cytokine in T cells, IL-17 [19]. SETDB1 was recruited by T cell stimulatory molecule OX40 to the IL-17 locus, repressing expression of IL-17 through H3K9 methylation to decrease Th17 cell function. Specifically, OX40 triggers RelB, an NF-κB protein, to bind to SETDB1 and another HMT, positioning them on the IL-17 locus for subsequent methylation. Finding ways to remodel the chromatin altered by SETDB1 around the IL-17 locus could be a promising therapy for autoimmune diseases induced by Th17.

Takikita and colleagues introduced SETDB1 as a key player in CD8+ T cell development within thymocytes [20]. In T cell specific SETDB1 knockout mice, analysis yielded decreased T cell counts, especially within CD8+ cells. Part of this deficiency was a consequence of upregulated FcγRIIB, a protein whose expression impedes extracellular signal-related kinases (ERK) activation. SETDB1 was thus shown to be critical to T cell development, not for direct regulation of lymphocyte establishment, but for suppressing abnormal expression of genes that could interfere with T cell maturation. Adoue and colleagues further highlighted the SETDB1 H3K9 methyltransferase role as an important mediator of CD4+ helper T cell differentiation [12]. Regularly, naive CD4+ T cells differentiate into either Th1 or Th2 cells. This study used CRE-mediated conditional knockout in CD4+ T cells. CD4+ T cells without SETDB1 exhibited greater acquisition

of Th1 lineage-specific phenotype evident by enhanced levels of IFN-γ upon induction of IL-12, a Th1 stimulating cytokine. In contrast, introduction of Th2 polarizing conditions correlated with reduction in Th2 specific gene signatures in cells lacking SETDB1. Furthermore, while Th2 T cells with intact SETDB1 remained committed to their lineage under Th1 stimulatory conditions, Th2 T cells lacking SETDB1 reprogrammed into Th1 state. The study suggests that SETDB1 suppresses ERVs near Th1 enhancers; therefore, the HMT is critical to produce Th2 cells. To summarize, SETDB1 holds a multifaceted role in T cells, decreasing production of IL-2 and IL-17, playing a critical part in T cell development, and mediating T cell lineage commitment (Figure 2).

SETDB1 Changes Leukocytes Activity to Suppress the Immune System

Besides the two aforementioned immune cells, leukocytes also include monocytes, macrophages, and dendritic cells. Monocytes are white blood cells that differentiate into macrophages and dendritic cells. Zhang and colleagues showed that SETDB1 represses transcription of CD1a, a membrane protein that regulates the presentation of antigens on T cells [21]. SETDB1 is first conscripted to the CD1a promoter by the td-piR(Glu)/PIWIL4 complex. After methylation of H3K9, SETDB1 recruits HP1β to sustain the chromatin modification. Thus, SETDB1 is able to have a suppressive effect on CD1a expression within monocytes and dendritic cells, therefore decreasing immune system efficiency. Future innovation of CD1a control could improve autoimmune disease treatment and aid in avoiding immune evasion due to abnormal CD1a expression.

Hachiya and colleagues later explored SETDB1 as an important chromatin modifier in macrophages, suppressing TLR4-mediated pro-inflammatory cytokine production [13]. The presence of SETDB1 at pro-inflammatory cytokine (ex. IL-6 and IL-12) promoter sites induces histone H3K9 methylation and blocks transcriptional activity and subsequent production. In this way, SETDB1 directly targets cytokines, whereas the previous example demonstrated a cytokine’s effect on SETDB1 recruitment. Activation of TLR4 receptors through lipopolysaccharide (LPS) leads to higher levels of these cytokines in macrophage cell line J774.1 when SETDB1 is knocked down by shRNA. Further, SETDB1 depletion promotes NF-KB p65 transcription factor recruitment at the IL-6 promoter, possibly through formation of a more open chromatin state for p65 transcriptional activity due to decreased methylation.

Tie and colleagues described SETDB1 as a regulator of innate immune genes alongside KAP1 (TRIM28) in a wide range of adult human cells as well as peripheral blood mononuclear cells (PBMCs), which include monocytes, lymphocytes, and macrophages [14]. Immune gene regulation was shown to be regulated by reactivation of ERVs. Utilizing human embryonic stem cells (ESCs) and differentiated cells (ex. 293T cells), KAP1 occupies sites enriched for ERVs. ENCODE data analysis suggested correlation between KAP1 binding sites and H3K9me3 chromatin mark as well as SETDB1. Depletion of either KAP1 or SETDB1 leads to a decrease in H3K9 methylation at the ERV sites following downstream activation of RNA-sensing pathway components (ex. MAVS), which upregulates ISGs and innate immune genes.

SETDB1 Interferes with the Immune System within Cancer

SETDB1 Alters Lymphocyte and Cytokine Expression

As we mentioned in the introduction, SETDB1 is commonly overexpressed in cancer cells. Zhou and colleagues studied the role of SETDB1 in breast cancer as a repressor of IL-6 expression and its association with antigen regulation [22]. They studied 159 breast cancer samples for transcriptional levels of SETDB1 by IHC assay and showed that SETDB1 expression inversely correlates with prognostic predictions. A bioinformatics analysis showed association between SETDB1 and six immune-related hub genes: IL-6, BMP4, CD74, PECAM1, HLA-DPA1, and HLA-DRA. Knockdown of SETDB1 correlates with increased expression of IL-6, reflecting the repressive nature of the gene. As we previously mentioned, Hachiya and colleagues suggested that IL-6 expression in macrophages is mediated by TLR4 and induced by SETDB1 depletion [13]; confirmation of direct SETDB1-mediated expression still needs to be confirmed in future studies. Xiang and colleagues characterized the mechanism associating SETDB1 overexpression with poor prognosis of multiple myeloma [23]. Higher levels of SETDB1 correlates with higher levels of beta-2 microglobulin, lactate dehydrogenase (LDH), bone marrow biopsy plasma cells, and lower hemoglobin levels. SETDB1 overexpression is also associated with increased CD56dim natural killer cells (named for its surface antigen and commonly found in peripheral blood) and decreased levels of infiltrating immune cells such as CD8+ T cells, dendritic cells, Th17 cells, and natural killer T cells. Meanwhile, SETDB1 induces expression of protein c-MYC to accelerate cancer progression, which in return allows c-MYC transcription factor to augment SETDB1 transcription. Another study by Lin and colleagues displayed the SETDB1 impact on different immune signatures and attributes in a wide variety of cancers [24]. Using the TISIDB database, they divided the immune environment of different cancers into different subtypes (ex. inflammatory, lymphocyte depleted, etc.). Interestingly, strong association of SETDB1 with different immune subtypes was observed in many different cancers including lung adenocarcinoma (LUAD), stomach adenocarcinoma (STAD), colon adenocarcinoma (COAD), and glioblastoma (GBM). Further, they showed that infiltration of cancer-associated fibroblasts (CAFs), which often play a role in tumor stroma and cancer progression have marked positive correlation with SETDB1 as well as other types of immune cells such as CD8+ T cells, CD4+ T cells, Tregs, and B cells. SETDB1 also has a positive correlation with a wide range of immune-checkpoint-related genes such as CTLA4, LAG3, CD80 and many others. Moreover, SETDB1 demonstrated a negative correlation with HLA-related genes in all cancers, with the exception of adenoid cystic carcinoma (ACC), clear cell renal cell carcinoma (KIRC), and cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC). Importantly, SETDB1 was found to have a positive correlation with microsatellite instability (MSI) and tumor mutational burden (TMB) in several cancers which often relate to response to immunotherapy.

SETDB1 Facilitates Tumor Immune Escape

Recent publications have reported that SETDB1 plays an essential part in allowing tumors to evade the immune system. In their work, Griffin and colleagues described SETDB1 among the top chromatin modifiers of 936 screened chromatin genes responsible for mediating

immune escape in tumors [25]. Utilizing Lewis lung carcinoma (LLC) and B16 melanoma mouse cancer models, they observed an increase in immune checkpoint therapy sensitivity when SETDB1 is targeted by CRISPR-Cas9 sgRNAs. This was evident by depletion of SETDB1 gRNAs in immunocompetent C57Bl6 mice relative to immunodeficient NOD-SCID-Il2rg^{null} (NSG) mice when tumors were treated with either Anti-PD1/CTLA-4 or GVAX/Anti-PD1 as well as observations of slowed down tumor growth. In their study, SETDB1 expression had strong positive correlation with its amplification in over 20 different cancers along with reverse correlation with many immune signatures ranging from TNF signaling to IFN-γ response. Knockout of SETDB1 triggered the activation of specific transposable elements (TEs). The study showed that TE activation then activates nearby genes involved in the interferon pathway, as well as NKG2D ligand expression responsible for activation of NK and CD8+ T cells. SETDB1 regulates the expression of the components of the MHC-I receptor, viral protein encoding TEs, and T cell response mechanisms. Future work needs to be done in understanding the role of SETDB1 TE regulation across types of tumors and cancers, which will strengthen anti-tumor therapies. In that same year, Zhang and colleagues confirmed SETDB1's role in promoting tumor immune evasion and weakening response to immune checkpoint therapy (ICB) through retroelement silencing [15]. However, their work determined another chromatin modifier KDM5B, an H3K4 demethylase, to be necessary in this mechanism. They utilized two cell lines in their experiments: YUMM1.7 and YUMMER1.7, the latter being a generation of the former and having additional mutations. The authors showed that KDM5B depletion in mouse melanoma cell line YUMM1.7 significantly slows down tumor growth in a CD8+ T cells dependent manner as well as making them more susceptible to ICB. RNA-seq analysis showed activation of type I interferon response in KDM5B KO YUMMER1.7, which was initiated by RNA sensing and cytosolic DNA sensing pathways. This process is an effect of ERV transcriptional activation from KDM5B depletion. ChIP-qPCR analysis of these retro-elements confirmed KDM5B presence at these loci. However, the H3K4me3 level was not affected as a result of KDM5B deletion but the level of H3K9me3 was significantly altered and decreased at these loci, which hinted to importance of SETDB1 H3K9 methyltransferase activity for the ERV suppressions. Interestingly, they showed that KDM5B is not only involved in recruitment of SETDB1 at ERV sites but also in dictating nuclear stability of SETDB1. KDM5B deletion largely affected the nuclear level of SETDB1 in YUMMER1.7 cells, which could be rescued and further increased by treatment of these cells with an MG132 proteasome inhibitor. Thus, ERV silencing by SETDB1 via KDM5B recruitment indirectly allows tumors to escape immune surveillance and KDM5B or SETDB1 targeting may improve immunotherapy outcomes. Hu and colleagues utilized an immune-escaped tumor model (KP-IE) of lung cancer to study key chromatin modifiers responsible for suppression of antigen presentation [26]. Knocking out SETDB1 resulted in enhanced tumor immunogenicity. This was evident through increase in expression of SINFEKLL peptide on the surface of tumor cells and reduced tumor growth in immune-competent B6/J mice compared to T cell deprived nude mice. This trend of tumor growth was evident in both lung cancer and in the MC38 colon cancer model. SETDB1 depletion correlated with elevated expression of ERVs. This not only increased tumor antigen expression but also derived activation of interferon immune response through dsRNA sensing pathway. ChIP-Seq identified H3K9me3 deposition at ERVs as the sole mechanism responsible for

their regulation, supporting the key role of SETDB1. SETDB1 later emerged as an inducer of PD-L1 expression in colon cancer in work by Tian and colleagues [27]. They reported that SETDB1 induces tumor immune evasion in CRC through upregulation of PD-L1 and hindering of CD8+ T cell infiltration in the tumor. SETDB1 uses the FOSB/miR-22/BATF3 axis to increase expression of PD-L1. Specifically, SETDB1 can promote methylation of FOSB promoters, thereby reducing transcription. FOSB is a transcription factor involved in regulating miR-22 transcription. FOSB transcriptional repression by SETDB1 reduces miR-22 level. BATF3 plays a role as an important transcription factor stimulating PD-L1 expression and can be a target of miR-22. Hence, reduction of miR-22 by SETDB1 positively affects BATF3 protein level in colorectal cancer where it directly promotes PD-L1 expression. This finding suggests that SETDB1-mediated regulation of PD-L1 is more complex than previously thought and needs to be separately validated and studied in different cancer types.

SETDB1 Disrupts the Type I Interferon Response

The type I interferon response has been established as an effective antitumor mechanism. However, recent publications have reported that SETDB1 may repress the pathway through various mechanisms (Figure 3). Studying acute myeloid leukemia (AML) cells, Cuellar and colleagues introduced SETDB1 as a significant contributor to cell viability and survival [28]. Utilizing a guide RNA library screening, SETDB1 was identified as the top hit of epigenetic and transcriptional modifiers in AML cell line THP-1 as evident by the greatest proportion of gRNA depletion per gene and total fold change in gRNA. RNA-sequencing determined type I interferon pathway and anti-viral response genes as the most upregulated in cells lacking SETDB1. As a result, several components of interferon-stimulated genes (ISGs) such as IFIT1-3, RIG-1, OAS3 and MDA5 were found to be enhanced. Furthermore, ChIP-seq on H3K9 methylation revealed loss of H3K9 methylation at KRAB zinc finger genes known to be repressed by SETDB1 and linked to ERV repression as well as repetitive segments of the genome when SETDB1 was mutated. However, no change of H3K9me3 observed on ISG related genes or loci specific to innate immune sensors. RNA-seq analysis on SETDB1 depleted THP-1 cells further showed upregulation in retro-TEs consisting of either LTR and non-LTR families such as LINEs and satellite repeats. Finally, disruption of cytosolic dsRNA-sensing pathway components by means of CRISPR-Cas9 mediated deletion such as MAVS, MDA5 and RIG1 demonstrated interruption in induction of type I interferon pathway and increased cell viability upon SETDB1 deletion. In a separate study, Kang and Min analyzed public transcriptome data of squamous-cell carcinoma (SCC) and lung adenocarcinoma (ADC). They reported that high SETDB1 expression correlates with depleted transcriptomic levels of genes related to the EMT, innate immune response, and autoimmunity and increased levels of genes involved in RNA interference and chromatin modification. Many years later, Pan and colleagues highlighted SETDB1 dependent immune cell infiltration as a mechanism responsible for increased sensitivity of patient’s tumors to radiotherapy in SKCM and NSCLC [29]. In their TCGA analysis of patients’ sub-cohorts, they first observed that NSCLC and SKCM patients expressing low SETDB1 have significantly longer progression-free survival compared to high SETDB1 expressing patients undergoing radiotherapy. Gene ontology analysis revealed innate immune response pathways as highly enriched in SETDB1 low patients compared to high ones. Moreover,

TIP online database and CIBERSORT bioinformatic analysis illustrated greater recruitment scores for immune cells such as T cells, NK, DC, and macrophages in low SETDB1 samples. When murine B16F10 cells were depleted of SETDB1, T lymphocytes had greater infiltration inside the tumor, suggesting that SETDB1 hinders T cell invasion. Irradiated human and mice melanoma cells showed increased ERV expression and activation with their levels being greater in SETDB1 knockout cells. Remarkably, SETDB1 levels showed a decreasing trend upon radiation, which was dependent on the proteasomal pathway. Enhanced ERV expression generated dsRNA induced type I interferon response, which was determined to be the ultimate factor responsible for tumor growth regression in mice in-vivo.

SETDB1 Activity Decreases ICB Effectiveness

Immune checkpoint blockade (ICB) is a therapy that targets specific checkpoint proteins to allow the immune system to work at full force to kill tumor cells; however, SETDB1 interference can decrease the success of ICB. In 2021, in an effort to target chromatin regulators to further enhance ICB efficacy in ovarian cancer, Lin and colleagues conducted a CRISPR-Cas9 gRNA screening in ID8 cells [30]. Their result proved SETDB1 as a critical histone modifier negatively regulating PD-L1 level working alongside TRIM28/KAP1 complex in ovarian cancer. This result disagrees with the previously mentioned paper that identified SETDB1 as an inducer of PD-L1, promoting immune escape in CRC [27]. However, patient TCGA data further illustrated SETDB1 negative correlation with CD8+ T cell infiltration in tumors as well as Granzyme B expressing activated T cells, which agrees with the CRC paper. Mechanistically, in ovarian cancer, the SETDB1 knockout developed mitotic defects in the G2-M phase resulting in formation of micronuclei. The micronuclei then stimulated ISG upregulation through cGAS-STING pathway, which resulted in increased PD-L1 expression. Later, Guo and colleagues reaffirmed SETDB1 regulation of PD-L1 levels and improved response to ICB when SETDB1 is downregulated in ovarian cancer [31]. In their study, however, inhibition by the drug Adavosertib/AZD1775 of Wee1-like protein kinase (WEE1), a negative regulator of CDK1/2 during the S/G2 phase of the cell cycle, suppressed SETDB1 expression. Specifically, reduced levels of SETDB1 happened transcriptionally due to decreased levels in FOXM1, a transcription factor regulated downstream by WEE1. SETDB1 downregulation in turn led to activation of ERVs which triggered the dsRNA sensing pathway and consecutively the activation of interferon signaling. This work showed that SETDB1 repression can activate IFN signaling not only through cGAS-STING cytosolic DNA sensing pathway but also through RNA sensing mechanism. However, what exactly determines this differential activation of these downstream pathways aside from SETDB1 remains to be investigated and may involve different levels of regulation.

SETDB1 Effects in the Antiviral Response and Innate Immune Response

Within its scope of immune regulation, SETDB1 plays a significant role in the antiviral pathways within the body. Rauwel and colleagues characterized the interaction between SETDB1, the co-repressor KAP1 (TRIM28), and the nonhistone chromatin protein HP1 to exert transcriptional silencing in human stem cells (HSC) experiencing human cytomegalovirus (HCMV) latency [32]. HCMV is a common virus that can cause life-long issues for immunocompromised individuals when reactivation of its latent state fails to be

fought off by the immune system. SETDB1 is recruited by the KAP1 bromodomain through SUMOylation to induce HCMV latency. HCMV leaves latency if KAP1 is specifically phosphorylated and loses the power to recruit SETDB1 for transcriptional silencing. Thus, these findings demonstrate the importance of SETDB1 in the establishment and maintenance of HCMV latency in collaboration with KAP1 and HP1α. Bogoi and colleagues identified overexpression of SETDB1 and overall greater DNA methylation in CD4+ T cells in HIV infected individuals [33]. RNA/DNA analysis of CD4+ T cells in 129 seropositive (having detectable HIV antibodies) patients and 34 seronegative patients deduced that higher overall levels of DNA methylation correlated with lower numbers of CD4+ and CD8+ T cells. Increased methylation of T cells can impede T cell function and hinder the efficiency of the T cell-mediated immune response. Bogoi and colleagues reported that the observed T cell ratios mimic that of an aging immune system. Zhu and colleagues illustrated the mechanism for silencing unintegrated retroviral DNA, which included SETDB1 and the HUSH complex [34]. A genome-wide CRISPR-Cas9 screen identified SETDB1 as an integral gene for silencing of the MLV-GFP reporter virus along with NP220, a DNA-binding protein, and the HUSH complex, a three-protein mechanism for silencing proviruses. Knockout of SETDB1 halted the silencing of unintegrated retroviral DNA in HeLa cells. However, when NP220 was knocked out, neither SETDB1 nor HUSH proteins became associated with viral DNA. Thus, NP220 must be the primary DNA-binding protein and takes initiative in recruiting SETDB1 and HUSH complexes to the viral DNA.

In the following years, the group Tovo and colleagues released a string of papers investigating SETDB1 in a variety of immunological topics. In 2021, they published a paper reporting the role of SETDB1 in moderate to severe pediatric COVID-19 cases in relation to endogenous retroviruses [35]. The team studied the peripheral blood of 64 pediatric patients with varying severity of COVID-19 infections. They observed that COVID-19 correlated with higher SETDB1 transcription levels. Additionally, they identified a relationship between SETDB1/TRIM28 overexpression and mRNA of human endogenous retrovirus (HERV) sequences. The increased expression of SETDB1 led to greater levels of formation of heterochromatin, inducing HERV silencing. Surprisingly, however, they reported higher levels of HERVs. They also noted a positive association between IFN-1/IFN-2 and HERV transcriptions, suggesting the interferons may be responsible for increased HERV transcription. Ultimately, they suggest that TRIM28 may regulate SETDB1 activation, with expression levels in COVID-19 infected children changing immune host defense and cellular homeostasis. In 2022, they illustrated the silencing effects of SETDB1 on endogenous retroviruses in pediatric food allergies [36]. Food allergies source their immunological issues in gastrointestinal dendritic cells and dermal Langerhans cells. Through qPCR of 32 children with food allergies, SETDB1 mRNA levels were reported to be higher in patients with known allergies than in control children, similar to their previous paper on COVID-19. Possible reasons for increased mRNA levels were not discussed. They report that abnormal SETDB1 expression in allergenic children may affect allergic reactions through their suppressive effect on DC and T cells as well as regulatory effects of HERVs. The effect of HERVs and SETDB1 in autism spectrum disorder was then described in a separate paper the same year [37]. Similar to the results of previous papers, their data showed that children on the autism spectrum expressed higher levels of both SETDB1/TRIM28 and HERVs

than neurotypical children. To address the repeated controversy between both having higher transcriptional levels, they propose that a higher concentration of inflammatory cytokines in cerebrospinal fluid and blood may be responsible for the activation of HERVs. Inflammatory actions of HERVs may be the cause of deleterious effects on early brain development that leads to autism spectrum disorder. Each of these three papers relayed the possible immune effects of increased SETDB1 expression and thus, regulation of HERVs in three distinct scenarios. They also served to address the disagreement between high expression of SETDB1 and HERVs and provided plausible explanations.

Final Remarks

Thus far, we have covered a broad range of the role of SETDB1 in the immune system in its relation to development and phenotypic changes in immune cells (Figure 2) and its effect in immune cell recruitment to tumor sites in cancer (Figure 3). Histone methylation by SETDB1 and establishing a “closed” chromatin state appears to be largely responsible for these effects. Although many genes can be suppressed transcriptionally through these epigenetic modifications, endogenous retroviral elements (ERVs) account for a significant portion of these genetic changes and immune cell functional attributes. These were shown to happen in two major forms: ERV dysregulation may lead to downstream effects on proximal gene promoters and functioning as their enhancer (ex. B cell development) [11] or may activate pathways such as RNA or DNA sensing pathways (ex. AML) [28], which may have important implications for cell survival or stimulation of the innate immune response. Large bodies of evidence also suggest the vital role of SETDB1 in T and B lymphocytes as well as macrophages and DCs both in their differentiation and development, lineage commitment and activity. This can be evident when disruption of SETDB1 level leads to abnormality in their numbers or development of certain autoimmune diseases such as autism [37].

A great number of literature findings portray SETDB1 as an attractive target for immunotherapy in a wide variety of cancers. Owing to its ability to regulate critical immune-related pathways such as type-I interferon signaling, PD-L1, and MHC-I class of receptors, its depletion can trigger a strong immune response against the tumor, involving activation and penetration of CD8+ T-cells and further enhancing the efficacy of immune checkpoint blockade therapy [25]. Activation of these pathways in cancer cells however proved to be complex involving diverse mechanisms of activation. In addition, different cofactors are involved in these processes such as TRIM28 or HUSH complex with each participating in a particular aspect of these regulations. As future research is done to understand the effects of SETDB1 on different types of T cells, work must also be done to identify the mechanisms of SETDB1 on Tregs that yield a positive correlation between gene expression and cell proliferation [22].

Continued research must be done to fully understand the effect of SETDB1 on the immune system in a wide range of cancers. Can SETDB1 be inhibited in isolation without detrimental genomic side effects? How can novel drugs selectively target SETDB1 overexpression in cancer cells without disrupting the essential role of SETDB1 in immune cells through off-target effects? Are there differences in the mechanisms altered by SETDB1 overexpression in different cancers and would these differences affect drug treatments? How

would immunotherapy be affected in different cancers when SETDB1 is targeted given the wide scope of SETDB1 regulation of immune checkpoint genes (ex. contradicting effects on PD-L1)? We have identified two factors to be critical in understanding the off-target challenge. (1) Finding the optimal level of decreased SETDB1 expression in cancer cells, without completely diminishing SETDB1 levels in immune cells. (2) Identifying differences in the repressing components that SETDB1 forms in cancer cells verses in immune cells. SETDB1 forms three major repressing complexes: HUSH, KRAB-ZFP and KAP1/TRIM28. These complexes are context dependent. For example, SETDB1-KAP1 complex contributes to ERV repression in leukocytes while KDM5B recruits SETDB1 to suppress ERVs in cancer models such as melanoma. Further studies to identify the components common in both cancer systems and immune cell systems will allow treatments to be properly tailored to minimize possible side effects. An additional strategy could be targeting the other SETDB1 repressing complex components to block SETDB1 function. For example, oncogene KDM5B is overexpressed in multiple cancer types and serves as a promising cancer target [38].

Regarding ERVs, how can we accurately determine the location of ERVs, considering their tendency for repetition. After all, ERVs represent 8% of human genome [39]. Further, which ERVs are most prominent in immune regulation, and can we map ERV integration in immune cell gene networks? These essential questions are some of our next steps in identifying therapeutic opportunities in cancer patients and other immune centric diseases.

We hope this review has efficiently presented a wide range of studies to better understand the function of SETDB1 in the immune system. We understand that this research will play a critical role in improving immunotherapy for patients moving forward and that future studies will continue to make significant progress for the medical community.

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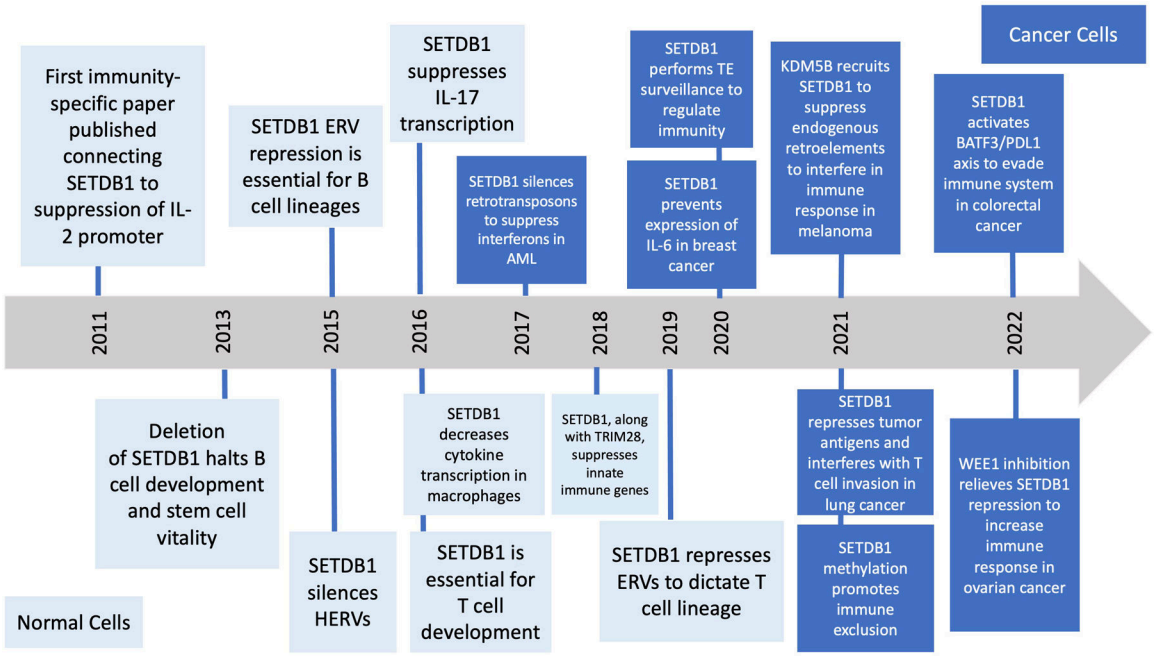

FIGURE 1.

A timeline of major discoveries and publications in SETDB1 studies regarding the immune system. Includes SETDB1 immunological functions in both normal cells and cancerous cells.

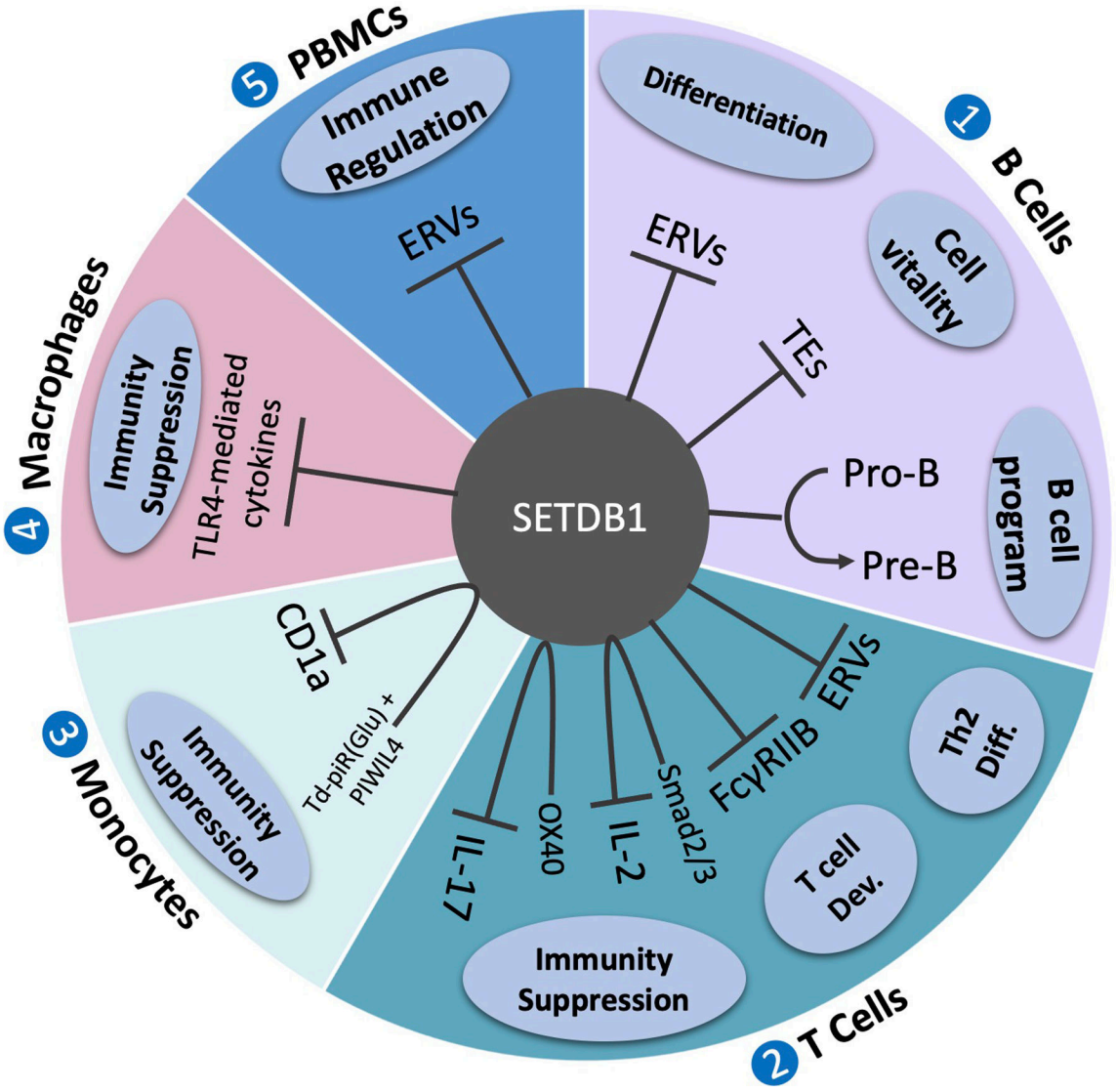

FIGURE 2.

Schematic diagram summarizing SETDB1 role in immune function within various leukocytes. **1.** Within B cells, SETDB1 suppresses endogenous retroviruses (ERVs) to ensure proper lineage differentiation; represses transposable elements (TEs) to maintain cell vitality [11]; and mediates the transition from Pro-B to Pre-B cells [11, 17], thus establishing a B cell program. **2.** In T cells, SETDB1 represses ERVs to allow differentiation and commitment of the TH2 lineage [12]; suppresses the protein FcγRIIB [20], allowing proper ERK function and T cell development. To suppress the immune system, Smad2/3 recruits SETDB1 to the IL-2 locus for methylation [18] and OX40 recruits SETDB1 to the IL-17 locus for methylation [19]. **3.** Within monocytes, Td-piR(Glu)PIWIL4 complex recruits SETDB1 for transcription repression of CD1a [21]. **4.** In macrophages, SETDB1 suppresses TLR4-mediated cytokines to repress the immune response [13]. **5.** In peripheral blood mononuclear cells (PBMCs), ERV suppression via SETDB1 regulates the immune system [14].

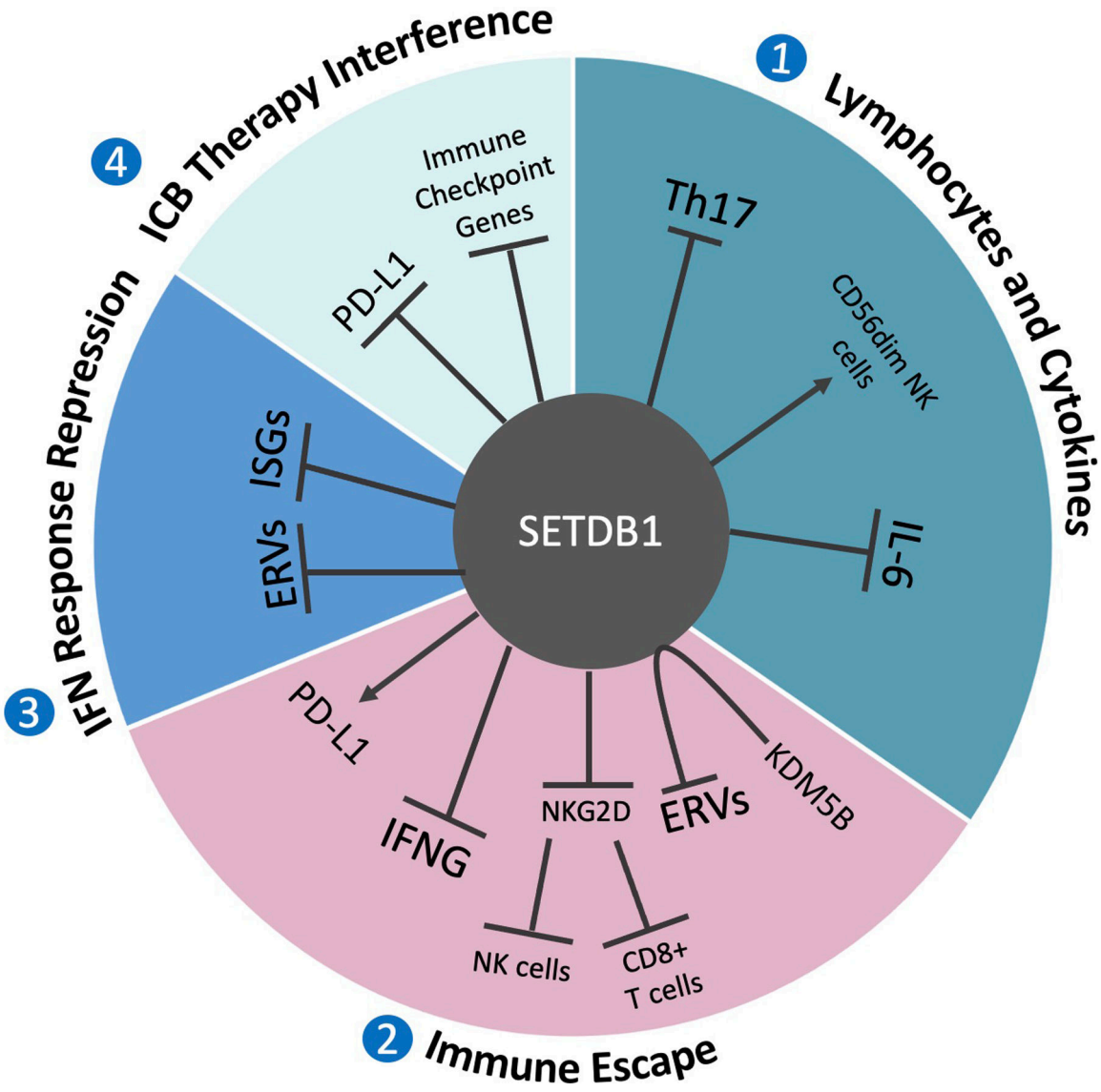

FIGURE 3.

SETDB1 systematically regulates transcription and expression of cytokines, lymphocytes, proteins, and transposable elements in cancerous cells. **1.** SETDB1 suppresses Th17 transcription and upregulates production of CD56dim natural killer cells [23]. SETDB1 suppresses IL-6 production [13]. **2**. KDM5B, a chromatin modifier, recruits SETDB1 for ERV suppression [15]. SETDB1 suppresses transcription of the NKG2D receptor to downregulate NK and CD8+ T cell production [25]. SETDB1 upregulates PD-L1 [27]. **3.** SETDB1 represses ERVs to inhibit the dsRNA induced IFN response. SETDB1 suppresses interferon stimulated genes (ISGs) [28]. **4.** SETDB1 represses PD-L1 [24, 31]. SETDB1 suppresses immune checkpoint genes, which interferes in ICB antitumor therapies [24, 31]. *Note that SETDB1 has been reported to both promote and repress PD-L1 transcription through different pathways and was studied in separate models. PD-L1 upregulation, studied in CRC models, is possible through inhibition of the FOSB-mediated miR-22 pathway,

which in turn activates BATF3/PD-L1 to upregulate PD-L1. PD-L1 downregulation, studied in ovarian cancer, is possible through suppression of the cGAS-STING pathway.