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Review Article

The MLL3/4 H3K4 methyltransferase complex in establishing an active enhancer landscape

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Enhancers are *cis*-regulatory elements that play essential roles in tissue-specific gene expression during development. Enhancer function in the expression of developmental genes requires precise regulation, while deregulation of enhancer function could be the main cause of tissue-specific cancer development. MLL3/KMT2C and MLL4/KMT2D are two paralogous histone modifiers that belong to the SET1/MLL (also named COMPASS) family of lysine methyltransferases and play critical roles in enhancer-regulated gene activation. Importantly, large-scale DNA sequencing studies have revealed that they are amongst the most frequently mutated genes associated with human cancers. MLL3 and MLL4 form identical multi-protein complexes for modifying mono-methylation of histone H3 lysine 4 (H3K4) at enhancers, which together with the p300/CBP-mediated H3K27 acetylation can generate an active enhancer landscape for long-range target gene activation. Recent studies have provided a better understanding of the possible mechanisms underlying the roles of MLL3/MLL4 complexes in enhancer regulation. Moreover, accumulating studies offer new insights into our knowledge of the potential role of MLL3/MLL4 in cancer development. In this review, we summarize recent evidence on the molecular mechanisms of MLL3/MLL4 in the regulation of active enhancer landscape and long-range gene expression, and discuss their clinical implications in human cancers.

Introduction

Precise gene regulation is required for the correct cellular identity in multicellular organisms. However, aberrant gene expression often leads to detrimental consequences such as developmental defect and cancer. Therefore, it is critical to illustrate how transcription factors/cofactors and the regulatory elements orchestrate transcriptional programs in normal and pathological conditions. Unique gene expression patterns are established by coordination of transcription factors (TFs) and cofactors for accessibility of RNA polymerase II (Pol II) to the *cis*-regulatory elements, such as promoters and enhancers [1–3]. While promoters are sufficient to recruit Pol II and initiate basal levels of gene expression, they require enhancers for full activity [4,5]. Enhancers can be bound by activating TFs, which respond to developmental and environmental signals, to increase the probability and/or level of target gene transcription [6,7]. Therefore, enhancers play a pivotal role in driving cell type- and tissue-specific gene expression and are capable of eliciting transcription of their target genes by communicating with the corresponding promoters over great genomic distances [8–11]. Emerging evidence suggests that deregulation of enhancer function could be the main cause of tissue-specific cancer development [12–16]. However, the prediction of enhancer locations is much more difficult compared to that of promoters since enhancers can locate at anywhere in the genome. Over the past decade, the epigenomic profiling technologies, such as chromatin immunoprecipitation (ChIP) coupled to next-generation sequencing (NGS), have enabled the annotation of enhancers and their properties across entire genomes and have shown that the genomic locations of enhancers typically display distinctive chromatin features associated with different

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transcriptional states [11,17,18]. One of the chromatin features used to distinguish enhancers and promoters is the posttranslational methylation of histone H3 at lysine 4 (H3K4). Mono-methylation of H3K4 (H3K4me1) is found at both inactive and active enhancers, whereas H3K4 tri-methylation (H3K4me3) localizes at the promoter regions of actively transcribed genes [19]. H3K4me3 at promoters is deposited by the SET1/MLL family lysine methyltransferases (KMTs), MLL1/MLL2 and SETD1A/SETD1B (also known as KMT2A/KMT2B and KMT2F/KMT2G, respectively). The other SET1/MLL family members, MLL3/MLL4 (also known as KMT2C/KMT2D), have been later identified as the major KMTs for enhancer-associated H3K4me1 [20–23]. Nevertheless, it is still poorly understood how MLL3/MLL4 contribute to the establishment of enhancer landscapes and/or enhancer–promoter communication and how they regulate enhancer activities. In this review, we describe the current knowledge about how MLL3/MLL4 establishes an active enhancer landscape for target gene activation and discuss novel insights into the roles of MLL3/MLL4 in cancer disease states.

MLL3/4 complexes function in enhancer activation

MLL3 and MLL4 share the core subcomplex WRAD (WDR5, RBBP5, ASH2L, DPY30) with other members of the SET1/MLL family, but also contain complex-specific subunits that include UTX/KDM6A, NCOA6, PTIP and PA1 [22,24,25] (Figure 1). The WRAD core subunits are critical for H3K4 methyltransferase activity in all SET1/MLL complexes [26,27], whereas the unique subunits could confer other functions or specificity for recruitment of the individual SET1/MLL complex to particular genomic regions [22]. MLL3/4 complexes are recruited to target enhancers through interactions with sequence-specific TFs, such as ligand-regulated nuclear receptors (NRs) and pioneer factors that activate *de novo* enhancers [28–30]. Since MLL3 and MLL4 form separate multi-protein complexes in mammalian cells, the binding of MLL3/4 to TFs is thought to be mediated through indirect interactions via other subunits within the complex. Below we discuss the unique complex subunits that were found to contribute to the recruitment of MLL3/4 on enhancers.

The recruited mechanisms of MLL3/4 complexes to enhancer regions

UTX

UTX is a histone demethylase that mediates H3K27 demethylation and thereby antagonizes the Polycomb proteins-mediated transcriptional repression [31–35]. UTX was found to interact with the retinoic acid receptor α (RARα) and that this interaction is essential for proper cell differentiation in response to retinoic acid [29,36]. Through the association within the MLL3/4 complex, UTX is thought to regulate the transition from inactive/poised ($H3K4me1^{+}H3K27me3^{+}$) to active ($H3K4me1^{+}H3K27ac^{+}$) enhancers by coupling its H3K27 demethylase

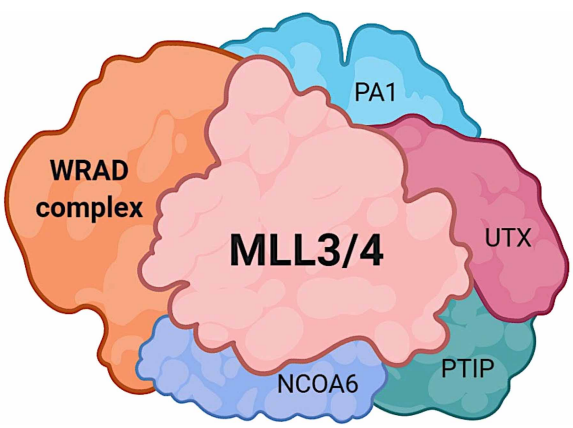

Figure 1. The MLL3/4 H3K4 methyltransferase complex.

Schematic representation of the MLL3/4 multi-protein complex. MLL3/4 proteins are catalytic components of the large SET1/MLL complex, which are known to catalyze mono-methylation of histone H3K4. The WRAD subunits (including WDR5, RBBP5, ASH2L, and DPY30) are shared by MLL3 and MLL4 with other members of the SET1/MLL family, with functions mostly relevant to their methyltransferase activities. Complex-specific subunits such as PA1, PTIP, UTX, and NCOA6 confer unique scaffolding and recruitment functions to MLL3 and MLL4, as well as loci-specificity through their interaction with secondary-associated proteins. The figures is created with BioRender.com.

activity and the H3K4me1-catalytic activity of MLL3/4 [34,37] (Figure 2). However, emerging evidence indicates novel catalytic-independent functions of UTX in early development and cancer [38–41], implying that UTX may activate transcription through other regulatory mechanisms besides histone demethylation. Indeed, UTX was found to associate with histone acetyltransferase (HAT) p300/CBP and facilitate recruitments of both p300/CBP and the MLL3/4 complex to target enhancers and thereby integrate their catalytic functions for establishing an active enhancer landscape ($H3K4me1^{+}H3K27ac^{+}$) [42]. This unique regulation appears to provide a mechanistic basis for the transition from *de novo* (unmarked) to active enhancers (Figure 2).

NCOA6

NCOA6, also named activating signal cointegrator-2 (ASC2), is a key coactivator of NRs in the NR pathways [43]. NCOA6 interacts directly with NRs through LXXLL motifs (NR boxes) and plays an important role for the recruitment of MLL3/4 complexes to the NR-targeted gene loci for H3K4 methylation [43]. NCOA6 can also interact with other TFs, such as the adipogenic factors PPARγ and facilitate the recruitment of MLL3/4 complexes to PPARγ target genes that contributes to PPARγ-dependent adipogenesis [44]. Interestingly, NCOA6 was found to interact with the transcriptional coactivator Yorkie (Yki), which facilitates the interaction of Yki with TFs and the subsequent regulation of the Hippo signaling pathway in *Drosophila melanogaster*. Both NCOA6 and Trr (a *Drosophila* homolog of MLL3/4) are functionally required for Hippo-mediated growth control and target gene expression, indicating that the MLL3/4-dependent H3K4 methylation is important for the Hippo signaling-mediated tissue growth control [45].

PTIP and PA1

Several lines of studies suggest that PTIP involves in transcriptional regulation through facilitating the genomic recruitment of MLL3/4 complexes [24,46–48]. PTIP was first discovered via its interactions with PAX2, a developmental regulator that specifies mesodermal cell fate determination and demarcates the midbrain–hindbrain junction [49,50]. By binding to PAX2, or a closely related PAX5, PTIP promoted the recruitment of MLL3/4 complexes and subsequent H3K4 methylation at PAX2/5-binding transcriptional regulatory regions [46,47]. PTIP was also shown to promote H3K4 methylation at the immunoglobulin heavy-chain (*IgH*) locus, which leads to immunoglobulin class switch recombination (CSR) in B-lymphocytes [48]. Interestingly, a stable PTIP-PA1 subcomplex was found to be sufficient for the transcriptional function of PTIP for IgH CSR, indicating that PTIP function in transcription can be separated from its association with MLL3/4 complexes [51]. Indeed, double deficiency in MLL3 and MLL4 displayed normal *IgH* germline transcription in B cells. The findings of PTIP and PA1 promoting H3K4me3 at *IgH* promoters further suggest that the PTIP-PA1 subcomplex functions independently from the modulation of H3K4me1 at enhancer regions [51].

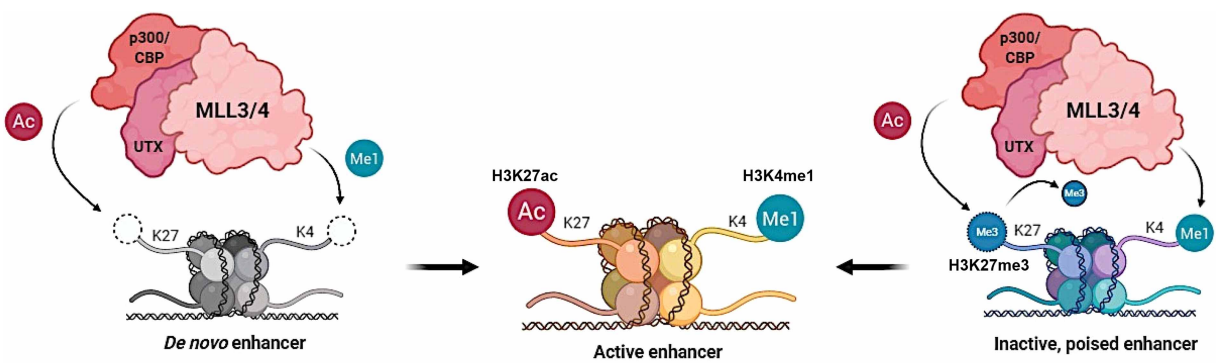

Figure 2. MLL3/4-mediated activation of *de novo* and poised enhancers.

Active enhancers are enriched in H3K4 mono-methylated and H3K27 acetylated histone marks ($H3K4me1^{+}/H3K27Ac^{+}$) which are deposited in one of two ways. (*Left*) Unmodified, *de novo* enhancers ($H3K4me1^{-}/H3K27Ac^{-}$) are simultaneously marked with H3K4me1 by the SET domain of MLL3/4, and with H3K27ac by its associated p300/CBP acetyltransferase. Notably, the H3K27 demethylase activity of UTX may be dispensable for this regulation. (*Right*) Inactivated poised enhancers marked with H3K4me1 and H3K27me3 ($H3K4me1^{+}/H3K27me3^{+}$) are activated through continued deposition of H3K4me1 by MLL3/4, and replacement of the repressive H3K27me3 mark with H3K27ac — a process achieved through the cooperative demethylating function of UTX and the acetylating function of p300/CBP. The figure is created with BioRender.com.

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Catalytic and noncatalytic-dependent function of MLL3/4 in enhancer activation

MLL3/4 are the major KMTs for H3K4me1 catalysis. Although H3K4me1 constitutes a central component of the enhancer chromatin landscape, whether MLL3/4 provoke enhancer activities mainly through the H3K4me1-catalytic activity is still obscure. In both biochemical and genetic studies, MLL3/4 were found to exert their transcription regulatory activities by binding to enhancer elements and promoting the recruitment of p300/CBP and the subsequent implementation of H3K27ac [42,52–55]. Loss of MLL3 and/or MLL4 resulted in a significant loss in H3K4me1 and H3K27ac, as well as the binding of Mediator and Pol II on active enhancers, resulting in diminished enhancer activities [42,52,53,56,57]. Direct associations between p300/CBP and MLL3/4 complexes are thought to be mediated through other complex subunits, including UTX, NCOA6, and ASH2L [42,58,59]. Loss of UTX could generally phenocopy the effects of MLL4 depletion on enhancer-associated active enhancer marks, decreasing both H3K4me1 and H3K27ac and downregulating nearby target genes [42]. While p300/CBP-mediated H3K27ac seems to play a dominant role in eliciting transcription [42,60], the function of MLL3/4-catalyzed H3K4me1 in transcriptional activation remains ambiguous. Using catalytically deficient MLL3/4 mutants in mouse embryonic stem cells (mESCs) or *Drosophila melanogaster*, Dorighi et al. [57] and Rickels et al. [61] found that H3K4me1 is largely dispensable for transcription, stem cell self-renewal, and *Drosophila* development. In contrast to severe transcriptional defects caused by MLL3/4 loss, loss of the MLL3/4 catalytic activity resulted in very little changes in mESC transcriptional programs [57]. Consistently, gene expression profiles for *Drosophila* adult brains or larvae wing imaginal discs expressing a catalytic-defective *Drosophila* MLL3/4 homolog (Trr) were highly similar to the profile in wild-type fly [61]. Therefore, MLL3/4 seems to exert a catalytic-independent function for enhancer regulation. Interestingly, an evolutionally conserved MLL3/4 domain was recently found to be sufficient to stabilize and recruit UTX to the genome and rescue the MLL3/4-null phenotypes in *Drosophila* and mammalian cells [62], raising the possibility that stabilization of UTX could be a central catalytic-independent function of MLL3/4. Nonetheless, the mechanism of action of UTX in such regulation warrants further investigation.

It remains puzzled whether H3K4me1 has any function within enhancers, or could just be a side-product of MLL3/4 as a hallmark of enhancer landscape. In conjunction with nucleosome pulldowns with mass spectrometry analysis, SWI/SNF (BAF) chromatin remodeling complex and other chromatin regulators (e.g. cohesin) have been identified as histone readers specifically for enhancer-associated H3K4me1 marks [56]. Using similar genetic approaches, Local et al. [56] found that loss of H3K4me1, either by MLL3/4-null or catalytically deficient mutants, resulted in lower binding of the BAF complex to enhancers. They further found that H3K4me1 greatly increased the nucleosome-remodeling activity of the BAF complex [56]. Since cohesin is known to facilitate enhancer–promoter looping [63], these findings implicate that H3K4me1 may actively involve in many aspects of enhancer regulation, from chromatin remodeling to the long-range enhancer–promoter interactions (Figure 3). Moreover, Wang et al. used the CRISPR-dCas9-based system to target p300 and/or MLL4 to well-known distal enhancers [42,60] and found that only MLL4 with the intact SET domain could promote p300-dependent long-range transcriptional activation [42]. The discrepancy between the catalytic and noncatalytic-dependent functions of MLL3/4 in enhancer activation could be due to several factors. For instance, it is possible that p300/CBP-mediated H3K27ac is sufficient to elicit transcription [42,60], while the fully enhancer activation requires H3K4me1 for promoting chromatin remodeling and enhancer–promoter looping [56,64] (Figure 3). It is also possible that MLL3/4 are essential for the recruitment of both p300/CBP and Pol II on enhancers [57], so that MLL3/4 loss has stronger effects than H3K4me1 loss. Since UTX is known to mediate the interaction between p300 and MLL3/4 complexes [42], it would be interesting to test whether UTX contributes to the recruitment of Pol II for the catalytic-independent function of MLL3/MLL4 [62].

Deregulation of MLL3/4 in enhancer dysfunction and human cancers

Considering the importance of MLL3/4 in the control of enhancer regulation and gene activation, deregulation of MLL3/4 may cause enhancer dysfunction and contribute to aberrant gene expression programs, thereby driving tumor malignancies [65,66]. Somatic mutations in *MLL3* and *MLL4* were first identified by whole-genome sequencing in medulloblastoma (MB) [67–70] and non-Hodgkin lymphoma [71–74]. Mutations in *MLL3* and/or *MLL4* were later found in numerous human cancers and cancer cell lines, including leukemia [75,76], bladder [77,78], lung [79–82], liver [83], prostate [84], breast [85–89], ovarian [90], gastric [91], pancreatic [70,92], renal [93], and

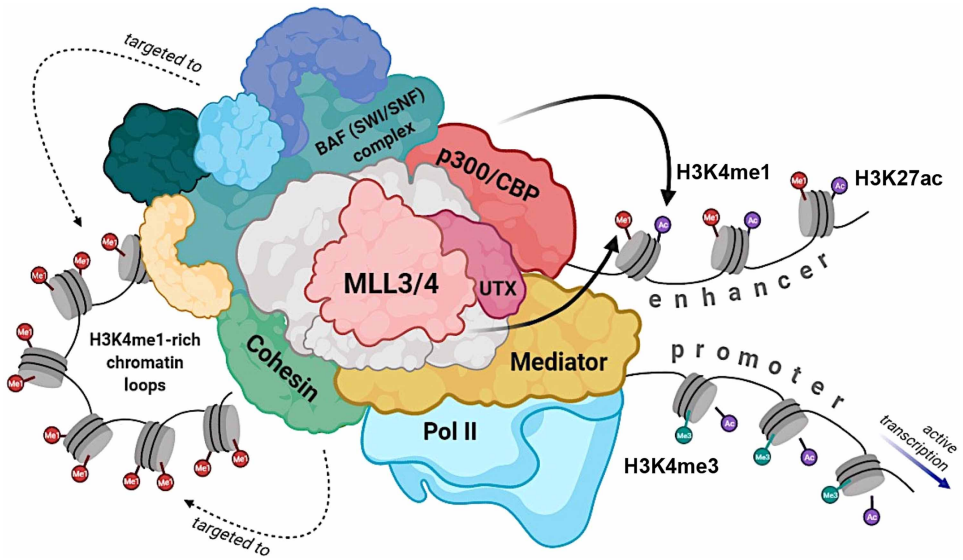

Figure 3. The proposed model of enhancer-mediated transcriptional activation by the MLL3/4 complex.
The MLL3/4 complex recruits p300/CBP (confers H3K27 acetyltransferase) through UTX to initiate and maintain the deposition of H3K4me1 and H3K27ac at enhancer regions. UTX possesses the H3K27 demethylase activity and is responsible to erase the repressive histone mark H3K27me3. At promoter regions, the Mediator coactivator complex recruits and stabilizes the pre-initiation complex (PIC), with RNA polymerase II (Pol II) at its core. Chromatin remodelers such as cohesin and the BAF (SWI/SNF) complex simultaneously target to these H3K4me1-enriched regions, facilitating the formation of enhancer–promoter loops that bring together all relevant cofactors, thereby leading to active transcription. The figure is created with BioRender.com.

colorectal cancers [94,95]. Most of the somatic mutations in *MLL3* or *MLL4* appear to be inactivating mutations, highly suggesting their roles as tumor suppressors during tumor initiation and progression (Table 1).

Hematopoietic cancers

MLL4 is one of the most recurrently mutated genes in both diffuse large B cell lymphoma (DLBCL) and follicular lymphoma (FL) that arise from germinal-center B (GCB) cells [71,72,96–98]. Most of the gene alterations are nonsense or frameshift mutations that may generate truncated MLL4 proteins with a deficient methyltransferase activity [96,97]. Indeed, Ortega-Molina et al. [97] and Zhang et al. [96] showed that genetic ablation of *MLL4* in murine GCB cells resulted in a significant reduction of H3K4 methylation at MLL4-bound enhancers, thereby provoking GCB cell malignancies. Moreover, MLL4 loss in cooperation with BCL2 overexpression greatly promoted murine B cell lymphomagenesis that recapitulates the pathological property of human DLBCL and FL [96,97,99]. These findings suggest that MLL4 act as a tumor suppressor whose loss-of-function mutations may promote lymphomagenesis by remodeling the enhancer landscape of lymphoma-initiating cells. Notably, *MLL3* is also mutated in lymphoma albeit at much lower frequencies [73,100]. These mutations appeared to not mutually exclude *MLL4* mutations, suggesting that MLL3 and MLL4 may exert distinct functions in lymphoma. However, it is still unclear whether MLL3 and MLL4 function nonredundantly in lymphoma, because MLL4 loss caused a global reduction of H3K4 methylation [96]. In addition, missense mutations occurring nearby or within the C-terminal SET domain of MLL4 affected its methyltransferase activity and, in some cases, caused the loss of protein expression [96]. Since the scaffolding function of MLL4 is known to be more important for transcription and enhancer activation [57,61,62], it remains uncharacterized whether the C-terminal missense mutations that affect MLL4 activity but retain its protein expression would have the similar functional consequence as the truncating mutations [96]. These questions warrant for more detailed investigations.

Monosomy 7 or large deletions of chromosome 7q often occur together with deletions in chromosome 17p in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) [101]. Through a combinatorial suppression of MLL3 (7q), NF1 (17q), and/or p53 (17q) in murine models, Chen et al. [76] observed a remarkable cooperativity in the initiation and progression of aggressive AMLs developed from p53-deficient hematopoietic stem and progenitor cells (HSPCs) when *MLL3* and *NF1* were simultaneously suppressed. Interestingly, suppression of *MLL3*

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Table 1. Summary of studies evaluating mutations of *MLL3* and/or *MLL4* in human cancers

Cancer type	Gene/s	Mutation type	Role/function	Techniques used	Refs
Diffuse large B cell lymphoma	MLL4	Nonsense Frameshift Missense Splice	Haploinsufficient tumor suppressor; Impaired H3K4 methylation	Whole exome sequencing; RNA-seq; Sequencing and IHC analysis of patient-derived tumor samples	[72–74,96]
Follicular lymphoma	MLL4	Nonsense Frameshift Missense Splice	Haploinsufficient tumor suppressor	RNA-Seq	[72]
Acute myeloid leukemia	MLL3	Deletion	Haploinsufficient tumor suppressor	Analysis of TCGA data	[76,101]
Medulloblastoma	MLL3, MLL4	Nonsense Frameshift Missense Splice	Tumor suppressor	Microarray sequencing; Whole exome sequencing	[67–69]
Glioblastoma	MLL3	Deletion	Haploinsufficient tumor suppressor?	Large-scale sequencing	[70]
Renal carcinoma	MLL4	Nonsense Frameshift Missense Splice	NS	SNPArray, PCR based exon resequencing	[93]
Hepatocellular carcinoma	MLL3	Missense	NS	Whole-genome sequencing	[83]
Prostate cancer	MLL3, MLL4	Nonsense Frameshift Missense	NS	Whole exome sequencing	[84]
Bladder cancer	MLL3	Nonsense Frameshiftv Missense Splice	NS	Whole exome sequencing	[78]
Colorectal cancer	MLL3	Nonsense Frameshift Missense Deletion	Tumor suppressor	CDS sequencing; Targeted MLL3 sequencing	[94,95]
Gastric adenocarcinoma	MLL3	Splice	NS	Whole exome sequencing	[91]
Breast cancer	MLL3, MLL4	Nonsense Frameshift Missense Splice Single nucleotide variants/structural variants	High levels of MLL4 are associated with poor prognosis in patients with breast cancer; Breast cancer driver gene; Deletion of MLL3 is significantly associated with shorter survival, and gain of this gene is significantly associated with longer survival; MLL4 mutations are functionally linked with poor patient survival	Database analyzed using KM survival analysis; Data analyzed from the cBio Cancer Genomics Portal; Whole-genome sequencing; Data analyzed from METABRIC data	[85–87,89]
Adult granulosa cell tumors (GCT) of the ovary	MLL4	Deletion Low IHC protein expression	Association between MLL4 inactivation and aGCT relapse	Whole exome sequencing and IHC staining of patient-derived tumors; WES of the KGN cell line (aGCT model)	[90]
Lung cancer	MLL3, MLL4	Nonsense Frameshift Missense Deletion	Tumor suppressor	Whole exome sequencing; Bioinformatics analysis of TCGA dataset	[66,78–82,103–107]
Pancreatic adenocarcinoma	MLL3, MLL4	Nonsense Frameshift Missense Splice	Patients wild type for MLL gene alterations have a significantly lower median survival compared with those with mutated MLL genes	Large-scale sequencing; Next-generation sequencing	[70,92]

along with *NF1* or *p53* did not induce or accelerate HSPCs to form tumor malignancy [76], indicating that the combinatorial suppression of these tumor suppressors (i.e. MLL3, NF1, and p53) is required for AML development. They also observed that p53-deficient HSPCs with approximate 50% of both MLL3 and NF1 repression have a great potential to leukemogenesis [76]. Thus, they further demonstrated that MLL3 plays as a haploinsufficient tumor suppressor that may explain recurring deletions of chromosome 7 and 7q in human hematopoietic malignancy [76]. It remains uncharacterized whether MLL4 also exerts tumor suppressor function in AMLs. However, MLL4 was shown to play an essential role in MLL1-rearranged leukemia. Santos et al. [102] tested the role of MLL4 in a well-established murine model that mimics AMLs containing a MLL1 translocation (i.e. MLL1-AF9) and found that loss of MLL4 greatly inhibits the development of MLL1-AF9-induced leukemia, suggesting a potential oncogenic role of MLL4 in certain AMLs. However, whether MLL3 and MLL4 exert distinct functions in the development of AMLs remains unclear and warrant for further investigations.

Solid cancers

Although *MLL3* and *MLL4* are amongst the most frequently mutated genes in human cancers, their mutation rates and actual functions in tumorigenesis could be different. Whole genome or exome sequencing revealed a high frequency of *MLL4*, but not *MLL3*, mutations in small cell lung cancer (SCLC) [103–106]. The investigation of human SCLC cell lines indicated a frequent loss of MLL4 protein via truncating mutations and the concomitant reduced levels of H3K4me1, suggesting that MLL4 deficiency may cause the dysregulation of transcriptional enhancers and transcription programs in SCLC [103]. In addition, somatic mutations of *MLL3* and/or *MLL4* were frequently found in non-small cell lung cancer (NSCLC), including lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) [66]. Interestingly, Yin et al. [107] identified deleterious *MLL4* mutations in NSCLC patients and found that the tumors showing reduced MLL4 expression were independent of mutation status, suggesting that *MLL4* expression in NSCLC tumors might be caused by reduced RNA transcription or stability. Interestingly, they found that only *MLL4* mutations but not the expression status are significantly associated with worse patients' survival outcome. Although this study supported a potential role of mutant MLL4 in driving lung tumor growth and progression, there is still no substantive conclusion due to the lower case number [107]. Nevertheless, a recent research revealed a tumor suppressor function of MLL4 in lung tumorigenesis. Alam et al. [79] demonstrated that lung-specific *MLL4* loss in a $KRAS^{G12D}$ mouse model promotes LUAD and extensively impairs global levels of H3K4me1 and H3K27ac at typical and super-enhancers. The MLL4-regulated super-enhancers include the super-enhancer for *PER2,* a circadian rhythm repressor that is also shown to regulate glycolysis [108]. MLL4 occupied the *PER2* gene locus in $KRAS^{G12D}$ lung cancer cells, while loss of MLL4 decreased *PER2* expression and thereby reduced PER2 occupancy in tumor-promoting glycolytic genes, leading to upregulation of these genes and increased glycolysis. Consistently, pharmacological inhibition of glycolysis benefited to suppress the tumorigenicity of human lung cancer cells with *MLL4*-inactivating mutations. This study indicated that MLL4 acts as a tumor suppressor for glycolysis-dependent lung tumorigenesis via inducing *PER2* expression [79]. Although it remains unclear about how MLL4 deficiency cooperates with aberrant KRAS activation to increase lung tumorigenicity, MLL4 deficiency has been reported to induce RAS signaling that contribute to the development of MB in mice. Dhar et al. [109] found that brain-specific *MLL4* loss in mice resulted in spontaneous MB through upregulation of the RAS and NOTCH pathways and downregulation of several tumor suppressor genes. The abnormal activation of oncogenic pathways in brain-specific loss of MLL4 could be attributed to the global diminution of active super-enhancers and broad H3K4me3 domains that often define active tumor suppressor genes (e.g. *DNMT3A* and *BCL6* in this situation) [109]. Notably, this tumor-suppressive mechanism of MLL4 in MB genesis could be specific to the brain tissue, because the loss of MLL4 in lung tumorigenesis did not induce either gene expression of RAS activators or decreased *DNMT3A* expression as well as a reduced global H3K4 tri-methylation [79].

In contrast to the tumor-suppressive function of MLL4 in lung cancer and MB, mutant p53 was found to associate with MLL4, which promotes the recruitment of MLL4 to a class of TNFα-responsive enhancers in colorectal cancer cells. This mutant p53-dependent MLL4 targeting was shown to regulate abnormal enrichment of H3K4me1 and H3K27ac at target enhancers and gene activation in response to TNFα-mediated chronic immune signaling [110]. In addition, MLL4 was found to interact with common estrogen receptor alpha (ERα) mutants and this interaction may facilitate the ability of ERα mutants to activate target gene transcription and promote breast cancer growth in resistant to endocrine therapies [111]. These findings imply a critical role of MLL4 in regulating the oncogenic gain-of-function activity of TFs. Notably, these studies did not exclude the possibility that MLL3 may also function as a tumor-promoting cofactor. Indeed, MLL3 was found to involve in the endocrine therapy resistance. Kim et al. found that MLL3 could regulate *ERα* expression through enhancer activation

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and thereby promote the proliferation of tamoxifen-resistant breast cancer cells. Depletion of MLL3 greatly enhanced the sensitivity of tamoxifen-resistant cells to fulvestrant-based endocrine therapy [112]. In addition, Gala et al. [113] revealed that depletion of MLL3 causes a significant loss of H3K4me1 and H3K27ac on selected ERα enhancers, as well as downregulation of estrogen-dependent gene expression, which suppresses the proliferation of ER-positive breast cancer cells. This finding is supported by the clinical outcome of breast cancer patients where MLL3 deficiency correlated with a poor patient survival [113,114]. Interestingly, Gala et al. also found that MLL3 loss promotes breast cancer cell outgrowth in hormone-depleted conditions. The development of hormone-independent outgrowth might not be caused by the replacement of MLL3 enhancer-regulatory activity by other KMTs (e.g. MLL4) because of the sustained H3K4me1 reduction in MLL3-deficient cells. Instead, it could be attributed to the reprogrammed ERα targeting in the genome where the unliganded ERα-mediated unique gene transcription may contribute to hormone-depleted cell proliferation. This study provided evidence to the dual effects of MLL3 in hormone-dependent or independent breast cancer growth [113].

Functional discrepancy between MLL3 and MLL4

The discoveries of MLL3 and MLL4 in sharing identical SET1/MLL complex proteins and having the same H3K4 mono-methylation activity have raised the possibility that their functions might be generally overlapping

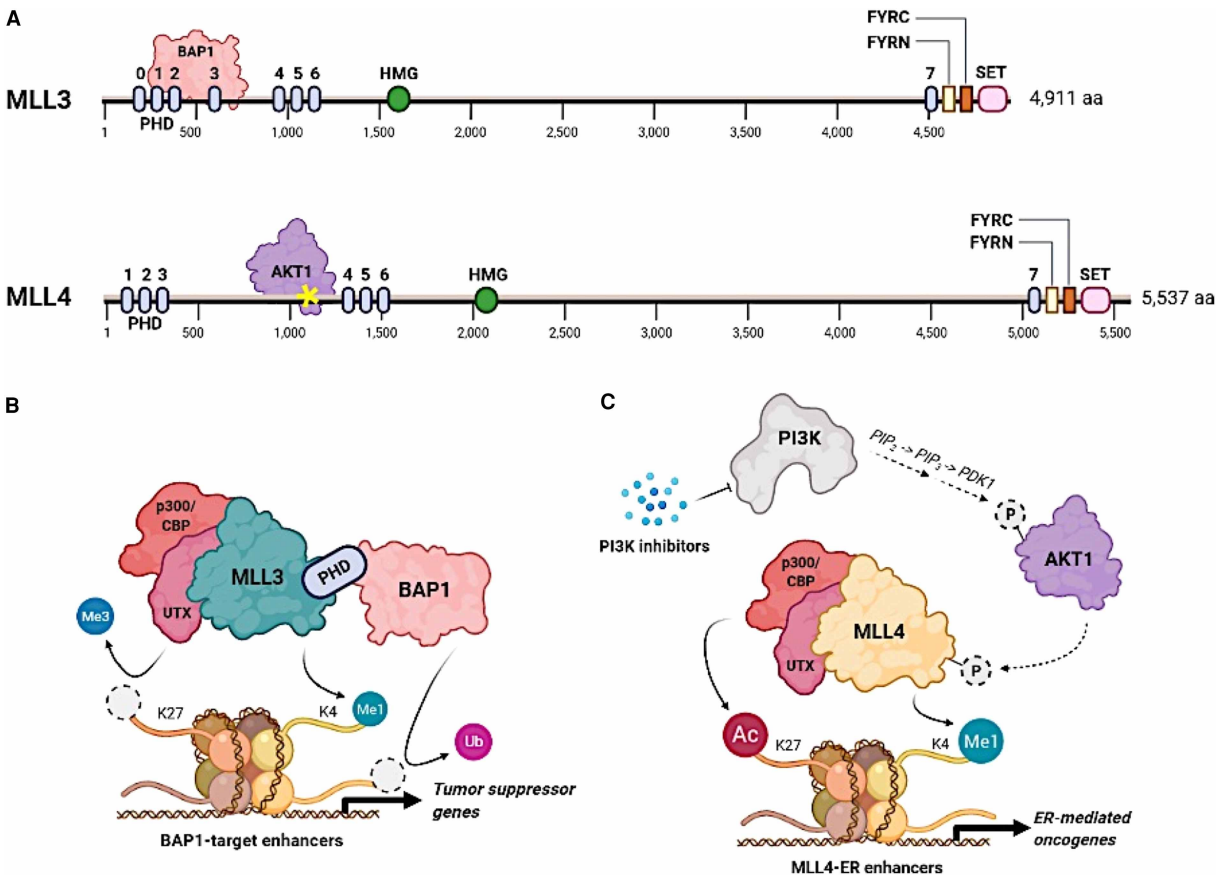

Figure 4. MLL3/4 share overlapping domains with complex-specific functions.

(**A**) The paralogous MLL3 and MLL4 proteins share identical PHD, HMG, FYRN/C, and the catalytic SET domain, varying only in sequence length and the additional PHD domain in MLL3. The deubiquitinase BAP1 interacts with MLL3 through its N-terminal PHD domains (1–3) while the kinase AKT1 targets to the RXRXXS/T motif (pictured in asterisk) upstream of the MLL4 PHD4 domain. (**B**) BAP1 associates with the UTX-containing MLL3 complex to demethylate H3K27 tri-methylation (Me3), methylate H3K4 mono-methylation (Me1), and deubiquitinate BAP1-targeted enhancers, resulting to activated expression of tumor suppressor genes. (**C**) AKT1 functions downstream of the PI3K-signaling pathway to phosphorylate MLL4 and attenuate its function. Upon PI3K inhibition, both PI3K and AKT1 are inactivated while MLL4 activity is restored, resulting to activated expression of MLL4 and ER-mediated oncogenes and evasion of PI3K inhibitor treatment. Figures are created with Biorender.com.

[24,25,44]. Indeed, mouse knockout models have revealed that MLL3 and MLL4 share partially functional redundancy [53]. Nonetheless, accumulating evidence, including the aforementioned examples, indicates that these two KMTs may exert distinct functions in gene regulation. An interesting cell model for studying this issue is the HCT116 human colorectal cancer cell line, which expresses wild-type MLL4 but has a homozygous frameshift mutation before the C-terminal SET domain of MLL3 [95]. In two independent studies, engineering either homozygous deletion of MLL4 or reintroduction of a functional MLL3 in HCT116 cells was found to negatively regulate cancer cell propagation, in which both cellular regulations are tightly connected to the enhancer-H3K4me1 regulatory activity of either KMT [115,116]. These findings suggest that although they may be functionally redundant in gene regulation for normal development, MLL3 could generally function as a tumor suppressor while MLL4 could, under certain cellular contexts, exert oncogenic effects for tumorigenesis. This functional discrepancy might be attributed to distinct protein interactions outside of the SET domain that confer to different target gene regulation (Figure 4A). However, the molecular mechanism(s) that discriminate the different functions between MLL3 and MLL4 are still obscure as they have very similar structural domains and organization [117]. Analysis of data from The Cancer Genome Atlas (TCGA) database, Wang et al. [118] identified two cancer-associated mutational hotspots within the first N-terminal PHD domain cluster of MLL3 while there were no obvious mutational hotspots within MLL4 in the similar region. This unique domain could mediate the association of BAP1 (a histone deubiquitinase) with MLL3, but not with, other SET1/MLL family of KMTs, including MLL4. Mechanistically, BAP1 appeared to facilitate the recruitment of UTX-containing MLL3 complex to BAP1-targeted enhancers and regulate tumor suppressor expression through H3K4 mono-methylation and H3K27 demethylation (Figure 4B). However, deletion of *BAP1* or the PHD-associated *MLL3* mutations in cancer cells reduced recruitment of MLL3 and UTX to target enhancers and thereby caused the downregulation of target gene transcription. These *MLL3* PHD mutations could be observed in numerous cancer types, especially enriched in lung cancer [118]. This difference may provide an explanation for how MLL3 and MLL4 are capable of forming distinct protein interactions and regulating different target genes. Another difference is that MLL4 was found to be inactivated through phosphorylation by AKT1 and SGK1, both kinases function downstream of the phosphoinositide 3-kinase (PI3K) pathway and share the same phosphorylation consensus motif (RXRXXS/T) [119,120]. Since PI3K inhibitors have shown clinical efficacy in breast cancers with elevated PI3K signaling but often develop therapeutic resistance because of ER activation [121], Toska et al. [122] revealed that PI3K inhibition may suppress AKT1 activity and in turn enhance MLL4 function to facilitate ER-dependent gene activation (Figure 4C). On the other hand, the expression of estrogen inducible kinase SGK1 could also be induced upon PI3K inhibition. Elevated SGK1 could directly phosphorylate MLL4 and attenuate its function, which leads to a global loss of H3K4me1 occupancy at ER target sites and causes downregulation of ER target genes. This ER-SGK1-MLL4 regulatory network may function as a negative feedback loop to restore homeostasis of the ER transcriptional output [123].

Conclusions

MLL3 and MLL4 methyltransferases play essential roles in establishing an active enhancer landscape for long-range target gene transcription, which is required for the correct tissue-specific development. MLL3/MLL4 may control gene transcription not only through their catalytic activities for the enhancer-associated H3K4me1 implementation, but also through scaffolding function that facilitate the recruitment of p300/CBP and Pol II on enhancers. These regulations might involve many direct or indirect protein–protein interactions. Importantly, the cancer genome sequencing data reveal their functional roles as tumor suppressors during tumor initiation and progression, while they might be capable of exerting oncogenic effects upon a specific cellular context in certain types of cancer. Although MLL3 and MLL4 share identical complex proteins and exert the same catalytic activity, a better understanding of the functional discrepancy between these proteins is important for understanding the etiology of MLL3/4-associated diseases and will benefit to disease treatment in the future.

Perspectives

- Precise enhancer regulation is required for the correct gene transcriptional programs during development, while enhancer malfunction through mutations in the regulatory factors, e.g. MLL3 and MLL4, may cause tissue-specific cancer development.

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- Although the cancer genome sequencing data support a key tumor suppressor role of MLL3 or MLL4 in a number of human cancers, there are certain cases in which these histone modifiers may function as oncogenic cofactors in driving tumorigenesis, which might be highly dependent on a specific cellular context.

- A better understanding of MLL3/4 oncogenic functions is relevant in the future of considering them as therapeutic targets for cancer treatment.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contributions

L.-H.W. and S.-P.W. conceived the idea for the review, researched the literatures and wrote the manuscript. M.A. E.A. researched the literatures, drew the figures, and collected the table. S.W. edited and revised the manuscript.

Abbreviations

AML, acute myeloid leukemia; CSR, class switch recombination; DLBCL, diffuse large B cell lymphoma; ERα, estrogen receptor alpha; FL, follicular lymphoma; GCB, germinal-center B; HSPCs, hematopoietic stem and progenitor cells; LUAD, lung adenocarcinoma; NRs, nuclear receptors; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; TFs, transcription factors.

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