



MLL5 (KMT2E): structure, function, and clinical relevance

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Abstract The mixed lineage leukemia (MLL) family of genes, also known as the lysine N-methyltransferase 2 (KMT2) family, are homologous to the evolutionarily conserved trithorax group that plays critical roles in the regulation of homeotic gene (HOX) expression and embryonic development. MLL5, assigned as KMT2E on the basis of its SET domain homology, was initially categorized under MLL (KMT2) family together with other six SET methyltransferase domain proteins (KMT2A–2D and 2F–2G). However, emerging evidence suggests that MLL5 is distinct from the other MLL (KMT2) family members, and the protein it encodes appears to lack intrinsic histone methyltransferase (HMT) activity towards histone substrates. MLL5 has been reported to play key roles in diverse biological processes, including cell cycle progression, genomic stability maintenance, adult hematopoiesis, and spermatogenesis. Recent studies of MLL5 variants and isoforms and putative MLL5 homologs in other species have enriched our understanding of the role of MLL5 in gene expression regulation, although the mechanism of action and physiological function of MLL5 remains poorly understood. In this review, we summarize recent research characterizing the structural features and biological roles

of MLL5, and we highlight the potential implications of MLL5 dysfunction in human disease.

Keywords Histone methylation · PHD finger · Cell division · Hematopoietic stem cell differentiation · Leukemia · Cancer

Introduction

The human *MLL5* gene (CCDS34723.1), termed full-length *HsMLL5* hereafter, was initially identified in a search for candidate myeloid leukemia tumor suppressor genes from a 2.5-Mb region of chromosome 7q22.1 that is frequently deleted in myeloid malignancies [1, 2]. *HsMLL5* gene covers 73 kb of genomic DNA, consisting of 25 coding exons, that is translated to a protein of 1858 amino-acid residues [1]. In addition to full-length *HsMLL5*, several alternatively spliced variants of *HsMLL5* have been identified, but only three of these transcripts (*HsMLL5* α , *HsMLL5* β , and *NKp44L*) are documented (Fig. 1). Full-length *HsMLL5* and these three isoforms all possess a single plant homeo-domain (PHD) zinc finger and a Su(var)3–9, Enhancer-of-zeste, and Trithorax (SET) domain, but have been reported to carry out diverse cellular functions.

The murine homolog of *HsMLL5*, referred to as *MmMLL5*, is located on chromosome 5 band A3. *MmMLL5* cDNA has 87.7% identity to *HsMLL5*, which is translated into protein sequences 88.6% identical to *HsMLL5* [3]. In addition, both *HsMLL5* and *MmMLL5* protein are localized in the nucleus, displaying a ubiquitous pattern of expression in embryonic and adult tissues [1, 3, 4]. Considering the high similarity in sequence and expression pattern, *HsMLL5* and *MmMLL5* are likely to function similarly in both species [3], albeit

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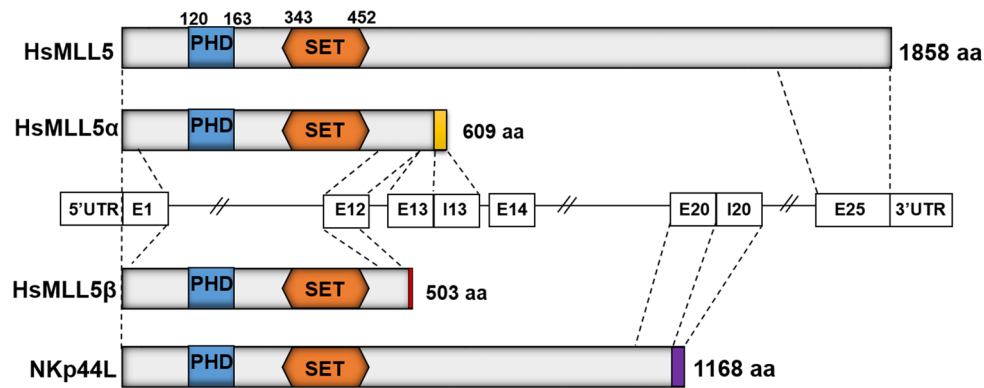


Fig. 1 Schematic representation of human MLL5 protein and its three isoforms with their mRNA organizations. Each protein has a conserved PHD finger and a single SET domain. Full-length *HsMLL5* gene consists of 25 coding exons, which produces a protein of 1858 amino acids. *HsMLL5α* is translated from coding exon 1 to exon 13 with an extra sequence located in the intron following coding exon 13 (labeled as I13) of full-length *HsMLL5*. This extra sequence translates into the last 35 amino acids of *HsMLL5α* (yellow square).

HsMLL5β has the same sequence as full-length *HsMLL5* from coding exon 1 to part of exon 12, where it is truncated by a 26-bp sequence. *HsMLL5β* protein has a single amino-acid difference compared with full-length *HsMLL5* (red line). *NKp44L* mRNA lacks coding exon 21 to exon 25, and contains an extra sequence located in the intron following coding exon 20 (labeled as I20). I20 translates into the last 12 amino acids, which are specific to *NKp44L* (purple square)

experimental evidence is lacking. Although MLL5 was initially categorized into MLL (KMT2) family, phylogenetic analysis demonstrated that MLL5 is distinct from the MLL1-4, SETD1A, and SETD1B group, but forms a family together with another mammalian protein, SET containing-domain 5 (SETD5) [5, 6]. Moreover, MLL5 appears to be the mammalian homolog of yeast SET3 and SET4 (termed as SET3/4 hereafter) as well as *Drosophila CG9007* (encoding the protein ‘UpSET’) on the basis of shared structural features in their SET domain [7–9]. Although the SET domain function of SETD5 and SET4 is largely undetermined, SET domains of UpSET, SET3, and MLL5 have been suggested to lack intrinsic methyltransferase activity that is a common characteristic of MLL (KMT2) family. In addition, MLL5 lacks the AT-hook and high-mobility group (HMG) homology domains required for DNA binding, which are present in other MLL family members. The versatile role of MLL5 in many cellular processes has been reported in increasing numbers of studies, although the precise mechanisms underlying MLL5 activity are still poorly understood.

Here, we describe the structure of MLL5 protein and discuss how the various protein domains confer its reported functions, specifically in controlling cell cycle progression, maintaining genomic stability, and regulating hematopoiesis and spermatogenesis (Fig. 2). In addition, we review the implications of MLL5 loss or dysfunction in human disease.

Structural insights into MLL5 protein

SET domain and methyltransferase activity

HsMLL5 protein (NP_891847.1) includes a SET domain, spanning amino acids Thr-323 to Ile-433 [5]. SET domains have been recognized as common elements in chromatin regulators, including protein lysine methyltransferases (KMTs) [10]. To date, numerous SET domain-containing proteins that catalyze methylation of histones and non-histones have been isolated and classified into several families based on their site- and state-specific methylation activities. In mammals, MLL (KMT2) family proteins possess a catalytic SET domain that methylates histone H3 on lysine 4 (H3K4) to promote chromatin accessibility and modulate transcription. However, the SET domain of MLL5 is unlike other MLL (KMT2) family members in sequence and structure. Typically, the MLL (KMT2) family proteins have a C-terminal SET domain that shares the conserved features of SET-N, SET-I, SET-C, and post-SET motifs. The latter provides several conserved residues that coordinate a zinc atom important for flexible cofactor turnover and intrinsic methyltransferase activity [11, 12]. In contrast, the MLL5 SET domain is located in the N-terminal region and lacks important elements required for methyltransferase activity. Mas-Y-Mas et al. performed sequence alignment of HsMLL5 with a panel of SET domain-containing proteins and found that HsMLL5 does not have the conserved

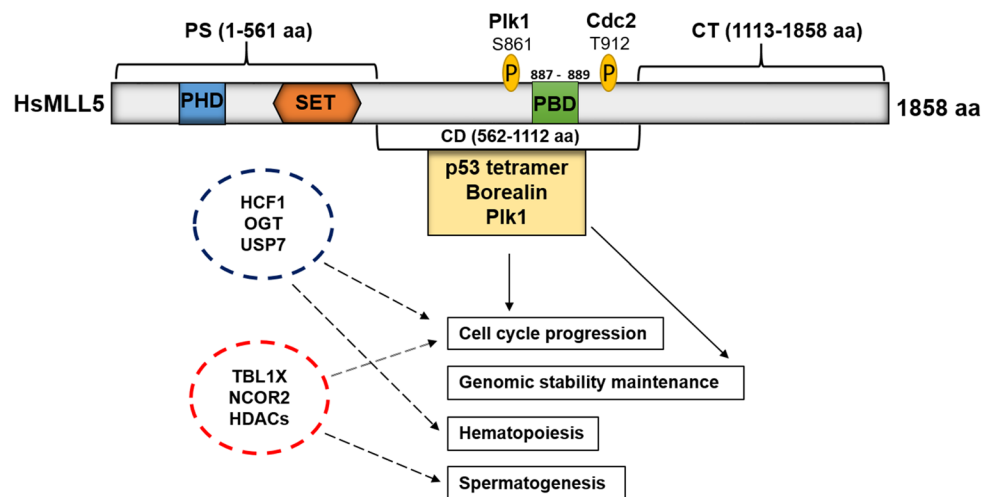


Fig. 2 Post-translational modifications and binding partners responsible for the biological functions of MLL5 protein. The 1858 amino-acid HsMLL5 protein can be divided into three regions: the PHD/SET domain (PS, 1-561 aa), central domain (CD, 562-1122 aa), and C-terminal domain (CT, 1113-1858 aa). Two phosphorylation sites (*yellow ellipses*) are indicated together with the protein

kinases known to phosphorylate HsMLL5. The Plk1 protein-binding motif (PBD, T887/S888/T889) is indicated by the *green square*. The CD domain is involved in interactions with p53 tetramer, Plk1, and Borealin. Putative partners, based on studies with MLL5 isoforms and orthologs, are also shown in *blue- and red-dotted ellipses*, respectively

XGXG and Y motifs [5]. Two adjacent amino acids (Asn-408 and His-409) within the SET domain's most conserved sequence motif are altered to arginine, akin to SETD5, MmMLL5, UpSET, and SET3/4 [3, 5]. Although the overall crystal structure of HsMLL5 SET domain is similar to previously determined SET domain structures, it displays an uncommon large loop which blocks the docking of lysine side chain substrate. Furthermore, the isolated HsMLL5 SET domain does not interact with either the methyl-donor SAM (S-Adenosyl Methionine) or any H3K4 histone peptides, and bacterially purified MLL5 fragment (residues Ile-262 to Arg-473) does not exhibit HMT activity towards histones isolated from calf thymus [5]. Consistently, Sebastian et al. were also unable to demonstrate H3K4 methylation using overexpressed full-length HsMLL5 immunoprecipitated from HEK293T cells [13]. Moreover, several lines of evidence have suggested that the methyltransferase activity of other MLL (KMT2) family members requires interaction with the common trithorax scaffold complex, WDR5-RBBP5-ASH2L [12]. However, HsMLL5 lacks the WIN (WDR5 interacting) motif required for interaction with this complex [5]. Consistent with this, HsMLL5 was not found to be associated with the WDR5-RBBP5-ASH2L complex [14, 15], casting further doubt on the intrinsic methyltransferase activity of HsMLL5.

Recent studies of HsMLL5 orthologs suggest that their SET domains, which exhibit high identity to HsMLL5, are also HMT-catalytically inactive. Using a truncated MmMLL5 construct (aa 1-450), Madan et al. were unable to detect *in vitro* HMT activity toward purified histones

and recombinant histone octamers [3]. Rincon-Arano et al. failed to detect any *in vitro* HMT activity for bacterial expressed SET domain of UpSET using purified histones as substrate [16]. Likewise, SET3 has also been shown to be HMT functional inactive [9]. It has been suggested that MLL5 might affect H3K4 methylation indirectly, for example, through regulating expression of the histone-modifying enzymes, lysine-specific histone demethylase 1 (LSD1), and SET7/9 in C2C12 murine myoblast cells [13]. Altogether, these studies suggest that MLL5 is well conserved, with the SET domain-lacking intrinsic methyltransferase activity, and its molecular functions remain to be elucidated.

The PHD finger as a H3K4 methylation reader

In addition to the SET domain, MLL5 also contains a PHD finger spanning Arg-120 to Cys-163. Unlike other MLL members which contain multiple PHD fingers, HsMLL5 contains only a single PHD finger with 100% identical to MmMLL5. PHD fingers are short (~65 aa) structurally conserved domains that function as epigenetic effectors in a wide range of chromatin remodeling proteins. Through binding to specific epigenetic histone marks, PHD fingers are known to recruit transcription factors, as well as nucleosome-associated complexes, to chromatin [17]. This raises the possibility that MLL5 may be recruited to chromatin through its PHD domain. To this end, two independent groups have characterized the molecular basis for the recruitment of MLL5 to chromatin. Using DNA adenine

methyltransferase identification (DamID)-based chromatin analysis in C2C12 cells, Ali et al. demonstrated that MLL5, together with RNA polymerase II, co-occupies promoters that display H3K4me3, suggesting that MLL5 may have the capacity to read this form of H3K4me3 modification [18]. Further crystallographic studies of the MLL5 PHD domain in complex with histone H3 peptides confirmed that MLL5 specifically binds to histone H3K4me3. However, this involves a non-canonical mechanism, whereby the H3K4me3 binding groove in the PHD finger of MLL5 contains only one aromatic residue, W141, rather than two to four aromatic residues typically contained in other PHD finger-containing proteins. Some discrepancies regarding the differential affinity for H3K4me3 and H3K4me2 have been described in the literature. Lemak et al. reported that the MLL5 PHD finger binds to H3K4me3 and H3K4me2 with similar affinity [19], whereas Ali et al. reported a five-fold stronger affinity of MLL5 PHD finger to H3K4me3. Such discrepancies could be attributed to differences in assay sensitivity or domain constructs. Nonetheless, studies by both groups clearly indicate that recognition of the H3K4me3 mark by the PHD finger can facilitate the recruitment of MLL5 to active transcription chromatin regions.

The PHD domain of MLL5 is highly conserved in HsMLL5, MmMLL5, UpSET, and SET3/4, suggesting that the PHD domain of UpSET and SET3/4 may also recognize methylated H3K4. Kim et al. reported that SET3 PHD domain binds to H3K4me2 facilitating its recruitment of chromatin [20]. SET3 has also been shown to interact with histone deacetylases, Hos2, and NAD-dependent Hst1 HDACs, forming a complex called SET3C. Together, SET3C plays essential roles in repression of sporulation genes expression [9]. Similarly, UpSET is found to be part of transcription-associated machinery, Rpd3/Sin3A-containing complex, exhibiting deacetylase activity [16]. Genomic-binding analysis in an embryonic *Drosophila* cell line (KC cell) revealed that UpSET stabilizes binding of histone deacetylase Rpd3 on active genes around their transcriptional start sites (TSSs). Strikingly, knockdown of UpSET in Kc cells increases histone acetylation on H3K9 and H4K16 and the level of H3K4me2 and H3K4me3 in the flanking regions of UpSET targeted promoters, correlating with a more open chromatin configuration. This consequently causes only subtle increase of UpSET targeted genes expression, but consistently upregulates silent genes neighboring UpSET target genes. HsMLL5 has also been suggested to be part of the NcoR repressor complex [21]. Moreover, female infertility caused by loss of UpSET can be rescued by overexpression of MmMLL5 [16]. It is thus possible that, like its orthologs, MLL5 may facilitate recruitment of NcoR-HDAC3 complex to transcribed chromatin, through the PHD finger-mediated recognition of

H3K4me2/3 marks, ensuring proper gene activation. MLL5 and its orthologs appear to be acting in histone deacetylation rather than methylation. Consistently, a recent study by Osipovich et al. demonstrated that MmSETD5 is associated with histone deacetylase-containing NCoR complexes and is not required for histone lysine methylation [22]. Loss of MmSETD5 increases histone acetylation at gene transcriptional start sites and near downstream regions, leading to dysregulated transcriptome. However, it is of note that SETD5 lacks PHD domain, and may be recruited to transcribed regions of active genes through direct interaction with the PAF1 complex, which may, in turn, directly associate with phosphorylated RNAP II and target transcriptional elongation. Although the molecular function of MLL5 in gene transcription regulation remains elusive, the above-mentioned studies may shed light on the role of MLL5 as a transcriptional regulator required to restrict active histone modifications within the promoter regions.

HsMLL5 Isoforms

HsMLL5 α

HsMLL5 α is an alternative splice variant of full-length HsMLL5, ubiquitously expressed in human tissues. HsMLL5 α is composed of 13 coding exons and is translated into a protein of 609 amino acids, substantially shorter than full-length HsMLL5. HsMLL5 α was initially identified in a complex containing host cell factor-1 N-terminal subunit (HCF1) and O-GlcNAcylation Transferase (OGT), and has been reported to exhibit mono- and di-H3K4 methyltransferase activity following O-GlcNAcylation by OGT at position Thr440 within the SET domain [23]. It is of note that this publication has recently been retracted. Nonetheless, another research group has independently demonstrated that HsMLL5 α is recruited to E2F1-responsive promoters by HCF1 to induce H3K4 trimethylation, resulting in activation of E2F1 target genes, such as *CCNA2*, *CDC2*, and *CDC6* in HEK293T and HeLa cells [15]. On the other hand, Sebastian et al. reported negative regulation of *CCNA2* by full-length MmMLL5 through binding to its proximal promoter, which contributed to maintenance of quiescence in C2C12 murine myoblasts [13]. Intriguingly, both studies reported decreased H3K4 methylation at the *CCNA2* promoter upon knockdown of MmMLL5 or HsMLL5 α , suggesting an epigenetic role of MLL5 on H3K4me3 dynamics. This dichotomy of MLL5-mediated regulation on the same gene indicates that MLL5 function in cell cycle progression may be cell-type/context-dependent. Recently, HsMLL5 α interaction with OGT and ubiquitin-specific protease 7 (USP7) was reported to protect HsMLL5 α from ubiquitination-mediated proteolytic

degradation [24]. HsMLL5 α , OGT, and USP7 are present as a ternary complex in which USP7 deubiquitinates HsMLL5 α , and OGT enhances HsMLL5 α stability through inhibiting its ubiquitination. In line with these physical interactions, the authors further revealed that HsMLL5 α protein levels, along with OGT and USP7, are elevated in human primary cervical adenocarcinomas, implicating HsMLL5 α involvement in cancer.

HsMLL5 β

HsMLL5 β is another truncated variant (503 amino acids) of full-length HsMLL5. The mRNA sequence of HsMLL5 β is identical to HsMLL5 from the start codon until partway through coding exon 12. *HsMLL5 β* is truncated by a 26-bp sequence, encoding a stop codon, followed by a polyadenine tail [25]. HsMLL5 β is specifically expressed in HPV16/18-positive cervical cancer cells where it is essential for transcriptional activation of the *E6/E7* oncogene via a physical interaction with transcription factor AP-1 at the distal region of HPV16/18-LCR. Mechanistic studies revealed that SET domain mutation (Y358A) of HsMLL5 β decreases HPV18-LCR promoter luciferase activity in HEK293T cells [25]. Moreover, O-GlcNAcylation of HsMLL5 β by OGT at position Thr-440 is required for the recruitment of HsMLL5 β /AP-1 complex to the HPV16/18-LCR and the subsequent initiation of *E6/E7* transcription [26]. Further investigations are required to understand the precise mechanism through which the HsMLL5 β /AP-1 complex interacts with other co-activators to initiate *E6/E7* transcription.

The restricted expression of HsMLL5 β in HPV16/18-positive cervical cancers provides an opportunity for targeted therapy, either by (1) inhibition of O-GlcNAcylation of HsMLL5 β to block its activity or (2) targeted silencing of its expression. Both OGT inhibition using azaserine and OGT knockdown using siOGT were found to inhibit cell proliferation in HPV16/18-positive SiHa and HeLa cells significantly [26]. Moreover, the same authors found that RNAi-targeted silencing of HsMLL5 β induced both apoptosis and senescence in HeLa cells [27]. Intratumoral injection of specific HsMLL5 β siRNAs has also been shown to inhibit cervical tumor growth significantly. Notably, HsMLL5 β silencing exhibits strong synergistic effects when used alongside gamma-irradiation (IR). These findings demonstrate the utility of HsMLL5 β as a biomarker and therapeutic target for HPV16/18-positive cervical cancers, warranting further investigation.

NKp44L

NKp44L was initially identified as a cellular ligand of NKp44 through monoclonal antibody library screening [28]. Recent

yeast 2-hybrid screening and sequencing analysis identified that NKp44L is, in fact, a novel isoform of HsMLL5 [29]. *NKp44L* mRNA shares the first 20 coding exons of *HsMLL5* with an additional and unique exon 21, encoding a specific C-terminal motif. NKp44L possesses a long 5'-UTR, which may be implicated in its preferential expression profile. NKp44L is undetectable in normal cells but found to be expressed in a broad panel of transformed cell lines as well as primary tumor samples obtained from patients with kidney and bladder malignancies. Surprisingly, differing from canonical nuclear localization of full-length MLL5 or other isoforms, NKp44L is located at the cell surface and cytoplasm. It has been suggested that the specific C-terminal motif of NKp44L is required for its cell surface localization and its interaction with NKp44. The authors also speculated that NKp44L might be secreted into the extracellular matrix or retained at the cell surface through sulfated glycosaminoglycan close to the C-terminal of NKp44L [29], but the exact mechanism is yet unexplored.

NKp44 is known as a major mediator of NK cell cytotoxicity, critical for an effective innate immune response against a variety of tumors and microbial pathogens. Anti-NKp44L antibodies were found to selectively inhibit the cytotoxicity of NK cells towards a range of target cells, such as HEK293, WM1361, H441, and MT2 cells [29]. Ectopic expression of NKp44L in NK-resistant EL4 cells sensitizes them to NK-mediated cytotoxicity, suggesting a role of NKp44L in conveying danger signals to NKp44-positive NK cells. Malignant tissues could, therefore, potentially be selectively targeted using anti-NKp44L monoclonal antibodies or through recognition by NKp44-positive NK cells [30, 31]. The same group also reported that misexpression of NKp44L on CD4-positive T cells is negatively associated with immunological reconstitution in HIV-infected immunological nonresponders (InRs) patients [28]. Expression of NKp44L correlates with depletion of CD4-positive T cells in InRs, and hence, development of therapeutic strategies to prevent NKp44L expression is expected to aid immune recovery in InRs. While Vieillard et al. revealed that the gp42 envelope protein of HIV-1 is responsible for inducing expression of NKp44L on CD4⁺ T cells in HIV-infected patients [32], further investigations are required to reveal the pathological significance of NKp44L stimulation and its intracellular trafficking to the cell surface of primary tumors.

Biological functions of MLL5

MLL5 regulates cell cycle progression

HsMLL5 is expressed throughout the cell cycle and its intracellular localization is tightly regulated during cell

cycle progression. Both HsMLL5 overexpression and knockdown inhibit cell cycle progression. Deng et al. reported that ectopic overexpression of HsMLL5 arrests HEK293T cells at G1 phase [4]. Similarly, Cheng et al. reported that siRNA knockdown of HsMLL5 decreases cell proliferation in both tumor and normal diploid cells, inducing dual-phase cell cycle arrest at both G1 and G2/M phases. Screening of a panel of cell cycle-related genes revealed a significant increase in p21 expression in *MLL5*-ablated cells [33]. p21 is known to inhibit Cyclin D/CDK4/6 complex formation, which is required for phosphorylation of the retinoblastoma protein (pRb), a key regulator of cell cycle progression. Upregulation of p21 also decreases phosphorylation of pRb, contributing to HsMLL5 siRNA-induced dual-phase cell cycle arrest. To dissect the mechanisms underlying G1/S cell cycle arrest following HsMLL5 depletion, the same authors investigated HsMLL5 function in S phase progression by applying various DNA-damaging agents to activate the G1/S checkpoint in human lung fibroblast WI-38 and three other human cancer cell lines (HeLa, U2OS, and HCT116) [34]. They found that camptothecin (CPT) specifically accelerates HsMLL5 protein degradation, leading to phosphorylation of p53 at Ser-392, which can be reversed by HsMLL5 overexpression. HsMLL5 interacts with tetramerized p53, and HsMLL5 elimination enhances chromatin accumulation of p53 tetramers. This results in activation of the p53 downstream targets and delays G1/S progression.

Using the same three cancer cell lines (HeLa, U2OS, and HCT116), Liu et al. demonstrated that phosphorylation of HsMLL5 on its central domain at Thr-912 by Cdc2 is crucial for mitotic entry at G2/M phase and such regulation is independent of cell-type [35]. HsMLL5 dissociates from condensed chromosomes upon phosphorylation during mitosis. After mitotic exit, HsMLL5 is then dephosphorylated and recruited back to the relaxed chromatin. Mitosis delay induced by HsMLL5 depletion is reversed upon ectopic expression of full-length HsMLL5 in HEK293T cells, but not the phosphorylation-dead mutant, demonstrating a critical role for phosphorylation of HsMLL5 in mitotic progression. Likewise, Ali et al. reported that MmMLL5 recruitment to chromatin is abolished as a result of phosphorylation of H3T3 and H3T6 during mitosis of C2C12 cells. Importantly, this phospho-methyl switch of histone H3 is conserved in UpSET and may serve as a mechanism to exclude MLL5 from chromatin [18]. Functional study is required to establish the role of this phospho-methyl switch in the future.

MLL5 also participates in the last step of cell division, i.e., cytokinesis. A genome-scale RNAi screen in HeLa cells by Kittler et al. indirectly suggested that MLL5, TBL1X, and NCOR2 form a repressor complex that regulates genes involved in cytokinesis [21]. In

addition, the yeast homologs, SET3 (homolog of MLL5), SIF2 (homolog of TBL1X), and ScSNT1 (homolog of NCOR2), form a histone deacetylase-recruiting SET3 complex (SET3C) and repress cytokinesis gene expression [36]. Given that NCOR2 is a corepressor of various transcription factors and that NCOR2 knockdown induces a defect in cytokinesis, the molecular action of MLL5 in cytokinesis warrants further investigation.

MLL5 maintains genomic stability

Spatial chromosome organization and spindle bipolarity are critical for faithful cell division and maintenance of genomic stability, and HsMLL5 is reported to play active roles in these processes. As described above, HsMLL5 depletion induces G1/S and G2/M arrest in different human cancer cell lines [33]. Furthermore, approximately 20% of total *HsMLL5*-depleted U2OS cells are found to exhibit aneuploidy, suggesting that HsMLL5 ablation causes genomic instability [37]. Induction of metaphase arrest results in chromosome misalignment and multipolarity in more than 80% of *HsMLL5*-depleted U2OS cells. Mechanistic studies revealed that HsMLL5 regulates the stability of the chromosomal passenger complex (CPC), a key controller of chromosome alignment and segregation. In particular, co-immunoprecipitation and *in vitro* pull-down assay demonstrated that HsMLL5 physically interacts with Borealin, a component of CPC. Overexpression of Borealin in HEK293T cells restored CPC expression but only partially reversed chromatin misalignment caused by HsMLL5 depletion, suggesting the presence of additional CPC-independent regulatory mechanisms [37].

HsMLL5 function in mitotic bipolar spindle formation has also been investigated. Zhao et al. monitored spindle assembly in *HsMLL5*-depleted U2OS cells using time-lapse microscopy and observed the appearance of new microtubule organizing centers (MTOCs), additional to the original two MTOCs [38]. These additional MTOCs are induced by aberrant cytosolic aggregation of PLK1, pivotal for microtubule nucleation from the centrosomes, which occurs in the absence of cytosolic HsMLL5. PLK1 mislocalization and formation of extra MTOCs can be reversed by overexpression of full-length HsMLL5 but not PLK1-binding site (S888A) mutant HsMLL5, indicating that cytosolic HsMLL5 interaction with PLK1 is required for preventing PLK1 aggregation and facilitating its assembly to the centrosome during mitosis. HsMLL5 was also found to be phosphorylated on Ser-861 by PLK1, but this was not essential for the interaction between HsMLL5 and PLK1 or for centrosomal localization of PLK1.

Role of MLL5 in hematopoiesis and spermatogenesis

To examine the function of MLL5 *in vivo*, a number of *Mll5*-deficient mouse models have been generated by three independent groups. In addition to partial postnatal lethality and retarded growth, all *Mll5*-knockout mice exhibit impaired hematopoiesis. Zhang et al. generated *Mll5*-deficient mice by deleting both the third and fourth coding exons and introducing a large insertion to prematurely terminate MmMLL5 expression [39]. They observed a 30% decrease in long-term repopulating hematopoietic stem cells (LT-HSC) and functional impairment of LT-HSC based on competitive repopulation assays. In these experiments, bone marrow cells from wild-type competitors were mixed with equal numbers of bone marrow cells from *Mll5*-deficient mice which were transferred into lethally irradiated recipients. Three months after transfer, hematopoietic cells from the spleens of recipients were repopulated with similar numbers of hematopoietic cells from both sources. The authors reported a marked under representation of hematopoietic lineage cells derived from *Mll5*-knockout mice. In addition, *Mll5* deficiency significantly reduced the number of common lymphoid progenitor (CLP) cells and disrupted myeloid differentiation. Madan et al. generated *Mll5*-deficient mice by excising the third and fourth coding exons [3]. Similar functional defects and decreases in LT-HSC were reported along with developmental defects in B and T lymphopoiesis. Given the critical role of MLL5 in cell cycle progression, it was hypothesized that the observed defect of LT-HSCs may result from impaired stem cell quiescence, survival, or differentiation. To investigate this further, BrdU was administered orally to *Mll5*-deficient mice for 7 days to label proliferating cells *in vivo*, upon which BrdU incorporation into both short- and long-term hematopoietic stem cells (LSK) was evaluated. Numbers of quiescent cells were reduced by 40–60% in *Mll5*-deficient mice compared with wild type, indicating that MLL5 is required for the maintenance of quiescent state of LT-HSC. Heuser et al. generated *Mll5*-deficient mice by disrupting the third coding exon [40], also observing impaired competitive repopulating capacity as reported by the other two groups. In particular, *Mll5*-deficient mice displayed increased susceptibility to bacterial infection due to a defect in terminal myeloid differentiation and neutrophil function. Although it has been suggested that MmMLL5 is not required for survival [3, 39, 40], a recent study by Osipovich et al. demonstrated that MmSETD5 deficiency causes embryonic lethality that results from a widespread impairment in the regulation of gene expression [22], which suggests non-redundant functions of MLL5 and SETD5. Readers are referred to a separate review [41] for a comprehensive description of these three *Mll5*-deficient mouse models.

While all three studies detected disrupted hematopoietic stem cell repopulation activity in the absence of MmMLL5 expression, the precise mechanisms and the key downstream effectors underlying these effects remain unknown. Heuser et al. examined the influence of the DNA-methyltransferase inhibitor, decitabine (DAC), and the histone deacetylase inhibitor, trichostatin A (TSA), on HSC differentiation and competitive repopulation capacity [40]. They demonstrated that loss of MmMLL5 is associated with higher sensitivity to DAC, as evidenced by depletion of HSCs due to induction of differentiation, suggesting a role for MmMLL5 in controlling HSC differentiation through DNA methylation. Recently, Tasdogan et al. reported that both homozygous and heterozygous loss of MmMLL5 results in accrued DNA damage and elevated level of reactive oxygen species (ROS) [42]. The toxic level of ROS is induced by activation of Ink4a/Arf locus as well as DNA damage-associated type 1 interferon (IFN-1) signaling pathway. The latter triggers mitochondrial accumulation of BH3-interacting domain death agonist (Bid), a critical effector in the DNA damage response. Knockout of IFN-1 receptor or Bid and oral administration of antioxidant N-acetyl-L-cysteine (NAC) diminishes ROS levels. Consequently, HSPC functions are restored, but it does not reverse the defects in double-strand breaks (DSBs) repair. These observations demonstrate that accumulation of ROS is the major cause of hematopoietic defects in *Mll5*-deficient mouse. Notably, this study suggests a direct effect of MLL5 in the DNA damage response warranting further investigations.

In addition to hematopoietic stem cell differentiation, MLL5 has also been implicated in spermatogenesis. Indeed, two of the above-mentioned *Mll5*-knockout mouse models exhibit male infertility [3, 40]. Yap et al. further revealed that male infertility was due to defects in terminal maturation and packaging of sperm [43]. They also identified multiple dysregulated genes involved in spermatogenesis in the absence of MLL5 expression, although the molecular mechanism through which MLL5 regulates these genes is still unknown. Interestingly, SET3 depletion results in reduced ascus formation due to deregulation of sporulation genes [9]. UpSET mutants were found to be female sterile due to upregulation of key regulators of oogenesis [16]. As mentioned above, UpSET is a component of the Rpd3-containing HDAC complex and takes part in fine-tuning of transcription by stabilizing the HDAC complex in chromatin and restricting active chromatin marks to promoter and transcribed regions. It would be interesting to study whether MLL5 might also associate with HDACs and regulate spermatogenesis-related genes in a similar manner.

MLL5 in human disease

Although the human *MLL5* gene was initially identified as a putative tumor suppressor gene located in 7q22, a screen of primary leukemia samples shows no evidence of altered *HsMLL5* expression or mutation within its exons [1]. Moreover, *MLL5*-knockout mice do not develop spontaneous tumors, and a 2-Mb segmental deletion of mouse chromosome band 5A3, the homolog of human chromosome band 7q22, does not result in spontaneous myeloid malignancies or abnormal hematologic parameters [44]. Thus, the myeloid tumor suppressor function of *MLL5* remains debatable. Nevertheless, *MLL5* has been reported to have potential prognostic significance in leukemia, and increasing evidence suggests that *MLL5* dysfunction is associated with several human diseases, including cancers, neurological disorders, atherosclerosis, and coronary artery disease. It is important to note that most of these studies focused on *MLL5* transcription levels and it is unclear whether full-length *MLL5* or its isoforms were involved.

MLL5 in leukemia

Low *MLL5* transcription levels in leukemic patients have been associated with shorter relapse-free and overall survivals. Damm et al. reported the prognostic impact of *MLL5* in 509 intensively treated patients with acute myeloid leukemia (AML) [45], determining that *MLL5* transcript level is an independent prognostic marker in cytogenetically normal acute myeloid leukemia (CN-AML) and core-binding factor leukemia (CBF-AML). Furthermore,

the same group showed that *MLL5* is a predictive molecular marker for sensitivity to decitabine (DAC), a DNA hypomethylating agent that has therapeutic effects in leukemia [46]. Patients with high *HsMLL5* expression levels displayed a significantly higher sensitivity to DAC as demonstrated by increased overall survival after at least three courses of DAC treatment. Consistent with this, a study using a *HOXA9*-immortalized mouse bone marrow cell model found that *MLL5*-knockout cells display reduced sensitivity and responsiveness to DAC-induced DNA demethylation [46]. Notably, no significant difference was observed in cell cycle arrest or apoptosis rates between *MLL5* wild-type and null murine cells upon DAC exposure. This suggests that the decreased sensitivity to DAC might be due to DNA methylation differences rather than deregulation of cell cycle control. The molecular basis of *MLL5* involvement in DNA methylation is discussed in more detail elsewhere [47]. The predictive value of *MLL5* expression has also been evaluated in 121 patients with acute promyelocytic leukemia (APL) who were treated with all trans retinoic acid (ATRA) and anthracycline-based chemotherapy [48]. APL patients with low *MLL5* expression have reduced remission rates and shorter two-year overall survival (OS).

MLL5 in cancer

Analysis of *MLL5* gene aberrance in human cancers as reported in the Catalogue of Somatic Mutations in Cancer (COSMIC) database revealed 348 somatic mutation cases out of 26,213 tested human tumors [49].

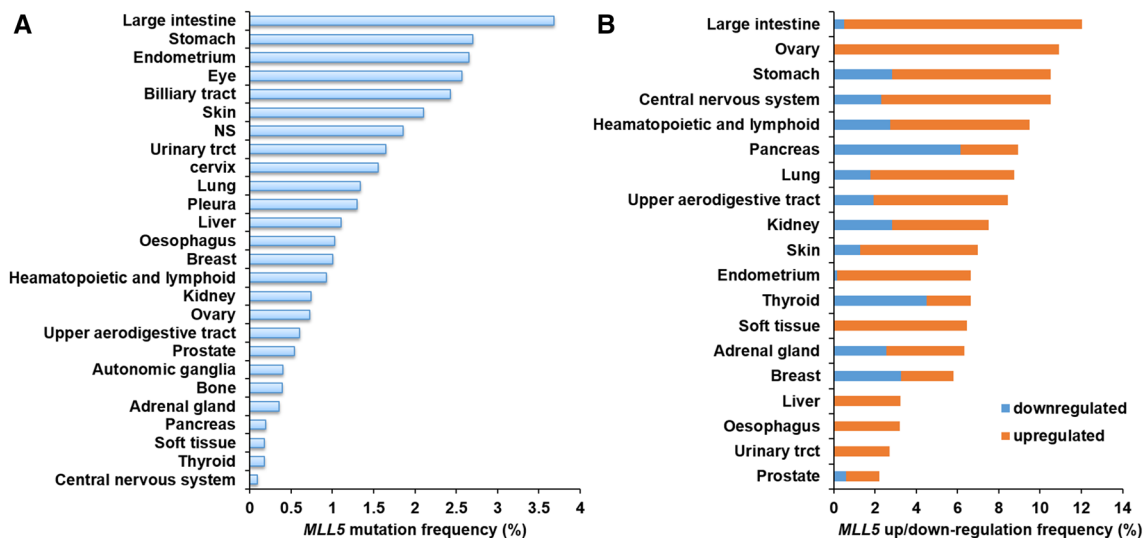


Fig. 3 *MLL5* gene alterations according to the Catalogue of Somatic Mutations in Cancer (COSMIC) database in human tumors. All informations are acquired using the keyword “*MLL5*” on 3 September

2016. All synonymous mutations are excluded. **a** *MLL5* gene somatic mutation rate; **b** *MLL5* gene overexpression and repression rate

These mutations were prevalent in a large range of solid tumors, particularly in the large intestine, stomach, and endometrium (Fig. 3a). The majority of identified *MLL5* gene mutations are missense mutations affecting the PHD and SET domains, accounting for 1.5% and 6.1%, respectively. It is unclear how these mutations affect *MLL5* protein function. *MLL5* gene expression profiles in the Oncomine database show upregulation in a variety of cancers [49], including those of the large intestine, ovary, central nervous system, and stomach, but downregulation in others, e.g., the pancreas, thyroid, and breast (Fig. 3b). Consistent with this, Rabello et al. found that the *MLL5* gene is the most prominently decreased gene in seven breast cancer cell lines tested, as well as in primary breast tumor samples [50]. These data hint at a possible role for *MLL5* in tumor suppression in some types or subtypes of cancers.

The involvement of *MLL5* in cancer development is also evidenced by gene deletion and amplification in multiple tumors. Hodge et al. reported that *MLL5*, along with all other genes located in chromosome band 7q22, show decreased expression in uterine leiomyomata (UL) [51]. Deletion of 7q22 has also been observed in lipomas [52] and endometrial polyps [53]. In contrast, a high incidence of copy number amplification (≥ 5 copies) of the *MLL5* gene is observed in 6.5% of oesophagus and 9% of ‘not specified’ (NS) cancers in the Oncomine database. It is likely that both deletion and amplification of *MLL5* will induce deregulation of gene expression, alteration of cell proliferation, and genome instability, contributing to carcinogenesis.

A recent study by Gallo et al. uncovered an antagonistic role for a small isoform of HsMLL5 (protein band around 90 kDa) and histone variant H3.3 in adult glioblastoma (GBM) [54]. The authors found that the small isoform of HsMLL5 is robustly expressed in GBM self-renewing cells, while H3.3 expression is downregulated. This suppresses GBM cell differentiation, which is associated with unfavorable outcomes. Mechanistic studies using next-generation sequencing (ATAC-seq) show that this small isoform of HsMLL5 orchestrates local chromatin compaction at the *H3F3B* locus, favoring repression of H3.3. In addition, they found that expression of this small isoform of HsMLL5 is negatively correlated with global H3K4me3, and as such, opposes the role of HsMLL5 as an H3K4 methyltransferase. Nevertheless, this study suggests that HsMLL5 is vital in regulating self-renewal and differentiation balance in GBM. Thus, HsMLL5 inhibitors may provide an opportunity for effective therapeutic treatment for malignancies with stem-like properties.

MLL5 in other diseases

Dong et al. reported that *MLL5* is strongly associated with Autism Spectrum Disorder (ASD) based on an exome sequencing study. ASD probands display multiple *de novo* insertion deletions (indels) of the *MLL5* gene that are likely to induce disruptive coding mutations, hence contributing to the risk for ASD [55]. In addition, *MLL5* gene rearrangement was reported in a patient with mental retardation and macrosomia [56]. This patient exhibited an interstitial 3.2-Mb deletion of 7q22.2–7q22.3, resulting in rearrangement of 15 genes. Considering the important role of *MLL5* in cell cycle regulation, it was proposed that the patient’s overgrowth symptoms might result from haploinsufficiency of *MLL5* and other cell cycle controllers. Mutations in PHD domain-containing proteins have also been linked to mental retardation [57], so it would be interesting to study the contribution of *MLL5* dysfunction to mental retardation. Another study by Bjorkegren et al. identified *MLL5* as a specific master regulator of atherosclerosis regression in mature lesions in response to plasma cholesterol lowering (PCL) [58]. Finally, Yuan et al. explored the association between *MLL5* gene polymorphisms and coronary artery disease in a Chinese Han population and identified two novel polymorphisms of *MLL5* gene which may contribute to disease manifestation [59].

Conclusions

MLL5/KMT2E is considered to be a distinctive member of the KMT2 family based on its structural homology. Emerging knowledge on the major *MLL5* isoforms, HsMLL5 α , HsMLL5 β , and NKp44L, as well as *MLL5* orthologs, has added further complexity to what is known about *MLL5* biology, in particular how its functions are tightly entwined with its structural features. In this review, we have summarized what is currently known about *MLL5*’s role in cell cycle progression, genomic stability maintenance, adult hematopoiesis, and spermatogenesis. The detailed molecular mechanisms underlying the regulation of target gene expression by *MLL5*, particularly those relating to post-translational modifications of *MLL5* and identification of its major interacting partners, still require further investigation. These studies will be fundamental to our understanding of the role of *MLL5* in various diseases, including cancer and neurological disorders. It is clear that *MLL5* has both prognostic and predictive value, and as such, deeper knowledge of *MLL5* biology will be beneficial for the development of new therapeutic strategies.

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Compliance with ethical standards

Conflict of interest The authors declare no competing financial interests.

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