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Original Article

The histone methyltransferase DOT1L: regulatory functions and a cancer therapy target

Matthew Wong[1], Patsie Polly[2], Tao Liu[1,3]

[1]Children's Cancer Institute Australia for Medical Research, Randwick NSW 2031, Australia; [2]Department of Pathology and Inflammation and Infection Research Centre, School of Medical Sciences, UNSW Australia, Kensington NSW 2052, Australia; [3]School of Women's & Children's Health, University of New South Wales, Randwick NSW 2031, Australia

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Abstract: DOT1L is a unique histone methyltransferase that targets the histone H3 lysine 79 (H3K79) residue for mono-, di- and tri- methylation. Histone H3K79 mono- and di-methylation results in active gene transcription, while H3K79 tri-methylation is associated with gene repression. DOT1L has a critical role in regulating gene transcription, development, cell cycle progression, somatic reprogramming and DNA damage repair. DOT1L interacts with Mixed Lineage Leukemia (MLL) fusion proteins, leading to enhanced H3K79 methylation, maintenance of open chromatin, overexpression of downstream oncogenes and leukemogenesis. Importantly, small molecule DOT1L inhibitors have been recently developed, and one of the DOT1L inhibitors is already under investigation in a Phase I clinical trial in patients with MLL fusion gene-driven leukemia.

Keywords: DOT1L, histone methylation, mixed lineage leukemia, gene transcription, DOT1L inhibitors

Introduction

Post-translational histone modifications have become a focus of research due to their ability to regulate gene transcription by modifying chromatin structure. Histone modifications can interact and crosstalk, forming a complex web of gene regulation called the histone code [1]. The four well-known histone modifications are acetylation [2], methylation [3], phosphorylation [4] and ubiquitination [5].

Histone methylation was the first post-translational histone modification identified by radiolabelling cell extracts [6]. It involves the attachment of a methyl group to a basic amino residue: lysine (K) [7] or arginine (R) [8]. Lysine residue methylation is the best studied, with lysine undergoing mono, di-, or tri-methylation on the -amine group.

Methyl groups are generally thought to have slowest turnover rate out of the four common histone modifications. However, methylation marks on different lysine residues have been shown to have differing turnover rates [9]. Mass spectrometry has identified many lysine residues in core histone proteins to be dynamically methylated and de-methylated [10]. The most comprehensively studied lysine methylation sites are located on the N-terminal tail (H3K4, H3K9, H3K27, H3K36 and H4K20) and in the histone core (H3K79) [11, 12].

Cross talk among different histone lysine residue methylation modulates gene transcription with H3K9 methylation overlapping with H3K4 de-methylation in regions of heterochromatin; and euchromatic regions show the opposite with H3K4 methylated and H3K9 demethylated [13].

Histone H3K4 mono-methylation (H3K4me), di-methylation (H3K4me2), tri-methylation (H3K4me3), H3K36me3, H3K79me, H3K79me2, H3K9me and H3K27me are linked to gene transcription [14-17]. Differing levels of methylation at the same histone position has been shown to have different effects with H3K9me2, H3K9me3, H3K27me2, H3K27me3 and H4K20me linked to gene repression [18-20].

Histone methyltransferases and histone demethylases

Histone methyltransferases target either lysine or arginine residues, with the majority belonging to the SET domain methyltransferase protein superfamily [21-24]. SET domain methytransferases function by transferring a methyl group from S-adenosyl-L-methionine (SAM) to the amino group of a lysine residue on the histone or non-histone protein, leaving a methylated lysine residue and S-adeno-L-homocysteine (SAH) as a by-product [21]. Further methyl groups are added progressively to achieve di- and tri- methylation.

Many histone methyltransferases have been shown to be involved in cancer and neurological diseases [25, 26]. One of the most well studied is Enhancer of Zest Homologue 2 (EZH2), a histone lysine methyltransferase belonging to the the Polycomb group (PcG) protein family. EZH2 is the active catalystic subunit of the Polycomb Repressive Complex 2 (PRC2), targeting histone H3 lysine 27 for mono, di and tri-methylation [19]. PRC2 is involved in a range of normal cellular processes, including cellular differentiation and stem-cell plasticity. Upregulation of EZH2 is a marker for aggressive prostate and breast cancers [27, 28]. Recurrent gain of function mutations have been identified at the Y641, A677 and A687 residues within the EZH2 catalytic domain [29, 30]. These mutations alter the substrate specificity of EZH2, increasing the conversion of H3K27 di-methylation to tri-methylation, while wild type EZH2 preferentially converts H3K79 mono-methylation to di-methylation. EZH2 gain of function mutations have been found in follicular lymphoma and Germinal Centre B-Cell like Diffuse Large B-Cell lymphoma [31]. A range of small molecular EZH2 inhibitors have been synthesized and shown good *in vitro* and *in vivo* efficacy against lymphoma cells [32-34].

Histone methylation was originally believed to be irreversible until the discovery of Lysine Specific Demethylase 1 (LSD1), also known as KDM1A [8]. Since 2004, a total of 15 lysine demethylases have been discovered [35], and have been separated into 2 families: the LSD family consisting of the amine-oxidase related enzymes LSD1 and LSD2, and the Jumonji C-terminal (JMJC) domain containing family [36, 37].

LSD1 converts mono- and di-methylated H3K4 into unmethylated H3K4 [38]. The catalytic mechanism of LSD family demethylases requires a lone electron pair on the lysine ε-nitrogen atom, meaning it cannot demethylate tri-methylated lysines [39]. LSD1 has been shown to require the removal of acetylated lysine residues on histone 3 before H3K4me2 demethylation can efficiently occur, due to LSD1 being a part of a complex that includes histone deacetylases [40, 41].

The JMJC protein domain has been found in 31 human proteins with 17 of these demonstrating demethylase activity [42]. The enzymatic mechanism of JMJC demethylases involves two cofactors, Fe(II) and 2-oxoglutarate binding to the JMJC domain and reacting with dioxygen to form an active oxoferryl intermediate that hydroxylates the ζ-methyl groups of the methylated lysine substrate [43]. This results in an unstable lysyl hemiaminal that breaks down to release methyl groups from nitrogen. This mechanism allows the mono-, di- and tri-methylation of lysine. Currently there are no known histone lysine demethylases that target H4K20 and H3K79 methyl marks.

The histone H3K79 methylatransferase: Disruptor of telomeric silencing 1-like (DOT1L)

Disruptor of telomeric silencing 1 (DOT1) was first identified through a genetic screen for proteins whose over-expression would lead to impaired telomeric silencing in yeast [44]. The DOT1 homolog gene, DOT1-like (DOT1L), has been found in a range of species, including drosophila [45], protozoa [46] and mammals [47] with mouse and human versions of DOT1L sharing an 88% similarity at the amino acid level [48, 49].

DOT1L is the only known histone methyltransferase that targets the histone H3 lysine 79 (H3K79) position, located on the nucleosome surface instead of the N-terminal tail where epigenetic modifications normally occur [48, 49]. It adds methyl groups in a non-progressive manner, requiring DOT1L to dissociate and reassociate to H3K79 as it adds methyl groups to generate mono-methylation (H3K79me), dimethylation (H3K79me2) and tri-methylation (H3K79me3) (**Figure 1A**).

Instead of a SET domain, DOT1L has an AdoMet binding motif similar to arginine and DNA meth-

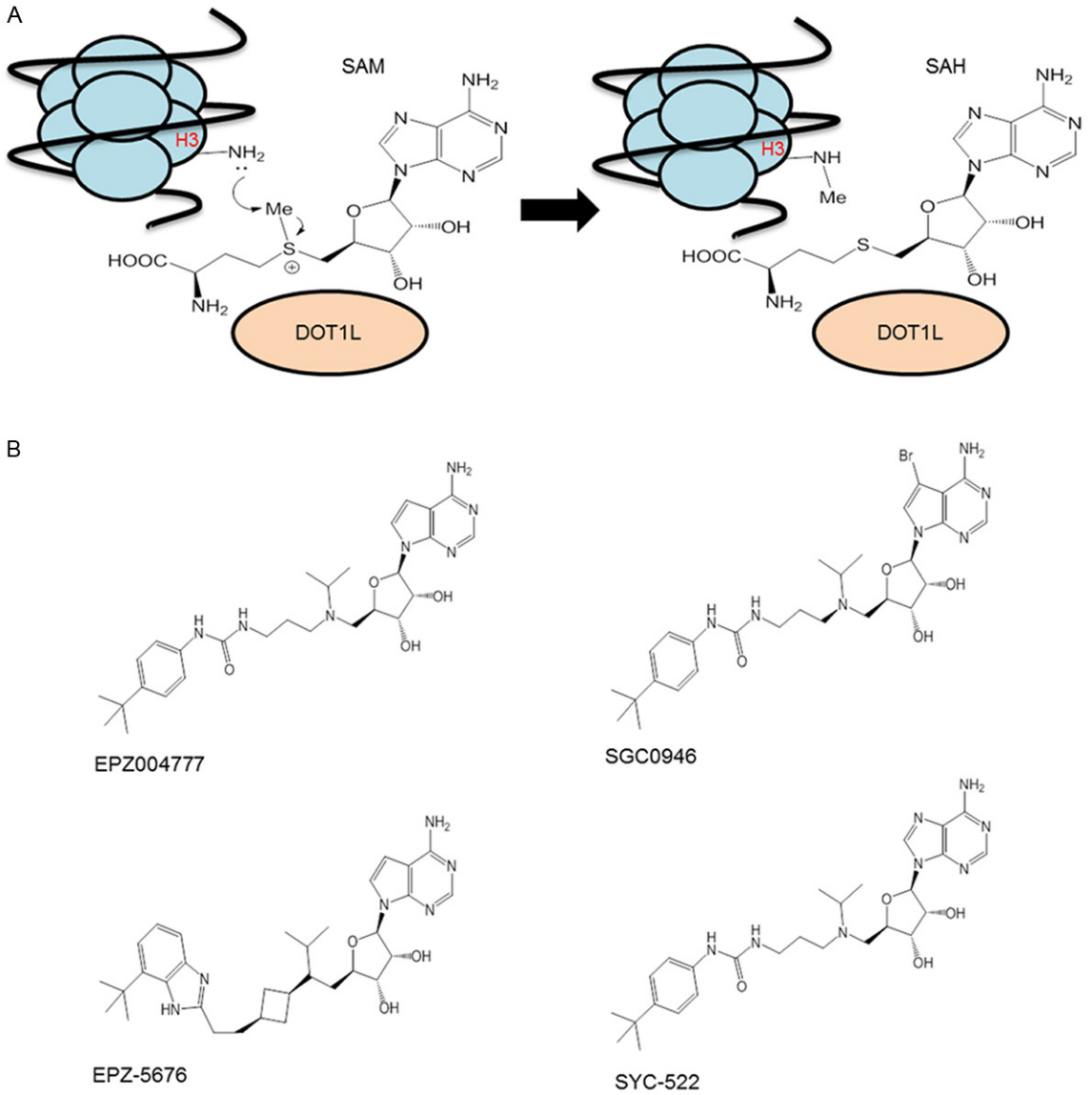

Figure 1. Chemical structures of DOT1L inhibitors. A. DOT1L catalyses histone H3K79 methylation by transferring a methyl group from its substrate S-adenosyl-L-methionine (SAM) to the amino group of a lysine residue on the histone. A methylated H3K79 residue and S-adeno-L-homocysteine (SAH) are produced, and DOT1L then dissociates. Additional methyl groups are added in a sequential and similar manner. B. Small molecular DOT1L inhibitors: EPZ004777, EPZ5676, SGC0946 and SYC-522. All are based on SAH backbone and target the SAM binding pocket of DOT1L.

yltransferases [50]. It is currently the only known non-SET histone methyltransferase protein [48, 49]. This makes DOT1L a key target for specific therapeutic treatments, with several small molecular inhibitors developed and one currently in clinical trials [51-53] (**Figure 1B**).

Study of the crystal structure of DOT1L has shown that the AdoMet binding pocket must be near a lysine binding channel and the C-terminus of the catalytic domain in order for nucleosome binding and enzymatic activity to occur [48]. This active site of DOT1L closely resembles catechol-O-methyltransferases and L-isoaspartyl methyltransferases, which are highly conserved in eukaryotic organisms [48].

Regulatory functions of DOT1L in gene transcription, somatic reprogramming, cell cycle regulation and development

The distribution of all three forms of H3K79 methylation on human histones has been studied using mass spectrometry, demonstrating

that H3K79me is the most abundant and correlates with the fraction of histone H3 modified by acetylation [54]. This suggests H3K79 methylation enrichment at active gene transcription sites. Further studies focusing on individual genes in mammalian cells have correlated H3K79me2 with transcriptional activation [55, 56]. Subsequent quantitative-chromatin immunoprecipitation (q-ChIP) studies of H3K79 methylation across the human genome reveal that H3K79me3 is present at higher levels in silent gene regions in comparison to active regions, linking it to gene repression [17]. This demonstrates the complex regulatory effects of DOT1L on a wide range of functions in eukaryotic organisms, with H3K79me and H3K79me2 associated with active gene transcription and H3K79me3 with gene repression in human cells.

DOT1L inhibition may enhance reprogramming in a broad range of cell types by facilitating the silencing of lineage-specific programs of gene expression. Inhibition of DOT1L by shRNA or small molecule inhibitors accelerates reprogramming, significantly increasing the yield of pluripotent stem cell colonies, and substitutes for KLF4 and c-Myc [57]. Genome wide analysis of H3K79me2 distribution reveals that DOT1L inhibition accelerates reprogramming due to loss of H3K79me2 among genes fated to be repressed in the pluripotent state [57, 58].

Methylation of H3K79, mediated by DOT1 and DOT1L, has been implicated in transcriptional elongation and cell cycle regulation. Nearly 90% of histone H3 in *Saccharomyces cerevisiae* (*S. cerevisiae*) bears H3K79me, H3K79me2 or H3K79me3. The level of H3K79me2 fluctuates between different stages of the cell cycle in *S. cerevisiae*, with a low level of H3K79me2 at the G_1 phase, gradually increasing at the S phase and peaking at the G_2/M phase, while H3K79me3 remains constant throughout the cell cycle [59]. *S. cerevisiae* mutants arrested at G_1 and G_2/M phases showed increased H3K79me3 levels, demonstrating that H3K79 methylation levels increase progressively over time in arrested cells [60].

H3K79me2 associates with gene promoter and coding regions during transcriptional activation of hepatic genes in G0/G_1 enriched human liver carcinoma cells [61]. The mechanism responsible for targeted DOT1L binding and consequent H3K79 methylation of actively transcribed genes involves DOT1L binding to the phosphorylated C-terminal domain of actively transcribing RNA polymerase II (RNAP II) [62]. Similarly human liver carcinoma cells arrested at the G_2/M phases have increased H3K79 methylation levels compared to G0/G_1 enriched cells, showing that this methylation mark is generated independently of gene transcription at the S phase [61].

H3K79 has been shown to control sexual differentiation in silk worms (*Bombyx mori)*, with higher H3K79me2 levels on the insulin-like growth factor II mRNA-binding protein (Imp) gene promoter in males than females [63]. RNAi-mediated depletion of DOT1L results in a total abolishment of male-specific Imp, showing H3K79 methylation regulates sex-specific splicing of Imp mRNA [63].

H3K79 methylation levels in *Drosophila* correlate to transcriptional activity [55]. The *Drosophila* ortholog of DOT1, grappa (gpp), has been identified as a dominant suppressor of pair-dependant silencing, necessary for the maintenance phase of Bithorax complex expression [64]. H3K79 methylation expression during embryogenesis is conserved in *Drosophila,* mouse, rat and human spermatids and also independent of H3K4 and H3K9 methylation [65]. During chromatin reorganisation in spermatids, H3K79 methylation is accompanied by H4 hyperacetylation and may be a prerequisite for proper histone to protamine transition [66].

DOT1L serves further developmental roles in a wide range of species. It is directly regulated during tadpole development in the model system *Xenopus tropicalis* by thyroid hormone receptor, which binds to a thyroid hormone response element in the DOT1L gene promoter region [67]. Embryos treated with DOT1L specific transcription activator-like effector nuclease show low H3K79 methylation and experience growth difficulties as tadpoles, ultimately leading to tadpole mortality [68].

DOT1L has been shown as a crucial regulator of early mammalian haematopoiesis by regulating the steady state levels of GATA2, a growth factor essential for early erythropoiesis [69], and PU.1, a transcription factor that inhibits erythropoiesis and promotes myelopoiesis [70]. DOT1L knockout mice display reduced GATA2 and increased PU.1 levels and early embryonic

death due to anaemia, indicating that DOT1L is essential for embryonic development and prenatal haematopoiesis [71]. Conditional DOT1L targeting strategies show DOT1L also playing a role in adult haematopoiesis maintenance, with DOT1L deletion in mice resulting in pancytopenia and failure of hematopoietic homeostasis [72, 73]. Taken together, these findings demonstrate that DOT1L plays a critical regulatory role in gene transcription, somatic reprogramming, cell cycle, development and haematopoiesis, via H3K79 methylation.

The role of DOT1L in DNA damage response

DOT1 was identified in a screen of radiation sensitive yeast mutants for DNA damage checkpoint defects, with DOT1 yeast mutants exposed to ionizing radiation becoming defective at the G_1 and intra-S phase checkpoint [74]. Checkpoint mediated arrest at the pachytene stage in *dmc1* and *zip1 S. cerevisiae* mutants was also shown to be DOT1-dependent, with loss of DOT1 leading to continued meiosis and the generation of unviable cells [75].

In the human osteosarcoma cell line U2OS, *in vitro* experiments established that the double-stranded DNA break repair protein 53BP1 bound most efficiently to H3K20me2 [76]. This was confirmed using a stable DOT1L knockdown model system, which showed that H3K79 methylation was not critical for 53BP1 recruitment to DNA damage sites [77]. However, H3K79me2 was shown to be required in an alternate pathway of 53BP1 recruitment in response to DNA damage during the G_1 and G_2/M cell cycle phases, when H3K20me2 levels dropped [78]. In addition, suppression of DOT1L inhibited recruitment of 53BP1 to sites of DNA damage in 293T cells [79]. Thus both H3K79 and H3K20 methylation are capable of 53BP1 recruitment in response to DNA damage repair, with each methylation covering different stages of the cell cycle.

In mouse embryonic fibroblast cells treated with UV, DOT1L reduction leads to UV hypersensitivity and reduced recovery of transcription initiation. DOT1L was found to promote an open chromatin structure to reactivate RNA Pol II-mediated transcription after DNA damage and was not involved in the nuclear excision repair pathway [80]. In addition, DOT1 was also shown to be required for cell cycle arrest in response to double stranded DNA breaks [75]. Thus, DOT1L-mediated H3K79 methylation may play a critical role in DNA damage signalling.

DOT1L and mixed lineage leukaemia

In humans, DOT1L is involved in the oncogenesis of several leukaemia subtypes, mostly characterised by chromosomal translocations involving the mixed lineage leukaemia (MLL) gene. MLL chromosomal translocations involving the cytogenetic band 11q23 produce a wide array of fusion proteins associated with Acute Lymophoblastic Leukaemia (ALL), Acute Myeloid Leukaemia (AML) and Mixed Lineage Leukaemia [81-83].

DOT1L has been found in the Elongation Assisting Proteins (EAP) complex along with RNA PII, factors AF4, AF6, AF9, AF10 or ENL [84-87], all known to be involved in chromosomal translocation-induced fusion with the MLL protein. DOT1L and AF10 have been isolated from yeast and mammalian two-hybrid assays as binding partners of ENL, which is a MLL fusion partner with transactivation abilities and known to associate with AF4 [88, 89]. AF4 stimulates kinase elongation by P-TEFb and interacts with AF9 and AF10 to recruit DOT1L to the Pol II elongation complex at ectopic loci, resulting in aberrant gene expression and MLL leukaemogenesis [84, 90]. The MLL-AF6 fusion protein requires H3K79 methylation for the maintenance of MLL-AF6 target oncogenic gene expression, with gene expression analysis and ChIP-sequencing finding high levels of H3K79me2 at MLL target gene promoters [87]. Abnormally high levels of H3K79 methylation on MLL target gene promoters in MLL leukaemia cells are indicative of aberrant DOT1L activity in these leukaemia cells due to MLL fusion oncoproteins recruiting DOT1L, causing overexpression of MLL target genes [91]. This has further been shown with inducible expression of the MLL-ENL fusion gene activating H3K79me2 on MLL target gene promoters, while inhibiting DOT1L binding leads to the MLL-ENL fusion gene losing its transforming ability [89].

A notable family of genes dis-regulated in leukaemia is the homeobox (HOX) gene family, which is highly expressed in multipotent haematopoietic stem cells (HSCs) but down-regulated once the HSCs have become differentiat-

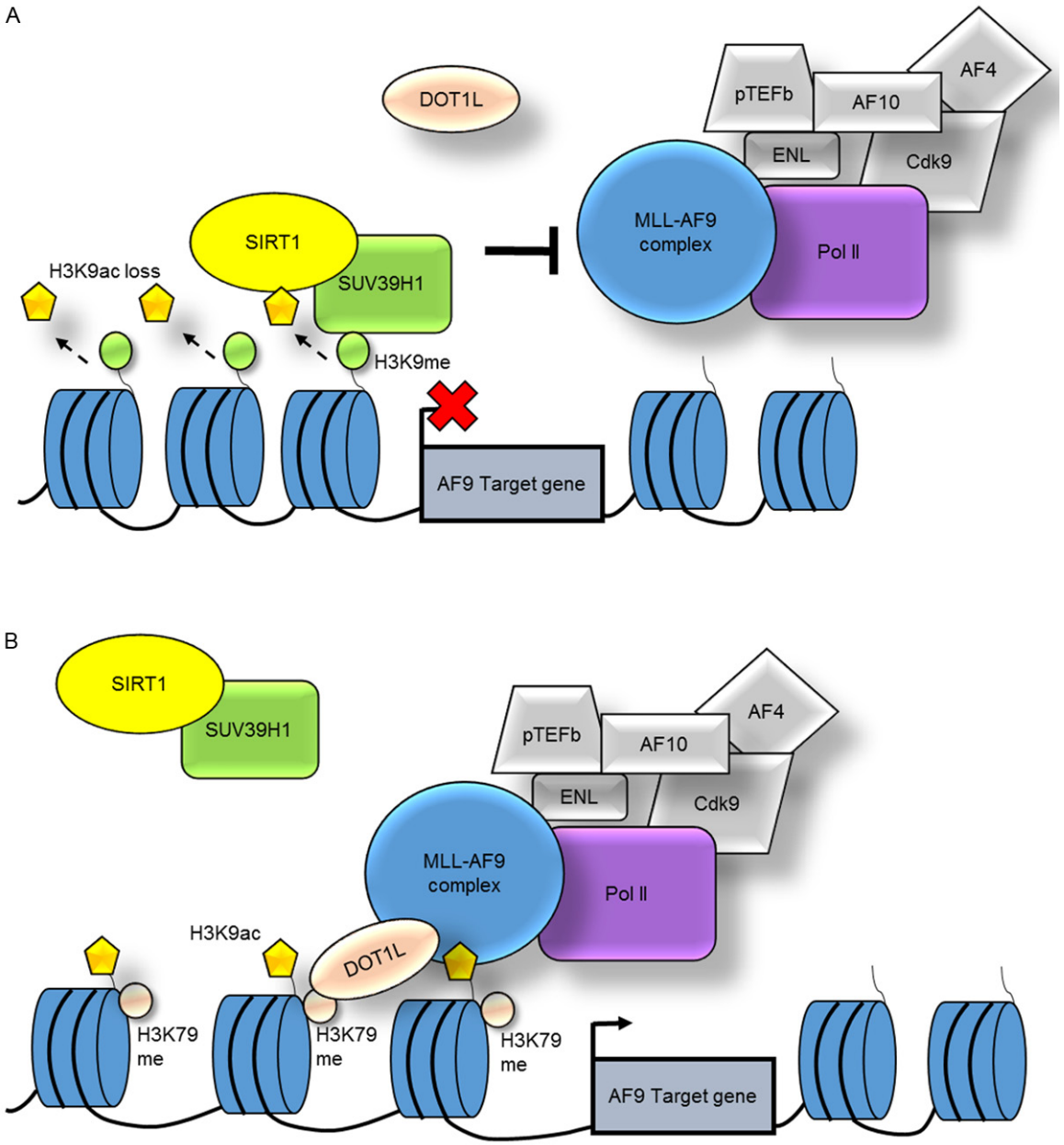

Figure 2. Model of possible MLL-AF9 and DOT1L-mediated gene transcription mechanism in MLL-driven leukaemia. A. SUV39H1 and SIRT1 increase H3K9 methylation and decrease H3K9 acetylation, preventing MLL-AF9 and Elongation Assisting Proteins (EAP) complex binding to gene promoters. B. DOT1L inhibits SIRT1 and SUV39H1 chromatin localization, thereby maintaining an open chromatin state with elevated H3K9 acetylation and H3K79 methylation and minimal H3K9 methylation at MLL fusion protein target gene promoters. MLL-AF9 forms a protein complex with DOT1L, recognises elevated H3K9 acetylation via its YEATS domain, and recruits the EAP complex containing RNA Pol II and other transcription factors at MLL fusion protein target gene promoters, leading to transcriptional activation.

ed [92, 93]. Epigenetic regulation of HOX loci is modulated by the MLL protein which introduces the H3K4me3 histone mark, resulting in HOX transcriptional activation [94]. DOT1L-AF10 interaction activates HOXA9 gene transcription and plays an important part in MLL-AF10-mediated leukaemogenesis, with AF10 being a DOT1L cofactor [95]. DOT1L also contributes to clathrin assembly lymphoid myeloid leukaemia protein (CALM)-AF10-mediated leukaemic transformation by preventing nuclear export of CALM-AF10 and by up-regulating HOXA5 gene expression through H3K79 methylation [96]. Consequently inhibiting DOT1L results in sup-

pression of MLL-AF10 and CALM–AF10 mediated transformation by down-regulating leukaemogenic genes such as HOXA and Meis1 [97].

H3K79 methylation by DOT1L is also crucial for the expression of critical MLL-AF4 target oncogenes such as HOXA in human MLL-AF4 leukaemia cells [85]. Experiments with conditional DOT1L knockout mice have found that H3K79me2 drives MLL-AF9 fusion gene-mediated leukaemogenesis, and DOT1L is required for up-regulation of HOXA and Meis1 gene expression and consequent initiation and maintenance of MLL-AF9-induced leukaemogenesis [86]. However, HOXA9/Meis1 and E2A-HLF MLL cell lines do not require DOT1L for oncogenic transformation [72], illustrating a crucial role for DOT1L in driving leukaemogenesis with specific MLL translocation oncogenes.

The mechanisms underlying DOT1L interaction with MLL-fusion proteins are still unclear, but studies have focused on identifying DOT1L protein binding sites. A 10 amino acid region of human DOT1L (865-874) has been identified as the AF9/ENL binding site, with four conserved hydrophobic residues within the binding site being shown to be essential for interactions with the C-terminal binding domain of AF9/ENL [98]. DOT1L binding to MLL-AF9 has been shown at three sites by nuclear magnetic resonance. Structure-guided point mutations of these binding sites lead to a graded reduction in DOT1L recruitment to MLL-AF9, with differential loss of H3K79me2 and H3K79me3 at MLL-AF9 target genes [99].

The YEATS domain of AF9 recognises H3K9 acetylation, providing a link between elevated H3K9 acetylation and DOT1L recruitment to target gene promoters [100]. Furthermore DOT1L inhibits chromatin localization of a repressive complex composed of the histone H3 deacetylase SIRT1 and the H3K9 methyltransferase SUV39H1, thus maintaining an open chromatin state with elevated H3K9 acetylation and H3K79 methylation and minimal H3K9 methylation at MLL fusion target gene promoters (**Figure 2**) [101].

DOT1L in other cancers

The complex regulatory role of DOT1L in MLL-driven leukaemia and physiologically normal eukaryotic organisms has led to interest in its possible function in various other cancer types. RNAi-mediated depletion of DOT1L in A549 and NCI-H1299 lung cancer cells resulted in chromosomal mis-segregation, cell-cycle arrest at the G_1 phase and senescence [102]. Overexpression of DOT1L reversed these RNAi-mediated phenotype changes in lung cancer cells.

DOT1L increased the tumorigenic potential of colorectal cancer cells by inducing NANOG, SOX2 and Pou5F1 gene expression [103]. High DOT1L gene expression and consequently increased H3K79me2 levels are predictors of poor patient survival [103], indicating DOT1L having a tumorigenic role in colorectal cancer.

DOT1L has also been recently linked to breast cancer with a study of a genomic database of over 1000 patient samples showing that higher levels of DOT1L expression correlated with breast cancer compared to normal breast tissues [104]. High DOT1L levels in breast cancer tissues correlated with approximately 20 pro-proliferative genes from the PAM50 gene set [104]. DOT1L interaction with c-Myc-p300, has been suggested to be critical for promoting EMT/CSC leading to an aggressive phenotype in breast cancer [105].

Chromatin immunoprecipitation assays have linked H3K4 and H3K79 methylation and H3 acetylation to the ability of the c-Myc transcription factor to recognise and bind to target gene promoters [106]. H3K4me2, H3K4me3 and H3K79me2 are generally associated with transcription machinery and these methylation marks precede and are independent of c-Myc binding at target gene promoters [107]. In contrast, H3K79 methylation has been shown to be non-essential for the maintenance or activation of Wnt pathway target gene expression in human colon adenocarcinoma cell lines, with H3K79 methylation showing no elevation in comparison to normal colon tissue [108].

Thus H3K79 methylation is a critical histone modification in a range of cancer types. The dynamic interplays among different chromatin post-transcription modifications control gene expression, and provide novel opportunities for targeted combination therapies in multiple cancer types.

DOT1L inhibitors

Molecular targeting of specific histone methyltransferase is emerging as a new direction for cancer therapy. Of the known histone methyl-

transferase enzymes, DOT1L is a promising target due to it being the only known H3K79 histone methyltransferase, its unique non-SET catalytic domain and its role in promoting and maintaining MLL leukaemogenesis [48, 49].

The first DOT1L specific small molecular inhibitor was EPZ004777, designed by Epizyme using a traditional ligand-based approach based on the DOT1L substrate SAM and the product SAH [51]. EPZ004777 displayed specificity against DOT1L with little reactivity against a panel of eight other histone methyltransferases. Modifications to EPZ00477 lead to the synthesis of EPZ5676 [52] and SGC0946 (**Figure 1B**) [109].

SGC0946 features an additional bromine atom at position 7 targeting a hydrophobic cleft present in DOT1L to improve the DOT1L inhibitor's binding affinity [109]. Another novel DOT1L inhibitor, SYC-522, was synthesized based on the structure of SAH with additional urea group, and showed specificity for DOT1L when tested against three representative histone methytransferases: PRMT1, PRMT4 and SUV39H1 [110]. These four DOT1L specific small molecular inhibitors are designed to occupy the SAM binding pocket, inducing DOT1L conformational changes and leading to the opening of a hydrophobic pocket outside of the SAM binding domain [52].

Anticancer efficacy of DOT1L inhibitors

The anticancer efficacy of allosteric DOT1L inhibitors has been tested *in vitro* and *in vivo.* The first reported DOT1L inhibitor, EPZ004777, demonstrated an IC_{50} of 400 ± 100 pM to inhibit DOT1L enzymatic activity in a biochemical radionucleotide assay [51]. Treatment with EPZ004777 caused apoptosis in MLL-rearranged leukaemia cells *in vitro* and blocked leukaemia progression in mice by suppressing the expression of HOXA cluster genes and Meis1 [51]. MLL-AF6 transformed mouse bone marrow cells also demonstrated a dose-dependent reduction in H3K79me2 and a reduction in cell number when treated with 10 μM EPZ004777 for 10 days [87]. DOT1L inhibition reduced the number of MLL-AF6 transformed cells at the S-phase, and increased apoptotic cell death [87]. *In vivo* administration of EPZ004777 by subcutaneously implanted osmotic pumps over 14 days resulted in a dose-dependent increase in the survival of mice xenografted with MV4-11 leukemia cells [51]. Pre-treatment of mice with EPZ004777 also decreased the *in vivo* spleen-colony-forming ability of MLL-AF10/CALM-AF10 transformed bone marrow cells [51].

EPZ5676 demonstrated a superior enzyme inhibition K_i value of ≤ 0.08 Nm compared to EPZ004777. EPZ5676 had an IC_{50} of 3 nM and 5 nM in MV4-11 and HL60 leukaemia cells, respectively [52]. It demonstrated synergistic anticancer effects when used in combination with cytarabine and daunorubicin to treat MOLM-13 and MV4-11 MLL-rearrangement leukaemia cells lines [111]. Continuous intravenous treatment of rats xenografted with MV4-11 cells with EPZ5676 for 21 days, leaded to dose-dependent leukemia regression with a 70 mg/kg dose leading to complete regression [52].

SGC0946 showed an IC_{50} of 8.8 ± 1.6 nM in reducing H3K79 methylation in cells, representing a significant improvement over EPZ-004777, which exhibited an IC_{50} of 84 ± 20 nM [109]. Treatment of MLL-rearranged MV4-11 and THP1 leukemia cells with the DOT1L inhibitor SYC-522 led to cell cycle arrest and cell differentiation, and treatment of primary MLL-rearranged AML cells resulted in up to 50% decrease in colony formation and promotion of monocytic differentiation [110]. SYC-522 was also tested in combination with existing chemotherapeutics: mitoxantrone, etoposide or cytarabine. Pretreatment with SYC-522 sensitized primary MLL-rearranged leukaemia cells to treatment with all three chemotherapy agents [112]. In addition, combination therapy with the SIRT1 activator SRT1720 and the DOT1L inhibitor EPZ004777 demonstrated enhanced antiproliferative activity against MLL-rearranged leukemia cells by supressing HOXA7 and Meis1 in vivo [101]. DOT1L inhibitors have also demonstrated anticancer effects against solid tumor cells. SYC-522 and EPZ004777 induced differentiation and inhibited proliferation, self-renewal and metastatic potential in a range of DOT1L overexpressing breast cancer cells [104].

Currently all reported DOT1L inhibitors have the common substructure of adenosine, making them competitive to the DOT1L enzyme substrate SAM and resulting in overall poor pharmacokinetic properties [51, 53]. This indicates

that improvements in the metabolic stability of DOT1L inhibitors are required before their use for human patient treatment.

Conclusions

Epigenetic modifications in cells play a major role in regulating the expression of genes important for development, haematopoiesis and cancer. Histone H3K79 methylation mediated solely by DOT1L is involved in somatic reprogramming, cell cycle progression, DNA damage response and tumorigenesis. Aberrant H3K79 methylation is associated with various types of aggressive MLL fusion gene-driven leukaemia. P-TEFb, ENL, AF4, AF6 AF9 and AF10 proteins have all been shown to be involved in the recruitment of DOT1L to MLL target gene promoters. H3K79me2 has been shown to be critical to MLL target gene expression via maintenance of open chromatin states around MLL target genes, with loss of H3K79me2 leading to loss of tumorigenicity in MLL-driven leukaemia. In addition, DOT1L disregulation has been linked to poor patient prognosis in lung, colorectal and breast cancers. These observations raise the possibility of DOT1L playing a major role in other forms of cancer and will be a subject for future inquiry.

The unique structural features of DOT1L have made it an emerging drug target with multiple allosteric small molecular inhibitors, based on the SAH structure showing selective inhibitory effects and low IC_{50} concentrations *in vitro*. Further chemical modifications to the first small molecular DOT1L inhibitor, EPZ004777, have led to the generation of EPZ5676 with improvements in metabolic stability and anticancer efficacy. These small molecular DOT1L inhibitors have shown synergy when used in combination with existing chemotherapeutics. Importantly, EPZ5676 is currently in Phase 1 clinical trials in leukemia patients (NCT02141828).

A limiting factor in DOT1L inhibitor discovery has been the complex biochemical assays necessary to determine loss of H3K79 methylation. Two new assays involving nanoparticle proximity and fluorescence polarisation respectively have been created for future DOT1L inhibitor screening and lead optimisation [113].

An alternative strategy for targeting DOT1L in MLL would be to target the DOT1L binding sites of the MLL-fusion proteins [98]. Targeting these MLL-AF9 and AF9/ENL binding sites in DOT1L could potentially result in fewer adverse effects than the conventional approach of targeting DOT1L enzymatic activity. Inhibitors targeting these binding sites could also have improved pharmacokinetic properties compared to existing DOT1L inhibitors based on SAH. Thus future DOT1L inhibitors have the potential to deliver better patient outcomes in treating MLL leukemia and potentially other forms of cancers where H3K79 methylation dis-regulation is present.

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Disclosure of conflict of interest

None.

Abbreviations

H3K79, histone H3 lysine 79; MLL, mixed lineage leukemia; H3K4me, histone H3 lysine 4 mono-methylation; H3K4me2, histone H3 lysine 4 di-methylation; H3K4me3, histone H3 lysine 4 tri-methylation; H3K36me3, histone H3 lysine 36 tri-methylation; H3K79me, histone H3 lysine 79 mono-methylation; H3K79me2, histone H3 lysine 79 di-methylation; H3K79me3, histone H3 lysine 79 tri-methylation; H3K27me, histone H3 lysine 27 mono-methylation; SET, (Su(var), Enhancer of zeste, Trithorax); SAM, S-adenosyl-L-methionine; SAH, S-adeno-L-homocysteine; EZH2, Enhancer of Zest Homologue 2; LSD1, Lysine Specific Demethylase 1; q-ChIP, quantitative-chromatin immunoprecipitation; AdoMet, S-Adenosyl methionine; ALL, Acute Lymophoblastic Leukaemia.

Address correspondence to: Dr. Tao Liu, Histone Modification Group, Children's Cancer Institute Australia, Lowy Cancer Research Centre, UNSW, Randwick, Sydney, NSW 2031, Australia. E-mail: tliu@ccia.unsw.edu.au

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