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# The emerging role of DOT1L in cell proliferation and differentiation: Friend or foe

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**Summary.** Cell proliferation and differentiation are the basic physiological activities of cells. Mistakes in these processes may affect cell survival, or cause cell cycle dysregulation, such as tumorigenesis, birth defects and degenerative diseases. In recent years, it has been found that histone methyltransferase DOT1L is the only H3 lysine 79 methyltransferase, which plays an important role in the process of cell fate determination through monomethylation, dimethylation and trimethylation of H3K79. DOT1L has a pro-proliferative effect in leukemia cells; however, loss of heart-specific DOT1L leads to increased proliferation of cardiac tissue. Additionally, DOT1L has carcinogenic or tumor suppressive effects in different neoplasms. At present, some DOT1L inhibitors for the treatment of MLL-driven leukemia have achieved promising results in clinical trials, but completely blocking DOT1L will also bring some side effects. Thus, this uncertainty suggests that DOT1L has a unique function in cell physiology. In this review, we summarize the primary findings of DOT1L in regulating cell proliferation and differentiation. Correlations between DOT1L and cell fate specification might suggest DOT1L as a therapeutic target for diseases.

**Key words:** DOT1L, H3K79 methylation, Cell proliferation, Cell differentiation

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## Introduction

From a single-cell fertilized egg to a multicellular embryo to an adult, it is actually a precisely regulated process of cell proliferation, division, migration, differentiation, and apoptosis. Among them, cell proliferation and differentiation are the basis and core of biological reproduction and growth (Jiang et al., 2021). Cell proliferation is achieved through the cell cycle, which is an orderly regulatory process involving multiple stages and factors, so that all types of cells rely on the needs of the body to proliferate or are in a static state (Le Bras, 2021). Cell differentiation refers to the process by which cells from the same origin gradually produce cell groups with different morphological structures and functional characteristics, and the result is that the cells are different in space, and the same cell is different from its previous state in time (Wang et al., 2021b). The essence of cell differentiation is the selective expression of the genome, which is turned on or off by different gene expressions, ultimately producing iconic proteins (Wang et al., 2021b). Abnormalities in cell proliferation and differentiation can both cause and promote disease. For example, in the normal development process, cell proliferation and differentiation are to ensure the normal growth and physiological function of organs and tissues, during cardiac development, the initial coronary vascular plexus expands and buds to form new blood vessels in the ventricular wall, and the original coronary endothelial cells after myocardial infarction significantly proliferate and promote neovascularization (Pu et al., 2020). However, defects in cell proliferation and differentiation can also lead to many diseases. Under normal circumstances, bone marrow hematopoietic stem cells have strong proliferation and differentiation capabilities, and it has been reported that some pathological conditions can lead to hematopoietic stem cell

proliferation defects, which in turn lead to bone marrow hematopoietic failure (Shao et al., 2021). Excessive cell proliferation can also lead to diseases such as tumors (Gaglia et al., 2022), and rheumatoid arthritis (Tu et al., 2023). Malignancy is a typical cell cycle abnormality. It has been found that during cell proliferation and differentiation, DOT1L and its homologs can induce the onset and progression of tumors or other diseases by regulating cyclin and differentiation regulators, affecting cell cycle progression (Hou et al., 2023).

As a telomere silencing interfering factor, DOT1L was screened for the first time through gene screening of proteins overexpressing in yeast (Singer et al., 1998). Later, DOT1 homolog gene, DOT1L- like (DOT1L) has been found in many species, including drosophila (List et al., 2009), protozoa (Janzen et al., 2006) and mammals (Jones et al., 2008). So far, the conserved histone methyltransferase DOT1L is the only known methyltransferase that can catalyze the mono-methylation, dimethylation and trimethylation of H3K79 (Min et al., 2003; Frederiks et al., 2008). The methylation modification site of H3K79 is located in the core of nucleosome, while the apparent modification sites of other histones are in the tail of histone, suggesting that DOT1/DOT1L functional modification has its particularity (Jones et al., 2008). DOT1L and H3K79 methylation have potential functions in normal physiological processes and disease progression, such as embryonic development, leukemia (Jones et al., 2008), gastric cancer (Song et al., 2020) and so on. Knockout of DOT1L resulted in significant proliferation defects of embryonic stem cells (ESCs) under differentiation stimulations, which indicated DOT1L could promote cell differentiation (Barry et al., 2009). Owing to the important role in cell cycle and cell proliferation, the abnormality of DOT1L activity leads to tumorigenesis, like in case of MLL-rearranged leukemia (Okada et al., 2005). Various studies have shown that DOT1L is an important epigenetic regulator that regulates cell proliferation and differentiation, thereby determining cell fate. However, further research is needed to thoroughly reveal the pathophysiological roles of DOT1L in cell fate. In this review, we summarize the major findings of DOT1L in regulating cell proliferation and differentiation in cancer, blood system, locomotor system, cardiovascular system, nervous system, immune system and other systems, providing a potential entry point for future research directions of DOT1L (Fig. 1).

## Biological function of DOT1L

Dot1 (yDot1) and the human homologous protein DOT1L (hDot1L) contain an α-helical N-terminal domain and a central open α/β structure, and the latter contains a typical structure of a class I SAM-dependent methyltransferase- a7-strand β-fragment (Min et al., 2003). The SAM-binding domain is a critical moiety binding the methyl source, resulting in the movement of methyl from SAM to the amino group of H3K79 (van Leeuwen et al., 2002; Daigle et al., 2013). The acidic recess between the two domains contains two basic residue binding pockets that can accommodate the outwardly protruding basic side chains around histone H3 Lys79 on the surface of the discoid nucleosome (Sawada et al., 2004). DOT1L is low during early mouse embryonic preimplantation development (Ooga et al., 2013) but widely expressed in mouse organs in adulthood (Zhang et al., 2004), which suggests the expression of DOT1L may be regulated by specific signals.

DOT1L-regulated H3K79 methylation is involved in multiple processes, including transcription elongation, DNA damage response, and cell cycle. H3K79 methylation correlates with RNAPII transcription in mouse 3T3 cells (Steger et al., 2008). In yeast, the Paf1 protein complex associated with elongation RNAPII

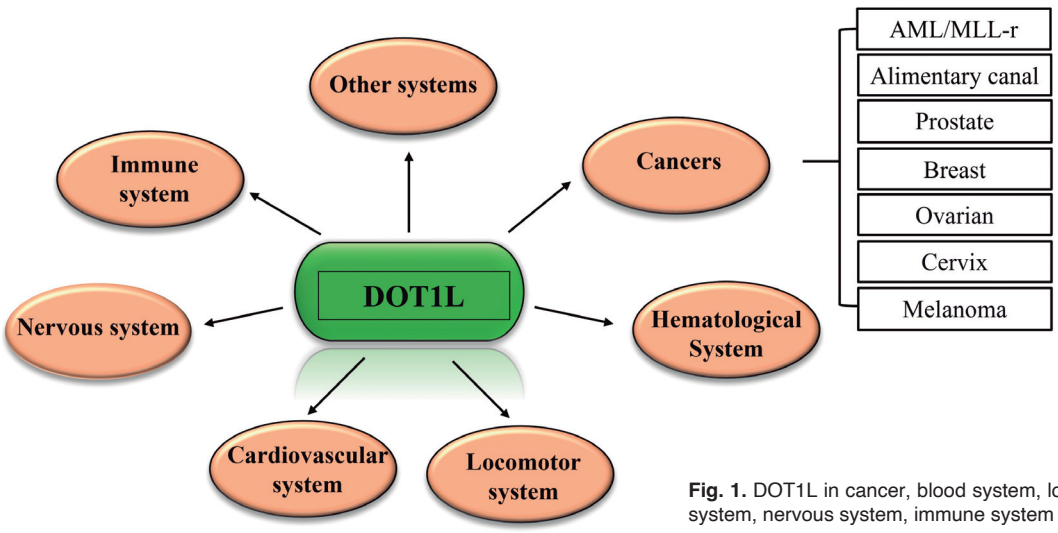


**Fig. 1.** DOT1L in cancer, blood system, locomotor system, cardiovascular system, nervous system, immune system and other systems.

might regulate H3K79 methyltransferase activity of Dot1, which is necessary for the methylation of lysines 4 and 79 in histone H3, and silencing of telomere-related genes (Krogan et al., 2003). It is found that the levels of H3K79me1, H3K79me2 and H3K79me3 are dynamically correlated with cell cycle and developmental stage (Singer et al., 1998). The role of Dot1/DOT1L-mediated methylation of H3K79 in cell proliferation depends on the type or nature of the tissue. Deletion of Dot1/DOT1L results in decreased cell proliferation in budding yeast, trypanosomes, mouse embryonic stem cells, and human cancer cells. Whereas DOT1L-null mouse ES cells undergo G2/M-phase block (Singer et al., 1998). DOT1-mediated H3K79 methylation (H3K79me) is required for two distinct phases of the Rad9-dependent DNA damage response, an early phase of G1/S checkpoint activation and a later phase of DNA repair in G2 phase. In the early stage of G1/S, DOT1L deletion leads to abnormal cell proliferation (Lazzaro et al., 2008). Furthermore, Dot1-dependent H3K79me plays a critical role in the repair of UV-induced DNA damage. Loss of these histone marks contributes to UV hypersensitivity (Long et al., 2020).

## Role of DOT1L in cancers

DOT1L plays an important role in the development and progression of leukemia, especially in MLL-rearranged leukemia (MLL-r). The role of transcription factors such as HOX (Chen et al., 2019) and MEIS1 (Meriç and Kocabaş, 2022) in the regulation of leukemia survival and maintenance is well recognized. DOT1L is involved in promoting proliferation of MLL-r cells. DOT1L deletion causes reduction of H3K79 methylation, which will decrease transcription of HOXA9 and Meis1, reducing the survival rate of leukemia cells (Kingsley et al., 2020; Richter et al., 2021). Meanwhile, Song et al. report that the hexosamine biosynthesis pathway (HBP) regulates the expression of MLL fusion leukemia HOXA9/MEIS1 and the proliferation of leukemia cells through DOT1L (Song et al., 2021). In addition, the high expression of DOT1L is also a crucial cause of other tumors. In neuroblastoma, DOT1L deletion reduces the mRNA and protein expression of N-Myc target genes ODC1 and E2F2, which in turn inhibits neuroblastoma cell proliferation (Wong et al., 2017). In mouse models, treatment with DOT1L inhibitors induces cell cycle arrest and cell apoptosis in myeloma and strongly inhibits tumor cell proliferation *in vitro* (Ishiguro et al., 2019). Liu et al found that DOT1L plays a role in HPV-induced cervical cancer. The hypermethylation of histone H3K79 regulated by DOT1L can lead to the activation of antioxidant response and the activation of Wnt signaling pathway, thereby inducing cervical tumor growth (Liu et al., 2018). In gastric malignant tumors, the expression of DOT1L was significantly increased, which was related to the degree of differentiation, lymph node metastasis and TNM stage. It was found that DOT1L regulates CDK4 and CDK6 through H3K79me2, leading to changes in cell cycle in G1, thus affecting the proliferation of gastric cancer cells (Song et al., 2020). In addition, DOT1L inhibition resulted in the reduction of H3K79me2 in the promoter of Wnt/β-catenin signaling genes, thereby reducing the activation of the Wnt/β-catenin oncogenic signaling pathway in colorectal cancer cells, increasing colon cancer cell apoptosis (Sun et al., 2022). DOT1L inhibition resulted in decreased MYC expression, promoting AR and MYC degradation, resulting in a significant decrease in prostate cancer cell viability (Vatapalli et al., 2020). Recent findings suggest that DOT1L is abnormally overexpressed in human breast cancer cells, and up-regulated DOT1L causes cancer cell proliferation and metastasis (Byun et al., 2020). The use of the DOT1L inhibitor fluoro-neplanocin A (F-NepA) can effectively down-regulate the level of H3K79me2 in triple-negative breast cancer (TNBC) cells by inhibiting DOT1L activity, and showed robust antiproliferative activity (Kurani et al., 2022). It has been reported that DOT1L is overexpressed in ovarian cancer, and high levels of DOT1L correlate with overall survival. Inhibition of DOT1L results in activation of cell death pathways and inhibition of cellular biosynthesis pathways, resulting in NK cell-mediated killing of ovarian cancer cells (Chava et al., 2021). Selective antagonist inhibition of DOT1L interference with ERα/DOT1L complex activity was found to lead to a dose-dependent decrease in Ovarian Cancer and Breast Cancer Cell proliferation, which may occur through G1 phase arrest and be mediated by significant changes in the cell transcriptome (Nassa et al., 2019; Salvati et al., 2019). It is worth mentioning that, unlike the above results, exposure to UV light in the absence of DOT1L is more likely to develop melanoma. DOT1L methylated H3K79 protects the body from UVR-induced melanoma by binding and recruiting XPCs to DNA damage sites for DNA damage repair (Torre et al., 2021). Taken together, these studies provide strong support for the hypothesis that DOT1L plays an important role in cell proliferation and differentiation. (Fig. 2).

## Role of DOT1L in hematological system

Increasing numbers of recent studies have indicated that DOT1L also plays an essential role in normal hematopoiesis and hematopoietic stem cells (HSCs) proliferation and differentiation. Knockout studies have found that DOT1L-deficient mice present severely impaired primitive and definitive hematopoiesis (Jo et al., 2011). Acute inactivation of DOT1L in adult mouse bone marrow for 7 days resulted in a rapid reduction in the number of myeloid progenitor cells, followed by a loss of long-term hematopoietic stem cells (Grigsby et al., 2021). Knockout of *DOT1L* gene in mouse embryonic cells can cause mouse death, and the cause of death is related to severe anemia and insufficient bone marrow cells, suggesting that DOT1L is essential for

hematopoiesis, especially for erythropoiesis (Heimbruch et al., 2021). Furthermore, DOT1L knockout embryos exhibited lethal anemia at day 11.5, indicating severe damage to fetal red blood cells. Analysis by RT-qPCR and CHIP showed that DOT1L exerts its role in erythropoiesis by activating GATA2, causing derepression of the transcription factor PU.1, thereby preventing erythrocyte differentiation (Malcom et al., 2021). However, deficiency of DOT1L in yolk sac-derived hematopoietic cells caused G0/G1 cell cycle arrest and apoptosis of cultured erythroid progenitors in the presence of growth stimuli *in vitro*, but had no effect on myeloid lineage growth and differentiation (Malcom et al., 2021). These findings imply that DOT1L is required for proper cell cycle progression and survival of erythroid progenitor cells, especially erythroid progenitors, during development.

### Role of DOT1L in locomotor system

Osteoarthritis and osteoporosis are widely prevalent and have far-reaching public health implications. There is increasing evidence that epigenetics, in particular, histone 3 lysine 79 methyltransferase DOT1L, plays an important role in the cartilage and bone biology (Sutter et al., 2021). First, DOT1L promotes the differentiation of mast chondrocytes in prenatal and postnatal mice and subsequent intrachondral osteogenesis (Jo et al., 2020).

Col10a1 is a marker of chondrocyte hypertrophy. Prenatal deletion of DOT1L in mouse chondrocytes leads to perinatal death and accelerates ossification and Col10a1 expression disorders. Deletion of DOT1L in

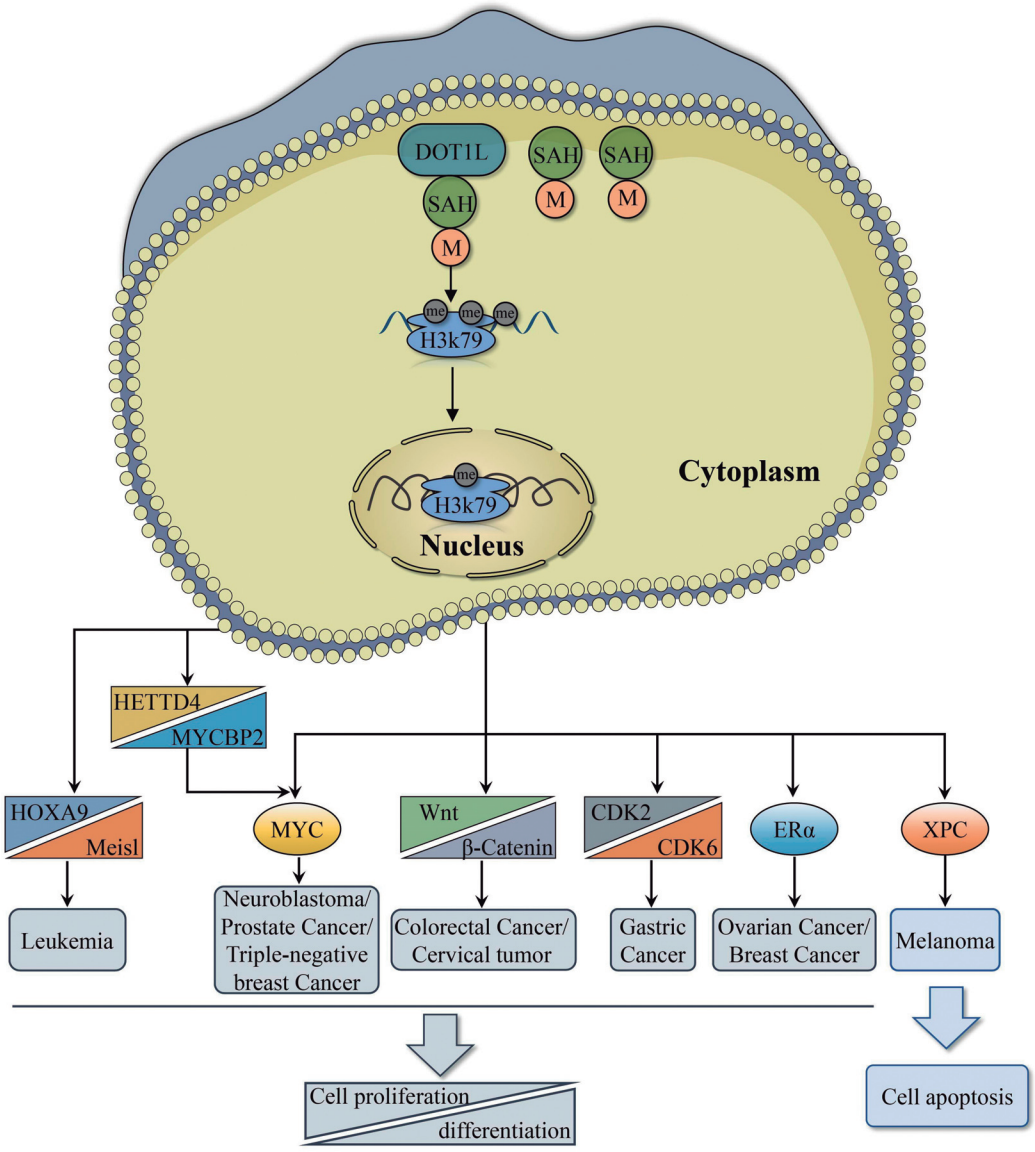


**Fig. 2.** DOT1L is a histone H3 lysine 79 (H3K79) methyltransferase that uses s-adenosylmethionine as a cofactor to unprocessed catalyze single, di- and trimethylation of H3K79. H3K79 methylation affects cell proliferation and differentiation by affecting transcriptional regulation, cell cycle progression, and DNA damage response, leading to tumor genesis and progression.

mouse chondrocytes after birth leads to reduced extracellular matrix production and destruction of growth plates, leading to developmental disorders in mice (Jo et al., 2020). In addition, hypoxia has been reported to increase the expression of DOT1L and methylation of H3K79 in chondrocytes by inducing hypoxia-inducing factor-1α (HIF1A), limiting the Wnt signaling pathway and thus preventing osteoarthritis (De Roover et al., 2021). New evidence suggests that DOT1L and H3K79me2 levels are upregulated during osteoclast differentiation. Inhibition of DOT1L increases osteoclast surface area, thereby reducing bone mass in mice (Gao and Ge, 2018). DOT1L is associated with cellular oxidative stress, inhibiting DOT1L to increase the production of pre-osteoclast reactive oxygen species (ROS) and autophagy activity, as well as osteoclast migration (Gao and Ge, 2018). These data suggest that the early expression of Dot1L in chondrocytes and osteoclasts provides necessary regulation of morphology, growth and stability of cartilage and bone, suggesting that Dot1L may become a new therapeutic target for cartilage and bone.

### Role of DOT1L in cardiovascular system

Cardiovascular developmental defects were observed in DOT1L knockout mouse embryos, suggesting that DOT1L may play a role in heart development (Pursani et al., 2018). Studies have shown that DOT1L may play a role in carotid intima. Huang et al. used balloon angioplasty to damage the common carotid artery in rats and induce neointima formation. DOT1L and its catalytic products H3K79me2 and H3K79me3 increased respectively, in injured (*versus* uninjured) carotid arteries at post-injury day 7 (Huang et al., 2020). In addition, Smooth muscle cells (SMC) proliferation is the main harmful factor promoting neointimal hyperplasia (IH). Either DOT1L silencing or inhibition in injured arteries can reduce proliferative marker proteins in smooth muscle cells, including proliferative cell nuclear antigen (PCNA) and cyclin D1. These results suggest that DOT1L plays a key role in epigenetic control of VSMC gene expression (Huang et al., 2020). Moreover, a feature of atherosclerosis is the formation of a nascent intima, that is, the proliferation of vascular smooth muscle cells (VSMCs). Inhibiting DOT1L in smooth muscle cells can directly regulate the methylation level of H3K79 in the NF-κB genomic region, thereby regulating the transcription of inflammatory factors CCL5 and CXCL10, reducing atherosclerosis burden and promoting lesion stability (Farina et al., 2022). AngII increases the viability and migration of cardiac fibroblast CFS and enhances the expression of fibrosis proteins (Chen et al., 2021). DOT1L is overexpressed in AngII-induced Myocardial infarction (MI) mice and CFS myocardial tissue. Suppression of DOT1L relieves myocardial fibrosis and eliminates activation of CFS. Mechanistic studies have shown that DOT1L mediates methylation of H3K79me2 on the (spleen tyrosine kinase) SYK promoter. Conversely, SYK upregulation reversed the inhibitory effect of DOT1L knockdown on CFS proliferation and fibrosis by activating the TGF-β1/Smad3 signaling (Li et al., 2022). As such, these studies present a strong rationale for continued mechanistic and translational investigation into DOT1L targeting for treatment of (re)stenotic vascular conditions.

### Role of DOT1L in nervous system

The proper function of the cerebellum is affected in various neurological diseases, including ataxia (Bhatia et al., 2022), schizophrenia (Yeruva et al., 2021), or Angelman syndrome (Maranga et al., 2020). In the germinal zones of the cerebellum there are cerebellar granule neurons (CGNs), which are one of the most abundant neuronal components of the central nervous system and receive the afferation and output of signals from different anatomical locations (Lanore et al., 2021). Bovio et al. have found that abnormal expression of DOT1L can lead to abnormal development of cerebellar granule cells. After knocking out DOT1L in mouse cerebellar granule cells, the outer granular layer became smaller and the precursors of granular neurons decreased. The proliferation and differentiation of granular progenitor cells in mouse after knockout DOT1L are impaired, resulting in smaller cerebellums, which exhibit mild ataxia (Bovio et al., 2019). Further studies have shown that DOT1L also has the effect of promoting the proliferation and development of progenitor cells in the cerebral cortex and the recognition of neuronal layers (Ferrari et al., 2020). Inhibition of DOT1L activity in NSCs in the cerebral cortex leads to cell proliferation and neuronal differentiation disorders. In addition, *in vitro*, DOT1L protects cortical neural stem cells from activation by ATF4-DDIT3-mediated endoplasmic reticulum stress (Roidl et al., 2016). During spinal cord development, DOT1L migrates and localizes dorsal and ventral interneurons by modulating transcriptional activation of Lhx2 (Gray et al., 2020). DOT1L is necessary for proliferation during retina regeneration. Kara et al. showed that inhibition of miR-216a upmodulates H3K79 methyltransferase DOT1L and activates Wnt/b-catenin signaling, thereby stimulating Müller glia reprogramming to promote cell proliferation (Kara et al., 2019). All in all, these results suggest that DOT1L activity is needed for the normal development and function of the cerebral cortex, cerebellum and spinal cord, providing new ideas for the treatment of neurologic lesions.

### Role of DOT1L in immune system

The immune system has the role of immune surveillance, defense, and regulation. This system consists of immune organs as well as immunoactive substances (Mccomb et al., 2019). The immune system is divided into innate immunity (also known as non-

specific immunity) and adaptive immunity (also known as specific immunity), of which adaptive immunity is divided into humoral immunity and cellular immunity (Ebrahimiyan et al., 2021). More recently, several reports have shown that epigenetic modifications can also modulate immunological and inflammatory processes, including innate immunity and antiviral responses (Chen et al., 2020b). The results showed that DOT1L expression was up-regulated when chicken macrophages (HD11 cells) were infected with chicken leukemia virus subset J (ALV-J). Targeting DOT1L with specific inhibitors can significantly reduce ALV-J replication (Chen et al., 2020a). Mechanically, ALV-J infection disrupts the activation of the IFN pathway mediated by inhibiting the expression of MDA5 (melanoma differentiation-related protein 5, MDA5) (Li et al., 2017), while knocking out DOT1L can preserve MDA5 and transcriptional activator 1 (STAT1), this tightly controls the innate immunity of the virus (Chen et al., 2020a). Kealy et al. found that knockout DOT1L in mice leads to defects in B cell development, ultimately leading to a decrease in peripheral mature B cells (Kealy et al., 2020). In addition, the lack of Dot1L in the B cells of mice can interfere with *in vivo* germination center (GC) establishment and the production of normal humoral immunity. From a mechanistic point of view, combined with epigenomics and transcriptomics analysis, DOT1L promotes the expression of proliferative and pro-GC procedures. In addition, DOT1L inhibits plasma cell differentiation by inhibiting the polycomb repression complex 2 (PRC2) target (Aslam et al., 2021). Cytotoxic T cell differentiation is guided by epigenomic adaptation. DOT1L marks the nucleosome core on the active gene, protecting the normal differentiation of CD8+ T cells. Mechanically, DOT1L controls CD8+ T cell differentiation by ensuring normal T cell receptor density and signaling. (Aslam et al., 2021). Furthermore, CD4+ T helper (Th) cell differentiation is controlled by lineage-specific expression of transcription factors and effector proteins, as well as silencing of lineage-promiscuous genes. Studies have shown that methyltransferase DOT1L promotes Th differentiation by activating CD4+ T cells and maintaining lineage integrity during infection and inflammation (Scheer et al., 2020). Taken together, DOT1L may be a central regulator of the immune system and might identify DOT1L as a potential therapeutic target for the treatment of diseases associated with immune dysregulation.

## Role of DOT1L in other systems

Brown and beige adipocytes are a type of thermogenic adipocyte that have great potential in the treatment of obesity and related metabolic diseases (Czech, 2020). Specific knockout of DOT1L in thermophilic fat cells leads to deinsistration of brown adipose tissue positive regulators, reduces obesity, improves homeostasis, and promotes adaptive thermogenesis *in vivo* (Shuai et al., 2021). Emerging evidence has suggested that loss of histone H3K79 methyltransferase DOT1L activates interstitial myofibroblasts and facilitates kidney fibrosis by upregulating endothelin1 through histone deacetylase 2 (Zhang et al., 2020). *DOT1L* gene expression was up-regulated during renal development in mice. DOT1L leads to defects in renal developmental regulators such as Lhx1, Pax2 and Notch through Six2, abnormal differentiation of renal progenitor cells, renal manifestations as congenital renal unit defects and cystic dysplasia nephropathy (Wang et al., 2021a). In addition, DOT1L was established as an epigenetic regulator of aging-associated secretion phenotype (SASP), whose expression is not coupled to aging-related cell cycle arrest. Mechanistically, H3K79 methyltransferase DOT1L is upregulated in Oncogene-induced senescence, and DOT1L mediates the occupation of IL1A site by H3K79me2/3, which is conducive to the subsequent expression of downstream SASP genes without changing other senescence phenotypes (Leon et al., 2021).

## Role of DOT1L inhibitors

Since the discovery of the key role of DOT1L in MLL leukemia, scientists have been committed to developing selective DOT1L inhibitors, which can not only be used as small molecule probes to study the biological function of DOT1L in cancer and other diseases, but also are expected to be further developed as novel anti-tumor drugs. Because DOT1L uses SAM as a cofactor to catalyze H3K79 methylation, most DOT1L inhibitors are mimics of SAM molecules (Chen and Park, 2019). These include compounds (EPZ004777 (Daigle et al., 2011), EPZ5676 (Waters, 2017) and SGC0946 (Cui et al., 2022)) that downregulate the MLL fusion target genes HOXA9 and MEIS1, selectively inhibit tumor MLL or MYC-driven cancer cell proliferation, and induce cell differentiation and apoptosis. However, these SAM-mimicking inhibitor compounds, which completely block DOT1L activity, lead to an increase in white blood cells and elevated levels of neutrophils, monocytes and lymphocytes, resulting in some side effects (Daigle et al., 2011). At the same time, it was reported that the DOT1L inhibitor EPZ5676 (Pinometostat) was used in the phase I clinical trial of MLL-r patients, and this study found that adverse events caused during treatment included febrile neutropenia (35%), cough (22%) and pneumonia (18%), and serious adverse reactions occurred in 35 patients (69%). The most common side effects may be related to infection, distributed as febrile neutropenia (25%), respiratory failure (12%), and pneumonia (10%), which may be due in part to decreased NF-κB activation caused by the drug, thereby preventing the induction of innate host responses and increasing pathogen amplification (Marcos-Villar and Nieto, 2019). These results suggest that patients with MLL-r leukemia may have an increased risk of infection when treated. Therefore, more

effective treatments, such as targeted therapies, which inhibit a protein that is essential for MLL-r leukemia but is dispensable for normal cells, are needed. In addition, the most commonly used treatment for AML patients, including those who develop MLL rearrangement, is currently chemotherapy with Ara-C and daunorubicin, or by hematopoietic stem cell transplantation (Lancet et al., 2021). Christine et al. (Klaus et al., 2014) studied the effect of DOT1L inhibitors combined with these chemotherapy drugs on the proliferation of MLL-r leukemia cells and found that the combination of EPZ5676 with Ara-C or daunorubicin produced strong synergistic antiproliferative activity in antiproliferative tests on MML-r leukemia cell lines MM4-11 and MOLM-13, which provided a theoretical basis for preclinical small molecule inhibitors combined with existing chemotherapy to treat mml-rearranged leukemia. At the same time, this also provides contemplation for the combination therapy of DOT1L inhibitors in other diseases.

## Conclusion

Using s-adenosylmethionine (SAM) as a cofactor, DOT1L unprocessed catalyzes the mono, di- and trimethylation of histone H3 lysine 79 (Wu et al., 2021). To date, H3K79 methylation is the only known histone lysine methylation without at least one corresponding histone demethyltransferase (Uğurlu-çimen et al., 2021). However, there is partial evidence that H3K79 methylation is reversible, for example, H3K79me2 levels in human cells can fluctuate with the progression of the cell cycle (Yang et al., 2022), but a loss of H3K79me2 has been observed during early development in Drosophila and mice (Shanower et al., 2005; Ooga et al., 2008). Previous studies have shown that monoubiquitination of histone H2B lysine 120 in these species directly stimulates DOT1L methyltransferase activity, promotes the methylation of H3K79 by DOT1L, and knocking out DOT1L leads to complete loss of H3K79 methylation, thereby affecting transcriptional regulation, cell cycle progression, and DNA damage response (Worden et al., 2019). Interestingly, studies have shown that DOT1L has less effect on embryonic stem cell self-renewal but is necessary to establish the correct expression signature of neural progenitor cells, while catalytic inactivation of DOT1L has less effect. Furthermore, DOT1L-controlled cell fate determination and transcriptional extension are independent of H3K79 methylation. This suggests that DOT1L can also perform catalytic functions independently of H3K79 methylation (Cao et al., 2020). Therefore, understanding the molecular mechanisms linking H3K79 methylation to intracellular interactions is critical to fully understanding the contribution of DOT1L and H3K79 methylation to genome function and integrity.

DOT1L is the only methyltransferase that can catalyze mono-, di- and tri-methylation of H3K79. DOT1L mediated H3K79 methylation is involved in tumor cell proliferation, development and immune response, whose processes are tightly regulated by the catalytic activity of DOT1L. We conclude that DOT1L is neither friend nor foe, but rather the obedient servant reacting to metabolic and environmental cues to benefit normal cells and promoting cell physiological development in normal physiological processes. However, during the tumor progression period, it tends to be upregulated, promoting the proliferation of cancer cells. However, the specific stimuli of DOT1L to promote normal development and malignant proliferation of cancer cells are still unknown. Studies have demonstrated that DOT1L expression is modulated by transcription factors, RNA, epigenetics modification, and indirect metabolic changes. Among them, we concluded that the type of metabolism the cell needs may be a critical factor affecting the function of DOT1L. Studies have found that glucose metabolism regulates the mechanism by which histone methylation affects the progression of malignant tumors through DOT1L (Song et al., 2021). In addition, oxygen-glucose deprivation reoxygenation-induced ubiquitin-modified enzyme cIAP1 (apoptosis inhibitor protein) promoter region H3K79me3 levels decreased (Wang et al., 2021c). Taken together, these findings allowed us to infer that glucose metabolism is a critical event in cell proliferation, assuming that DOT1L is a switch in this process.

Further understanding of the molecular processes of cell fate regulation must address the paradox of pro-proliferative effects of DOT1L in leukemic cells and its anti-proliferative effects in some cancer cells. Clarifying the potential connections among DOT1L, glycolysis, and cell proliferation, might solve this puzzle. DOT1L as a novel cell proliferation regulator and identification of underlying disease. Its therapeutic targets provide new avenues for unlocking cell proliferation abnormalities and differentiation in a variety of diseases. Given that DOT1L appears to be one of the key regulators of leukemia, the nucleoside DOT1L inhibitor EPZ5676 has been developed and successfully entered a Phase I clinical trial in 2013, certified as an "orphan drug" for the treatment of MLL-rearranged leukemia. In addition, non-nucleoside DOT1L inhibitors have been reported. In a broader context, the current results provide validation of small molecule inhibition of DOT1L as a treatment modality for cancer and other diseases in which genetic alterations in these epigenetic enzymes drive pathogenesis. Continued research into the pharmacological mechanisms of DOT1L inhibition will lead to new drugs for patients with DOT1L abnormal disease. In addition, according to the above literature, DOT1L inhibitors in combination with chemotherapy drugs may also provide additional benefits for patients with MLL-r leukemia. These preclinical data provide the basis for rational hypotheses that must ultimately be validated in human clinical trials, suggesting whether other chemotherapy drugs in combination with DOT1L inhibitors should be considered clinically for testing in other tumors or other diseases.

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