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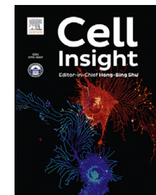
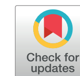


Review

NSD family proteins: Rising stars as therapeutic targets



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ABSTRACT

Epigenetic modifications, including DNA methylation and histone post-translational modifications, intricately regulate gene expression patterns by influencing DNA accessibility and chromatin structure in higher organisms. These modifications are heritable, are independent of primary DNA sequences, undergo dynamic changes during development and differentiation, and are frequently disrupted in human diseases. The reversibility of epigenetic modifications makes them promising targets for therapeutic intervention and drugs targeting epigenetic regulators (e.g., tazemetostat, targeting the H3K27 methyltransferase EZH2) have been applied in clinical therapy for multiple cancers. The NSD family of H3K36 methyltransferase enzymes—including NSD1 (KMT3B), NSD2 (MMSET/WHSC1), and NSD3 (WHSC1L1)—are now receiving drug development attention, with the exciting advent of an NSD2 inhibitor (KTX-1001) advancing to Phase I clinical trials for relapsed or refractory multiple myeloma. NSD proteins recognize and catalyze methylation of histone lysine marks, thereby regulating chromatin integrity and gene expression. Multiple studies have implicated NSD proteins in human disease, noting impacts from translocations, aberrant expression, and various dysfunctional somatic mutations. Here, we review the biological functions of NSD proteins, epigenetic cooperation related to NSD proteins, and the accumulating evidence linking these proteins to developmental disorders and tumorigenesis, while additionally considering prospects for the development of innovative epigenetic therapies.

1. Introduction

Epigenetic dysregulation, encompassing aberrant DNA methylation, histone modifications, and chromatin states, is a causative factor in various disorders, including developmental disease and diverse carcinomas (Cavalli & Heard, 2019; Millán-Zambrano et al., 2022; Zhao et al., 2021). The reversibility of epigenetic modifications makes them promising targets for therapeutic intervention, and this recognition has spurred substantial efforts towards the development of drugs targeting these modifiers. Noteworthy achievements include the approval by the US FDA of several epigenetic drugs for clinical application, including azacitidine, an inhibitor of DNMTs (DNA methyltransferases) employed in treating MDS (myelodysplastic syndromes), tazemetostat targeting H3K27 methyltransferase EZH2 for epithelioid sarcoma, and belinostat, an inhibitor of HDACs (histone deacetylases) used in peripheral T-cell lymphoma (Hoy, 2020; Kaminskas et al., 2005; Lee et al., 2015).

The nucleosome, serving as the fundamental functional unit of chromatin, comprises a histone octamer with two copies of each core histone (H2A, H2B, H3, and H4) and enfolds approximately 147 base pairs of DNA (Tessarz & Kouzarides, 2014). Chromatin can be subdivided into heterochromatin and euchromatin, respectively representing a highly condensed and transcriptionally repressed state or a relatively open and transcriptionally active state (Millán-Zambrano et al., 2022). The transition between these two states is regulated by reversible covalent modifications including DNA methylation and histone modifications including methylation, acetylation, and ubiquitination (Zhao et al., 2021).

Histone methylation, a prevalent epigenetic modification, exerts a profound influence on chromosome accessibility, gene transcription, and genome stability. The levels of methylation are controlled by the action

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of methyltransferases ('writers') and demethylases ('erasers'). Additionally, distinct effector proteins ('readers') recognize specific methyllysines. The specific impacts of histone methylation marks are linked to the degree of histone methylation, the specific sites modified within the histones, and the position of the target nucleosome in the genome (Topchu et al., 2022). Previous studies have illustrated associations between specific methylation marks and transcriptional states. For instance, H3K4me3, H3K36me2, H3K36me3, H3K79me3, and H4K20me1 are often linked to transcriptional activation, while H3K9me3 and H3K27me3 are correlated with transcriptional repression (Millán-Zambrano et al., 2022). Histone methyltransferase enzymes are subdivided into two groups: the SET (su (var)3–9, enhancer of zeste and trithorax) domain containing proteins (e.g., NSD1/2/3, EZH1/2, and G9a) and the 7βS (seven-beta-strand) domain containing proteins (e.g., DOT1L and KMT9) (Husmann & Gozani, 2019).

H3K36 is a residue located on the histone tail, and is susceptible to mono-, di-, or tri-methylation. H3K36me1 is broadly distributed in the genome, signifying an intermediate modification without exerting a direct transcriptional regulatory function. In contrast, both H3K36me2 and H3K36me3 are associated with active transcription, with H3K36me2 concentrated in intergenic and regulatory regions, while H3K36me3 is predominantly located in intragenic regions (Li et al., 2019; Topchu et al., 2022). The NSD (nuclear receptor–binding SET domain) family proteins, including NSD1 (KMT3B), NSD2 (WHSC1/MMSET), and NSD3 (WHSC1L1) have been identified as enzymes for mono- and di-methylation of H3K36 (Husmann & Gozani, 2019). In humans, dysregulation of NSD proteins has been associated with developmental defects and cancers (Husmann & Gozani, 2019), prompting the development of an increasing number of inhibitors specifically designed to target NSD proteins (Ma et al., 2023; Shrestha et al., 2021). Notably, KTX-1001, a specific inhibitor of NSD2 targeting the SET domain, has progressed into the Phase I clinical trials to treat relapsed or refractory multiple myeloma as of February 2023 (ClinicalTrials.gov identifier: NCT05651932) (Ma et al., 2023). This review presents the known physiological and pathological impacts of NSD-catalyzed H3K36 methylation and considers current and potential strategies for targeting NSD family proteins.

2. The domain organization and enzymatic activities of NSD proteins

In humans, the genes encoding NSD proteins are respectively located on chromosome 5q35.3 (NSD1), 4p16.3 (NSD2), and 8p11.23 (NSD3) (Topchu et al., 2022), and alternative splicing gives rise to isoforms for each of the NSD proteins (Fig. 1). The NSD proteins contain an enzymatic SET domain facilitating the transfer of methyl groups from SAM to the substrate histone (Topchu et al., 2022) they also have domains including a HMG (high-mobility-group) box, PWWP (proline-tryptophan--tryptophan-proline) domains, and PHD (plant homeodomain) zinc fingers, which are involved in NSD proteins' interactions with chromatin and partner proteins (Topchu et al., 2022; Vougiouklakis et al., 2015).

The HMG box is able to bind DNA in a non-sequence-specific manner and enhance NSD2's affinity to DNA (Murphy et al., 1999). The PWWP domain exerts dual functions: it can bind both DNA and methyl-lysine of histones (Wu et al., 2011). The PHD-C5HCH module folds into a PHD-PHD-like structure, with H3 peptide binding on the surface of PHD, providing the basis for the recognition of unmodified H3K4 and tri-methylated H3K9 by NSD3 (He et al., 2013). Upon translation, NSD methyltransferases adopt an auto-inhibitory state, which is sustained by a loop connecting the SET and post-SET domain (Li et al., 2021; Qiao et al., 2011). The PHD zinc finger of NSD proteins can recognize histone-lysine marks and is located closely with the SET domain and the lysine substrates, leading to DNA unwinding and allowing the SET domain to bind DNA, which releases the auto-inhibitory state and enables methylation of H3K36 (Li et al., 2021). Beyond catalyzing H3K36 methylation, studies have shown that RE-IIBP (another isoform of NSD2) has an altered substrate preference for H3K79 and H3K27, in HEK-293T and HeLa cells, respectively (Kim et al., 2008; Woo Park et al., 2015). WHISTLE, another NSD3 isoform, preferentially catalyzes methylation of H3K4 and H3K27 in NIH3T3 cells (Kim et al., 2006) (Fig. 1).

3. The interactions of NSD proteins with other epigenetic modifiers

It is now clear that the combinatorial arrangement of histone modifications creates a "histone code", considerably expanding the informational capacity beyond that of the genetic code (Margueron et al., 2005). Epigenetic crosstalk amongst DNA methylation and histone modifications—including exclusion relationships—dictate dynamic transitions between transcriptionally active or transcriptionally silent chromatin states (Greenberg & Bourc'his, 2019; Millán-Zambrano et al., 2022). Clarifying the mechanisms underlying interactions between NSD proteins and partners responsible for other modifications (e.g., H3K27 methylation, H3K27 acetylation, and H2AK119 ubiquitination) can deepen our understanding of NSD proteins' biological functions and provide insights for identifying potential drug targets. The following

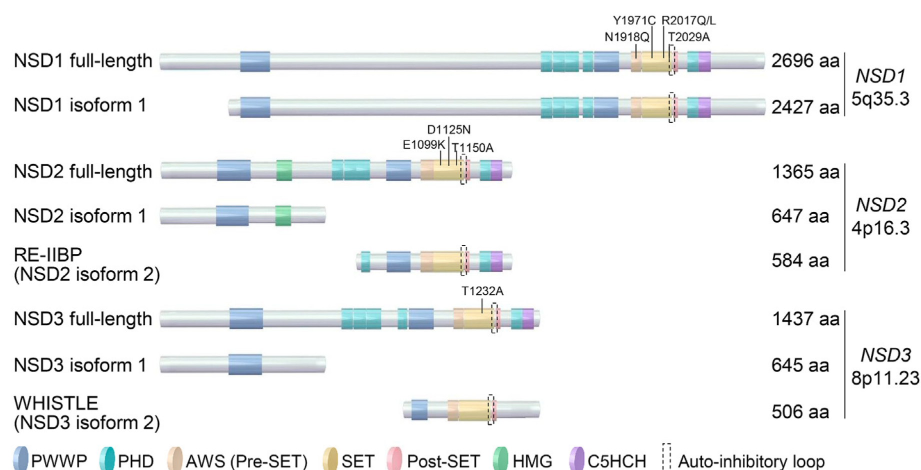

Fig. 1. The domain structures of NSD proteins and high-frequency mutations in human disease. PWWP, proline-tryptophan-tryptophan-proline domain; PHD, plant homeodomain; AWS, associated with SET domain; SET, suppressor of variegation, enhancer of zeste, and trithorax domain; HMG, high-mobility-group box; C5HCH, Cys-His-rich domain.

content presents known interactions between NSD proteins and various epigenetic regulators.

3.1. Histone methyltransferases

It has been reported that NSD-catalyzed H3K36me2 does not co-occur with H3K27me3 (a marker of transcriptional repression) at most chromosomal loci (Fig. 2A) (Popovic et al., 2014; Schmitges et al., 2011; Shirane et al., 2020; Streubel et al., 2018; Yuan et al., 2011). EZH2, the catalytic subunit of PRC2 (polycomb repressive complex 2), is known to deposit H3K27 methylation (with an unmodified H3K36 accommodated in the EZH2-DNA interface) (Finogenova et al., 2020). The existence of NSD-catalyzed H3K36me2 permits allosteric inhibition of EZH2 by impeding the interaction between the active site of EZH2 and H3K27, which prevents H3K27me3 deposition and maintains transcriptionally active states. Such regulation has been detected in mesenchymal progenitor cells in sarcomas (Lu et al., 2016).

3.2. Histone acetyltransferases

Recent studies have revealed that NSD2 overexpression leads to expansion of H3K36me2, which increases chromatin accessibility, thus promoting the binding of the genome organizer CTCF (CCCTC-binding factor) and various transcription factors (e.g., AP-1). Increased CTCF binding in the genome can weaken compartmentalization, and AP-1 can recruit p300/CBP to catalyze H3K27 acetylation, making chromatin more accessible (Fig. 2B), which in turn drives compartment switching from B (closed chromatin) to A (open chromatin) and promotes the expression of oncogenes, for example during the development of multiple myeloma (Lhoumaud et al., 2019).

3.3. Histone deubiquitinases

A recent study of myelomagenesis reported that NSD2 interacts with the proteasome component PSMD14 on chromatin and that NSD2-directed H3K36me2 and PSMD14-catalyzed H2AK119 deubiquitination are functionally coordinated during the transcriptional activation of target genes linked to NF-κB (IκB kinase/nuclear factor κB) signaling (Fig. 2C) (He et al., 2023). Consistently, previous structural analyses have noted the close spatial proximity of H2AK119 to H3K36 within the nucleosome core particle (Bi et al., 2016; Di Croce & Helin, 2013), and it is known that the H3K36me2 writer activity of NSD proteins is reduced in the context of elevated H2AK119ub levels (Li et al., 2021; Sato et al., 2021; Yuan et al., 2013).

3.4. DNA methyltransferases

Beyond histone modifiers, NSD1 has been reported to recruit the DNA methyltransferase DNMT3A to intergenic regions, thereby contributing to the maintenance of DNA methylation (Fig. 2D) (Xu et al., 2020). Genome-wide analyses have revealed that DNMT3A colocalizes with NSD1-catalyzed H3K36me2 at non-coding regions of euchromatin, and the PWWP domain of DNMT3A is known to recognize NSD1-catalyzed H3K36me2 (Dukatz et al., 2019; Weinberg et al., 2019).

4. Roles of NSD proteins in cell differentiation and development

Cell identity is attained by establishing and sustaining specific gene expression profiles through the orchestration of lineage-specific transcription factors and epigenetic regulation (Cheng et al., 2020; Furlan et al., 2023). Specifically, NSD proteins have well-established functions in multi-lineage differentiation (Barral et al., 2022; Sun et al., 2023). The following content presents the known impacts of NSD proteins on chondrogenic differentiation, erythroid differentiation, lymphocyte differentiation, and gametogenesis.

4.1. Chondrogenic differentiation

Both NSD1 and NSD2 have been linked to chondrocyte differentiation of mesenchymal progenitor cells (Lu et al., 2016; Shao et al., 2023). NSD1 promotes expression of SOX9 and OSR2, transcription factors known to function in chondrogenic differentiation, specifically by depositing H3K36me1 and H3K36me2 at promoters. NSD1 deficiency impedes chondrogenic differentiation, thus inhibiting skeletal growth and fracture healing, and increasing the risk of osteoarthritis (Shao et al., 2021, 2023). Studies of mouse mesenchymal cells *in vitro* have shown that knockout of Nsd1 and Nsd2 resulted in a decreased (genome-wide) H3K36me2 level, leading to redistribution of H3K27me3 and ultimately suppressing the transcription of multiple genes linked to regulation of mesenchymal differentiation (Lu et al., 2016; Rajagopalan et al., 2021).

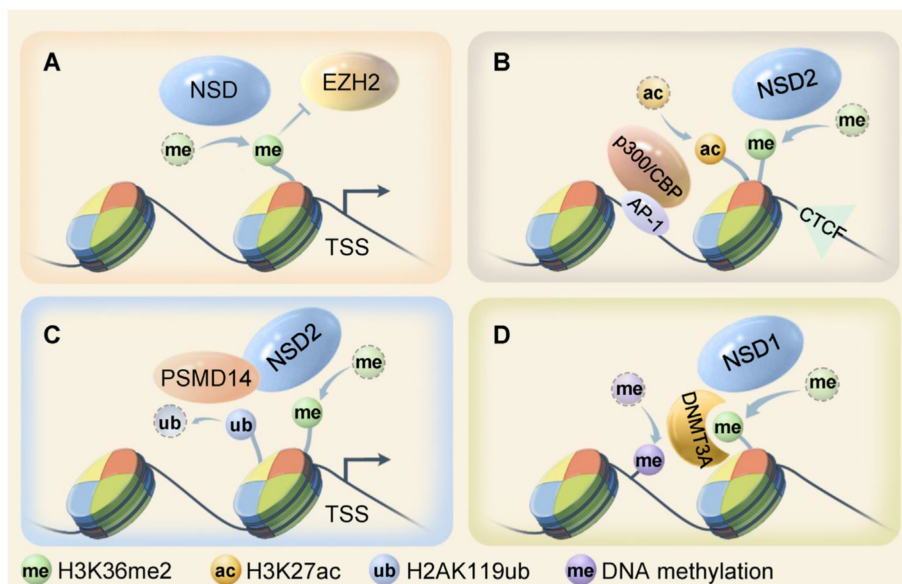

Fig. 2. Interactions between NSD proteins and other epigenetic modifiers. Histone methylation, histone acetylation, histone ubiquitination, and DNA methylation are regulated through the interplay between NSD proteins and other epigenetic modifiers. (A) NSD protein-catalyzed H3K36me2 prevents EZH2-catalyzed H3K27me3. (B) NSD2-deposited H3K36me2 provides a preferential environment for transcription factor (e.g., AP-1) and CTCF binding. AP-1 can recruit p300/CBP to catalyze H3K27ac and CTCF weakens chromatin compartmentalization, which regulates the 3D genome and promotes gene transcription. (C) NSD2 interacts with PSMD14 as well as NSD2-directed H3K36me2 and PSMD14-catalyzed H2AK119 deubiquitination products, which are functionally coordinated to promote gene transcription. (D) NSD1-catalyzed H3K36me2 recruits DNMT3A to intergenic regions and contributes to the maintenance of DNA methylation. TSS, transcription start site.

4.2. Erythroid differentiation

The transcription factor GATA1 is well-known to promote erythroid development by upregulation of erythropoiesis-related multiple genes (e.g., *Hbb* and *Alas2*) (Moriguchi & Yamamoto, 2014). It has been reported that reduced expression of NSD1 suppresses terminal erythroid maturation (Tauchmann et al., 2020). Causally, NSD1-catalyzed H3K36 methylation can inhibit interactions between GATA1 and multiple transcriptional corepressors including SKI and NCOR1/2, increasing GATA1 occupancy on promoters of target genes linked to erythropoiesis (Leonards et al., 2020). Recent studies have shown that alteration of DNA methylation patterns impacts the hematopoietic differentiation landscape, and GATA1 may physically interact with DNMT3A (Izzo et al., 2020; Leonards et al., 2020), indicating that an NSD1-H3K36 methylation-DNMT3A-DNA methylation axis may contribute to GATA-regulated erythroid differentiation.

4.3. Lymphocyte differentiation

NSD1 has been shown to promote B cell development and GC (germinal center) formation (Zhai et al., 2022). Loss of NSD1 leads to increased expression of multiple genes linked to germinal center formation and lymphopoiesis (e.g., *Rap1b* and *Arid3a*), thus activating BCR (B cell receptor) signaling and promoting B1 cell differentiation in the peritoneal cavity and spleen (Zhai et al., 2022). In the early stages of T cell activation, CD28-induced upregulation of NSD2 has been shown to stimulate expression of BCL6, a master regulator of Tfh (follicular helper T) cells generation, thus promoting Tfh cell differentiation (Long et al., 2020).

4.4. Gametogenesis

NSD1-catalyzed H3K36me2 is known to be required for *de novo* DNA methylation in mouse prospermatogonia, with NSD1 deficiency leading to widespread reduction of DNA methylation and defects in spermatogenesis (Shirane et al., 2020). In oocytes, NSD1 lacks the N-terminal PWWP domain, so H3K36me2/3 in those cells is dependent on SETD2 (Xu et al., 2019). Accordingly, there are distinct patterns of DNA methylation between mouse prospermatogonia and oocytes, with over 80% of the genome methylated in mature sperm vs. approximately 40% in oocytes (Shirane et al., 2020).

5. Dysregulation of NSD proteins in human disease

Genetic abnormalities of *NSD* genes involving mutations, amplifications, and fusions are frequently detected in human diseases including developmental disorders and neoplasms (in both solid tumors and hematological malignancies) (Li et al., 2019; Morishita & di Luccio, 2011; Vougiouklakis et al., 2015). Understanding the pathological implications of dysregulated NSD proteins can guide development of innovative and potentially individualized cancer therapies. The following content focuses on the dysregulation of NSD proteins in developmental disorders, solid tumors, and hematological malignancies.

5.1. Developmental disorders

NSD1 functions as an enhancer-enriched coactivator, initiating developmental transcriptional programs linked to the multi-lineage differentiation of embryonic stem cells (e.g., towards nervous, cardiovascular, and genitourinary fates) (Sun et al., 2023). Loss-of-function mutations (Fig. 1) or deletions of *NSD1* have been identified in neurodevelopmental disorders or overgrowth disorders, including Sotos syndrome and Beckwith-Wiedemann syndrome (Baujat et al., 2004; Tatton-Brown & Rahman, 2007). It has been reported that H3K36me2 regulates the distribution of DNMT3A-deposited DNA methylation in neocortices, particularly non-CG types, facilitating the expression of neuronal genes linked to axon guidance, ion transport, and synapse assembly, while simultaneously repressing non-neural genes (e.g., those implicated in skeletal muscle, pancreas, and kidney development) (Zheng et al., 2023). NSD1 depletion in neocortex leads to alterations in all four regions and to a rewiring of cortico-thalamic-cortical circuits, causing discernible defects in spatial memory, motor learning, and coordination (Hamagami et al., 2023; Zheng et al., 2023). *NSD1* duplication has been detected in a growth retardation disorder known as Silver-Russell syndrome (Sachwitz et al., 2017). A previous study reported that Nsd2 coordinates with cell-type-specific transcription factors (e.g., Sall1, Sall4, and Nanog) in embryonic stem cells, as well as Nkx2-5 in embryonic hearts, to regulate developmental programs (Nimura et al., 2009). *NSD2* haploinsufficiency has been associated with WHS (Wolf-Hirschhorn syndrome), with the relevant cases characterized by a distinctive craniofacial phenotype and growth restriction (Wiel et al., 2022). Additionally, frameshift mutations in *NSD2* have been linked to Rauch-Steindl syndrome, a development delay disorder with characteristic facial features that is distinct from WHS (McConkey et al., 2022).

5.2. Solid tumors

Dysregulation of NSD proteins has been reported in various solid tumors, including HNSCC (head and neck squamous cell carcinoma), LUSC (lung squamous cell carcinoma), LUAD (lung adenocarcinoma), HCC (hepatocellular carcinoma), breast cancer, prostate cancer, and colorectal cancer (Brennan et al., 2017; Farhangdoost et al., 2021; Topchu et al., 2022; Zhang et al., 2019). In HNSCC and LUSC, inactivating mutations of NSD1 (Fig. 1) contribute to promoting tumorigenesis and inhibiting anti-tumor immunity (Brennan et al., 2017; Choufani et al., 2015; Farhangdoost et al., 2021). Overexpression of NSD1 can promote cell proliferation, migration, and invasion by activating the Wnt/β-catenin signaling pathway during HCC tumorigenesis (Zhang et al., 2019). Aberrantly high NSD2 levels have been reported to promote tamoxifen resistance in breast cancer, specifically through metabolic reprogramming of glycolysis and the pentose phosphate pathway (Wang et al., 2016). In prostate cancer, elevated NSD2 levels have been associated with an immunosuppressive microenvironment and with an altered EMT (epithelial-mesenchymal transition) and metastasis (Aytes et al., 2018; Ezponda et al., 2013; Want et al., 2021). Notably, NSD2 can increase the expression of MHC-I, thereby promoting antigen presentation and T cell infiltration, and downregulation of NSD2 is associated with impaired antitumor immunity in colorectal cancer (Ren et al., 2022). A hyperactive mutant $NSD2^{E1099K}$ has been reported to promote LUAD by initiating multiple oncogenic transcriptional programs, including activating KRAS signaling (Sengupta et al., 2021) (Fig. 1). Elevated NSD3 has been shown to transcriptionally activate cell cycle-related genes (e.g., *CDC6* and *CDK2*), which can promote cell proliferation and survival in HNSCC (Saloura et al., 2016). *NSD3* amplification is one of the more common molecular alterations in LUSC (Yuan et al., 2021). The clinically relevant $NSD3^{T1232A}$ mutant variant, which exists a relieved auto-inhibitory state (with increased catalytic activity) (Fig. 1), has been shown to contribute to LUSC progression by promoting the expression of multiple oncogenes involved in mTOR signaling and MYC-associated pathway (Yuan et al., 2021). NSD3 has also been reported to undergo translocations, giving rise to carcinogenic fusion proteins such as NSD3-NUT in midline carcinoma (French et al., 2014).

5.3. Hematological malignancies

Loss of *NSD1* can drive acute erythroblastic leukemia pathogenesis (Leonards et al., 2020). Mutations in *NSD2* have also been reported in various malignancies, including ALL (acute lymphocytic leukemia), MM (multiple myeloma), and MCL (mantle cell lymphoma) (Fig. 1). In ALL, an E1099K mutation in the SET domain of NSD2 has been shown to contribute to hyperactivation by destabilizing the auto-inhibitory loop, which can maintain active chromatin compartments and further affect

the 3D genome, promoting transformation and glucocorticoid resistance (Jaffe et al., 2013; Li, Hlavka-Zhang, et al., 2022; Narang et al., 2023; Oyer et al., 2014; Swaroop et al., 2019). The E1099K mutation of NSD2 was also detected in MM and MCL, conferring a proliferation advantage in all these disease contexts (Beà et al., 2013; Jain & Wang, 2019; Oyer et al., 2014). There are also reports that the D1125N and T1150A mutations of NSD2's SET domain can enhance interactions with nucleosomes, resulting in increased NSD2 enzymatic activity and tumorigenesis (Azagra & Cobaleda, 2022; Beà et al., 2013; Jaffe et al., 2013).

Studies of hematological malignancies have detected deleterious impacts from chromosome translocations of *NSD* genes. Chromosome rearrangement and *IgH* enhancer hijacking in MM have been linked to *NSD2* upregulation, with the aberrantly high NSD2 levels apparently driving myelomagenesis (Manier et al., 2017). In AML (acute myeloid leukemia), the recurrent t(5;11) (q35;p15.5) translocation is understood as a driver event resulting in the fusion of NSD1 with NUP98 (nucleoporin 98) (Cerveira et al., 2003; Wang et al., 2007). NUP98-NSD1 has been demonstrated to bind regulatory elements of the proto-oncogenes (e.g., *HOXA* and *MEIS1*), maintaining H3K36 methylation and recruiting p300/CBP to catalyze histone acetylation, thus promoting gene expression and immortalization of myeloid progenitor cells (Wang et al., 2007). The NUP98's FG (phenylalanine-glycine) repeat domains is responsible for formation of NUP98-NSD1 nuclear condensates, the constituents of which include SMARCA5, a nucleosome remodeling factor complex member. The interaction between NUP98-NSD1 and SMARCA5 contributes to the maintenance of the altered cellular characteristics in hematopoietic cells (Jevtic et al., 2022). Notably, a study of radiation-associated MDS showed that a translocation resulted in generation of a NUP98-NSD3 fusion protein, although the function of the fusion protein remains unclear (Taketani et al., 2009). Despite the distinct chromosomal locations of NSD1 (5q35.3) and NSD3 (8p11.23), both NSD1 and NSD3 form fusions with the same "fusion partner", NUP98. Remarkably, MDS is characterized by the abnormal proliferation and differentiation of hematopoietic stem cells, with the potential to progress to AML (Li, Hu, et al., 2022). Further investigation into the biological functions of these two fusion proteins is warranted.

6. NSD proteins as therapeutic targets

As mentioned above, NSD proteins are associated with a variety of malignancies and have become targets for drug development. However, to date there are no small molecule inhibitors of NSD proteins approved by the US FDA (Shrestha et al., 2021). Beyond small molecule inhibitors, oligopeptides, PROTACs (proteolysis-targeting chimeras), and nucleic acid agents are under investigation for targeting NSD proteins (Table 1). The following content presents the development of diverse NSD inhibitors.

6.1. Small molecule inhibitors

The development of small molecules inhibitors for NSD proteins is challenging, owing to the auto-inhibitory conformation of the enzymatic SET domains. Additionally, achieving ligand selectivity among NSD proteins is difficult owing to their conserved catalytic SET domain (Huang et al., 2020; Shrestha et al., 2021). Finally, difficulty in obtaining crystal structures capturing complexed catalytic domain inhibitors—and the attendant lack of understanding about lead compound structures and binding modes—also limited the development of NSD inhibitors (Zhang & Zha, 2023).

6.1.1. Targeting the SET domain

By targeting the SET domain, the enzymatic activity of NSD proteins can be inhibited by either interfering with the binding site for the cofactor SAM or disrupting the histone tail binding pocket (Zhang & Zha, 2023). Sinefungin and MCTP-39 exhibit structural similarities to SAM, displaying both competitive and non-specific inhibitory activity against

Table 1
The selective inhibition for NSD proteins.

Type	Target	Specificity	Name	References
Small molecule inhibitors	SET domain	Non-specific	Sinefungin	Tisi et al. (2016)
			MCTP-39	Chinnaiyan et al. (2014)
			BIX-01294	Morishita et al. (2017)
		NSD2	DA-3003-1	Coussens et al. (2018)
			PF-03882845	Coussens et al. (2018)
			Chaetocin	Coussens et al. (2018)
			TC LPA54	Coussens et al. (2018)
			ABT-199	Coussens et al. (2018)
			LEM-06	di Luccio (2015)
			LEM-14	Shen et al. (2019)
	PWWP1 domain	NSD2	MR837	Ferreira de Freitas et al. (2021)
			UNC6934	Dilworth et al. (2022)
			Compound 38	Li, Yang, et al. (2022)
		NSD3	BI-9321	Böttcher et al. (2019)
	PHD zinc finger	NSD1	Mitoxantrone	Berardi et al. (2020)
			Quinacrine	Berardi et al. (2020)
			Chloroquine	Berardi et al. (2020)
Oligopeptides	SET domain	NSD2	PTD2	Morrison et al. (2018)
PROTACs	PWWP1 domain	NSD2	MS159	Meng et al. (2022)
			UNC8153	Hanley et al. (2023)
			UNC8732	Nie et al. (2023)
		NSD3	MS9715	Xu et al. (2022)
Nucleic acid agents	mRNA	NUP98-NSD1	LNP/siRNA formulation	Mohanty et al. (2020)

NSD proteins (Chinnaiyan et al., 2014; Tisi et al., 2016; Zhang & Zha, 2023). BIX-01294 exerts an inhibitory effect on NSD proteins by targeting the histone tail binding pocket within the SET domain (Morishita et al., 2017).

NSD2 mutants are frequently detected in malignancies, and five small molecule inhibitors (DA-3003-1, PF-03882845, chaetocin, TC LPA54, and ABT-199) have been found to exert similar inhibitory effects on NSD2 wild-type and mutants (e.g., E1099K and T1150A). Unfortunately, all five compounds show poor selectivity, inhibiting several other methyltransferases, including NSD1/3 and SETD2 (Coussens et al., 2018; Zhang & Zha, 2023). Based on the aforementioned nonspecific inhibitor BIX-01294 and docking simulation studies, two specific inhibitors of NSD2 have been developed (LEM-06 and LEM-14); these exert minimal inhibitory effects on NSD1 and NSD3; however, as the IC_{50} (half maximal inhibitory concentration) of LEM-06 and LEM-14 are 0.8 mM and 132 μM *in vitro*, respectively, they appear unsuitable for clinical application against malignancies (di Luccio, 2015; Shen et al., 2019).

6.1.2. Targeting the PWWP1 domain

Targeting domains that regulate NSD-protein/DNA interactions is also a strategy to develop NSD inhibitors. The N-terminal PWWP1 domain preferentially binds H3K36me2, and this interaction stabilizes NSD proteins on chromatin, providing a promising target for inhibition

(Zhang et al., 2021). Previous studies have reported that small molecules including MR837, UNC6934, and compound 38, can bind the PWWP1 domain of NSD2 and block its interaction with H3K36me2 (Dilworth et al., 2022; Ferreira de Freitas et al., 2021; Li, Yang, et al., 2022). BI-9321 targeting the PWWP1 domain of NSD3, can interfere with its binding methylated lysine and inhibit the growth of AML cells *in vitro* (Böttcher et al., 2019).

6.1.3. Targeting the PHD zinc finger

Intervention strategies targeting the PHD zinc finger of NSD proteins are also under development. NMR (nuclear magnetic resonance) and spectral analysis have indicated that the PHD zinc finger of NSD1 can be disturbed by three compounds, which reduce the interaction of NSD proteins with the zinc finger domain of the transcriptional repressor NIZP1. The compounds include a type II topoisomerase inhibitor mitoxantrone, which has been used to treat AML, and two antimalarial drugs, quinacrine and chloroquine, which have been considered as anticancer agents (Berardi et al., 2020). However, the specific targeting of NSD1 by these compounds *in vivo* remains to be further investigated.

6.2. A peptide inhibitor

Previous studies have reported that NSD2 acts as a di-methyltransferase towards H3K36 when presented with nucleosomes, but it preferentially di-methylates H4K44 when presented with octamers (Li et al., 2009). Screening of a peptide library covering histone protein sequences revealed that PTD2 (a norleucine-containing peptide derived from the histone protein H4 sequence surrounding residue K44) is an inhibitor targeting NSD2. PTD2 exerts inhibitory activities against both NSD2 and NSD3, with IC_{50} values of 22 ± 2 μM and 3.2 ± 0.2 μM, respectively (Morrison et al., 2018).

6.3. PROTACs

PROTAC strategies have been explored for the targeted degradation of NSD proteins. There are small molecules known to selectively bind the PWWP1 domain of NSD proteins with high affinity and to inhibit binding H3K36me2-containing nucleosomes, yet which fail to disturb NSD enzymatic activity. These inhibitors were used in the development of PROTACs (Hanley et al., 2023; LegaardAndersson et al., 2023; Meng et al., 2022; Nie et al., 2023; Sun et al., 2022; Xu et al., 2022).

UNC6934 is a high-affinity and selective NSD2 binder; based on the structure of UNC6934, MS159 was developed to degrade NSD2 in a CRBN- (a E3 ubiquitin ligase component) and proteasome-dependent manner (Meng et al., 2022). UNC8153 (derived from UNC6934) specifically targets NSD2 dependent on a Cullin-RING family E3 ubiquitin ligase; and the degradation efficiency and durability of UNC8153 to NSD2 is enhanced compared to UNC6934 (Hanley et al., 2023). UNC8732 can promote FBXO22-mediated NSD2 degradation in ALL harboring E1099K mutation in NSD2, further inhibiting proliferation and reversing drug resistance (Nie et al., 2023).

Regarding NSD3 degradation, a study reported that linking an NSD3 antagonist (BI-9321, which binds its PWWP1 domain) with an E3 ligase VHL ligand (MS9715) can effectively reduce the growth of NSD3-dependent hematological cancer cells (Xu et al., 2022). PROTACs thus appear promising for the specific degradation of NSD proteins. Research to optimize characteristics including target protein ligands, linkers, and E3 ligase ligands would advance PROTAC-based strategies to modulate NSD protein activity in the context of various disorders.

6.4. Nucleic acid agents

A study reported that a LNP (lipid nanoparticle)/siRNA formulation targeting NUP98-NSD1 fusion gene can prolong the survival of AML PDX (patient-derived xenograft) mice. As it spans the translocation junction of NUP98-NSD1, and given that such fusions appear only in leukemic cells, the LNP/siRNA approach does not obviously affect the normal hematopoiesis, making it a promising approach to disturb malignancies with fusion genes (Mohanty et al., 2020).

Given the instability and defects of targeting specific cells with siRNA *per se* (Hu et al., 2020), further studies should focus on modifications and on delivery systems to advance such approaches towards clinical relevance. Additionally, given that loss of function mutation or haploinsufficiency of NSD proteins is closely associated with developmental disorders (Tatton-Brown & Rahman, 2007; Wiel et al., 2022), and considering that mRNA-based therapeutics are becoming a powerful strategy for a variety of diseases (Qin et al., 2022), further studies might focus on developing mRNA drugs to directly upregulate the expression of NSD proteins.

6.5. Targeting NSD-interacting proteins

Recent studies have indicated the involvement of NSD proteins in phase separation, although the underlying mechanism(s) remain unclear (Liu et al., 2021; Peng et al., 2023). Further elucidating the LLPS (liquid-liquid phase separation) properties and identifying the IDRs (intrinsically disordered regions) of NSD proteins would likely offer valuable insights to guide the development of inhibitor agents. Studies have also shown that the BET (bromodomain and extraterminal) protein BRD4, which binds acetylated histones, can recruit NSD3 to chromatin, and BRD4 depletion results in reduced NSD3-catalyzed H3K36 methylation (Rahman et al., 2011). Exploiting this molecular understanding, bromodomain inhibitors (e.g., ZEN003694) have been developed to reduce NSD3 modification activity. Notably, ZEN003694 has been advanced into Phase II clinical trials for treating a subset of LUSC cases featuring NSD3 amplification or mutation in November 2022 (ClinicalTrials.gov identifier: NCT05607108). This advancement underscores the potential of targeting epigenetic regulators that interact with NSD proteins as a promising therapeutic strategy for addressing malignancies associated with NSD abnormalities.

7. Perspectives

Despite the homologous protein structures and similar H3K36 methylation functions of NSD proteins, it remains unclear whether (and to what extent) they have redundant function(s) depending on specific cellular contexts. Loss-of-function mutations and deletions of NSD proteins are frequently observed in development disorders; however, in tumors, gain-of-function mutations, amplifications, and fusions of NSD proteins are common and are known to promote oncogenesis. Obtaining a deeper understanding of the complex interplay between NSD proteins and other epigenetic modifiers in normal and cancer cells would likely provide insights to inform the development of innovative therapeutic interventions. Advances with protein-protein interaction inhibitors could also inform drug discovery efforts targeting NSD proteins. A particularly exciting recent development is the elucidation of a positive feedback loop that continuously activates the enzyme activity of NSD2 and NSD3 (Jiang et al., 2022; Saloura et al., 2017). The auto-inhibitory state of NSD proteins is known to be released to catalyze H3K36 methylation upon binding chromatin (Li et al., 2021). Thus, better understanding the upstream mechanism(s) regulating the expression or/and enzyme activity of NSD proteins would almost certainly aid the development of specific NSD inhibitors.

Dysregulation of NSD proteins is closely related to human diseases, including development disorders and tumors, and NSD proteins have emerged as promising targets, and there has been rapid progress in developing small molecular inhibitors, oligopeptides, PROTACs, and nucleic acid agents. Notably, NSD proteins have been reported to regulate gene transcription through rewiring epigenetic modifications on promoters, enhancers, and intergenic regions (Fang et al., 2021; Popovic et al., 2014; Weinberg et al., 2019), and NSD2 in particular has been shown regulate 3D genome organization (Lhoumaud et al., 2019; Narang

et al., 2023). Thus, further explorations of the effects of NSD proteins on long-range interactions (e.g., enhancer–promoter contact) in gene expression regulation seem likely to yield insights into the functions of NSD proteins in epigenetic regulation.

Declaration of competing interest

The authors declare no conflicts of interest that pertain to this work.

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