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Review Article

Histone methyltransferases: novel targets for tumor and developmental defects

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Abstract: Histone lysine methylation plays a critical role in epigenetic regulation of eukaryotes. To date, studies have shown that lysine residues of K4, K9, K27, K36 and K79 in histone H3 and K20 in histone H4 can be modified by histone methyltransferases (HMTs). Such histone methylation can specifically activate or repress the transcriptional activity to play a key role in gene expression/regulation and biological genetics. Importantly, abnormities of patterns or levels of histone methylation in higher eukaryotes may result in tumorigenesis and developmental defects, suggesting histone methylation will be one of the important targets or markers for treating these diseases. This review will outline the structural characteristics, active sites and specificity of HMTs, correlation between histone methylation and human diseases and lay special emphasis on the progress of the research on H3K36 methylation.

Keywords: Histone lysine methylation, histone methyltransferases, epigenetic modification

Introduction

Nucleosome, a complex formed by repeated winding and folding of both DNAs and histones, is a basic structural unit of chromosome. Formation of nucleosome firstly needs a histone octamer (as surrounded by a segment of approximately 200 bp DNA) consisting of 2 copies each of the core histones H2A, H2B, H3, and H4 [1, 2]. Then, approximately ~147 bp of the aforementioned 200 bp DNA directly wrapped around the histone octamer comprises a core particle, called "core DNA", which is difficult to be digested and decomposed by nucleases; whereas approximately 20-80 bp DNA acts on the "linker DNA" connecting two neighboring nucleosomes where H1 (a linker histone) binds to [3-5]. The terminal tails of the nucleosomal core histone, N-terminal tails, are subject to various external modifications because they are freed externally. Modifications known to date include methylation, acetylation, phosphorylation, ubiquitination and ADP-ribosylation, which can modulate the affinity between DNAs and histones to alter the chromatin structure conditions (including causing looser or tighter chromatin) or function as a regulator of gene expression similar to genetic codes in DNA (now termed "histone code") by regulating the binding characteristics of transcription factors to DNA sequences. However, such histone modifications, together with DNA methylation and RNA modification, constitute the epigenetic modification [6-8]. In recent years, histone methylation has been a research hotspot in epigenetics, and also a focus of molecular biology, genetics and oncology [9-12]. However, histone methylation mainly occurs on arginine and lysine residues of histones H3 and H4 that are mainly regulated by histone methyltransferases (HMTs). Of the 24 sites of histone methylation ever discovered, there are 17 lysine and 7 arginine residues. Lysine residues can be modified by mono-, di- and tri-methylation, whereas arginine residues by mono- and di-methylation [13, 14]. This article is trying to outline histone lysine methyltransferases with respect to structures, active sites and specificity, and lay special

Table 1. Histone methyltransferases, target sites, binding domains and biological functions

Target sites	Histone methyltransferases	Binding domain	Biological functions
H3K4	SET1	Chd1	Transcriptional activation
	MLL	WDR5	Transcriptional elogation
	SET7/9	JMJD2A	Transcriptional scilencing
	SMYD2	MBT	
	SMYD3	PHD finger	
	EZH2		
	ASH1		
H3K9	SUV39h1	HP1	Transcriptional activation
	SUV39h2	CDY1	Transcriptional repression
	G9a	JMJD2A	DNA methylation
	EZH2		Heterochromatic silencing
	GLP		Euchromatic silencing
	RIZ		
	SETDB1		
	ASH1		
H3K27	EZH1	Pc	Transcriptional scilencing
	EZH2		Euchromatic silencing
	NSD2		X-inactivation
	G9a		
H3K36	NSD1	Eaf3	Transcriptional scilencing
	NSD2		Transcriptional elogation
	NSD3		Transcriptional regulation
	ASH1		
	SetD2		
H3K79	DOT1L	53BP1	DNA repair
			Demarcation of euchromatin
H4K20	SUV4-20H1	Crb2	Transcriptional scilencing
	SUV4-20H2	JMJD2A	Transcriptional activation
	NSD1		Transcriptional regulation
	ASH1		Heterochromatic silencing
	SET9		Cell cycle-dependent silencing
	SET8/PR-SET7		Mitosis and cytokinesis

emphasis on the relevant research progress of histone H3 lysine 36 (H3K36) methylation so far.

HMTs

Structural characteristics of HMTs

Histone methylation is mainly regulated by a series of HMTs containing highly conserved core SET, cysteine-rich pre- and post-SET domains. SET domains were named after the initials of the three genes first discovered which express such domains, namely, Suppressor of variegation 3-9 (Su(var) 3-9), Enhancer of zeste (E(z)) and Trithorax (Trx) [15-18]. The catalytic domain in the SET domain is in charge of determining the catalytic activity of HMT; the pre-SET domain functions as a maintainer of the structural stability of the protein; whereas the post-SET domain offers a hydrophobic channel to participate in composition of parts of active sites of the enzyme [19-22]. Because the presence of SET domain makes HMTs different from other methyltransferases, it has its own unique folding structure. The SET domain is a peptide chain containing 130 amino acid residues, with high conservation. In this domain, N- and C-termini separately coil up and circle round to constitute two non-adjacent spatial conformations with 3-4 short folds; then a short helix containing 9 rings links with the spatial conformations of N- and C-termini. The vast majority of C-termini of the SET domain coil up into a "pseudoknot-like" structure. This topological structure passes through a short helix containing 9 rings and then links to other sides in the side chain of the C-terminus of the SET domain, forming the core SET domain which contains the most conservative motifs (ELXF/YDY and NHS/CXXPN) in the SET domain [23-25]. In brief, the "pseudoknot-like" structure at the C-terminus of the SET domain is constituted by the fragments of the C-terminus passing through the terminal sequence of the protein and extending forward to form ring structures. Moreover, there are also inserts in the SET domain, termed iSETs, which differ in length and specifically recognize the active substrates and cofactors of HMTs [26, 27]. Furthermore, unlike the SET domain, pre- and post-SET domains on both sides of it are not highly conserved [28, 29].

Types of HMTs

SET domain, an important domain of the HMTs, is a 130 amino acid-long peptide chain, which was first defined in 1998 [30]. To date, the SET

domain has been found in the great majority of studies of organs of eukaryotes. There are 157 human-derived SET domain containing proteins in the SMART database and 93 in the Pfam database [31-35]. It is well known so far that SET domain containing proteins are majorly divided into seven families (SUV39, SET1, SET2, EZ, RIZ, SMYD and SUV4-20) and other rare members (i.e., SET7/9 and SET8) [36-39]. SET domain containing proteins in the same family share not only highly similar SET domains but also similar motifs beside this domain.

Target sites of HMTs

Histone methylation commonly occurs on lysine and arginine residues of H3 and H4 (**Table 1**). Of the 24 sites of histone methylation ever discovered, there are 17 lysine and 7 arginine residues, which are regulated by histone lysine methyltransferases (KMTs) and arginine methyltransferases (RMTs). Lysine residues can be modified by mono-, di- and tri-methylation [13, 14]. Post-methylation biological effects vary from sites of histone lysine residues, by which the gene transcription can be either activated or repressed. For example, methylation modifications at histone H3 lysine 4 (H3K4) and H3K36 can activate the gene transcription, whereas those at histone H3 lysine 9 (H3K9), histone H3 lysine 27 (H3K27), histone H3 lysine 79 (H3K79) and histone H4 lysine 20 (H4K20) can repress it [39-43].

Diversity and specificity of HMTs

Histone methylation is a very complicated process. Because its sites are diverse, it can either specifically activate or repress the transcriptional activity of the gene. Methylation modification comes in many forms, including mono-, di- and tri-methylation. Thus it can be seen that diverse sites and patterns of methylation lead to tens of thousands of patterns of methylation modifications, increasing the complexity and diversity of gene expression regulated by histone methylation and also highlighting the importance of histone methylation in epigenetic regulation. Because HMT is also specific to the active site of the enzyme, it can possess its own specific substrate and target site. As for its specificity of the active site, the current mainstream view is that iSET, the insert in the SET domain, specifically recognizes the substrate and cofactor of the HMTs and function as an antigenic determinant, causing different HMTs to specifically recognize diverse substrates [26, 27]. In addition, a few studies have reported HMTs may also recognize the same motif in different substrates, i.e., SET7/9 which can methylate not only H3K4 but p53 and TAF10 because these three substrates share the same motif, K/R-S/T-K [44]. Thus, the unique three-dimensional structure of the HMTs is characterized by specific recognition of the substrate and also exhibits the diversity of histone methylation.

Biological functions of histone methylation

Histone methylation is a part of epigenetic modification, but it possesses an extremely wide range of biological functions and plays important roles in transcriptional regulation, gene expression, heterochromatinization, genomic imprinting and X-inactivation [45-50]. Methylation at H3K4 and H3K36 can activate the gene transcription and that at H3K9, H3K27 and H4K20 can repress it; however, that at H3K79 is not involved in gene transcription and regulation directly but DNA damage/repair, where error localization occurs in P53BP1 and Crb around the DNA damage area and thus affects the process of DNA damage/repair when H3K79 methylation is repressed [51]. To date, it has been found that two members of the SUV39 family HMTs, SUV39h1 and SUV39h2, play a key role in heterochromatinization. When they mutate simultaneously, the methylation level of H3K9 will decrease by 50% or so and lead to disorders and deficits of separation of chromatin in neonatal mice during mitosis [52]. Moreover, SUV39h1 is a transcriptional repressor in mammals, which is co-localized in the transcriptionally silenced heterochromatin with the transcriptional repressor. When H3K9 is methylated by SUV39h1, HP1 can bind to the histone H3. It follows that SUV39h1 can recruit HP1 to the site of the heterochromatin during methylation of H3K9, playing a vital role in heterochromatinization [53]. Genomic imprinting is a phenomenon independent of the Mendelian inheritance, by which certain homologous alleles exhibit monoallelic expression during expression in a parent-of-origin-specific manner. Such genetic model is controlled monophyly-dependently. However, HMTs play a vital role in such genomic imprinting. It will lead to a loss of genomic imprinting of certain homologous alleles in genetic processing where there is a lack of functions of HMTs

[54]. To date, scholars have found in studying the rat chromosome 7 that in order to maintain the genomic imprinting there also involves an important pathway-histone methylation-besides the important regulatory effect on DNA methylation [55]. In the dosage compensation mechanism for sex determination in organism, sex determination mainly depends on Xist, a non-coding RNA, and X-inactivation. Trimethylation of H3K27 (H3K27me3) and monomethylation of H4K20 (H4K20me1) are closely related to the Xist expression; DNA methylationis closely associated with X-inactivation, whereas maintenance of X-inactivation status is strongly related to the Xist expression. Thus it follows that X-inactivation is co-regulated by methylation of both histones and DNAs [56]. To sum up, histone methylation is strongly related to genetics and molecular biology.

Correlation between histone methylation and diseases

The structural feature, namely, HMTs containing SET domains, has determined a very close relationship between HMTs and human tumorigenesis (**Table 2**). It is found that members of the RIZ family mutate in hepatocellular carcinoma, breast carcinoma, spinal cord tumor, neuroblastoma, lung carcinoma, colon carcinoma and bone tumor, resulting in loss of activity of histone H3K9 methyltransferase. Loss of enzyme activity of RIZ family results in prolonged cell cycle G2/M phase and repression of apoptosis. It can be speculated that methylation at H3K9 by the RIZ family can repress the tumorigenesis [57-59]. Also, some studies have found that two members of the SUV39 family, SUV39h1 and SUV39h2, are closely related to geneses of B-cell lymphomas and non-Hodgkin's lymphomas (NHLs), and that unstable gene expression of SUV39h1- and SUV39h2-knockout mice results in improper chromosome segregation and final genesis of B-cell lymphomas in mice. However, decrease in SUV39h1 and SUV39h2 methylation affects the interaction between SUV39h1/SUV39h2 and proteins of glioma retinae, thereby failing to regulate the gene expression of cyclinE in a normal manner and resulting in uncontrolled cell proliferation and thus promotion of carcinogenesis [60]. Moreover, mutation of mixed-lineage leukemia (MLL), the SET1 family member, results in the pathogenesis of leukemia; expression of the histone methyltransferase EZH2 increases aberrantly in patients with breast cancer, lymphoma and prostate cancer; histone methyltransferase SMYD3 also increases aberrantly in hepatocellular carcinoma and colorectal carcinoma cells [61]. Besides the fact that HMTs have a close relationship with human tumorigenesis, its role in the spectrum of human diseasehas attracted more and more attention. Recent studies also found that compared with normal healthy controls, histone methyltransferase Set7 was overexpressed in the peripheral blood mononuclear cells (PBMCs) of patients with type 2 diabetes and was closely linked to chronic inflammatory responses in organism, suggesting that overexpression of Set7 has a key effect on dysfunction of blood vessels in patients with type 2 diabetes [62]. In addition, foundational research found that mutation of H3K4 methyltransferase MLL2 may result in hyperglycemia and insulin resistance and progress to nonalcoholic fatty liver [63]. To sum up, with the gradual improvement of the research on histone methylation and the definition of functions of different sites of histone methylation, relationships between the aberrant methylation at all sites and tumors or other diseases will be demonstrated gradually. This will provide novel strategies and ideas for clinical diagnosis and treatment of tumors and other human diseases.

H3K36-specific methyltransferases

Recent studies have found that methylation of H3K36 plays important roles in expression and regulation of genetic information, including gene transcription and regulation, alternative splicing, dosage compensation, DNA replication, repair and methylation, and gene inheritance and expression [64-68]. Therefore, this article is to lay special emphasis on types of H3K36-specific methyltransferases, patterns of H3K36 methylation, roles of H3K36 methylation in gene transcription and regulation, and the correlation between H3K36 methylation and human diseases.

Types and modifications of H3K36-specific methyltransferases

HMTs add methyl groups on the certain lysine or arginine residue through active adenosylmethionine in order to regulate the patterns of histone methylation [69]. To date, in vitro and in vivo studies have demonstrated that there are

Table 2. Histone lysine methyltransferases and related cancer or related developmental defects

H3K4 methyltransferases	Related cancer	Related developmental defects
SET1	prostate cancer, T-cell acute lymphoblastic leukemia, leukemia	retarded growth
MLL	acute myeloid leukemia, acute lymphoblastic leukemia, acute promyelocytic leukemia	Wiedemann-Steiner Syndrome, Kabuki syndrome, hematopoiesis defects
SET7/9	gallbladder cancer	impaired muscle differentiation
SMYD2	renal cell tumors, breast cancer, gastric cancer, acute lymphoblastic leukemia, esophageal squamous cell carcinoma	impaired skeletal and cardiac muscles development
SMYD3	glioma, gastric cancer, prostate cancer, breast cancer, medullary thyroid carcinomas, hepatocellular cancer	impaired early embryonic lineage commitment, impaired heart morphogenesis, impaired skeletal and cardiac muscles development
EZH2	prostate cancer, colorectal cancer, breast cancer, hepatocellular carcinoma, lung cancer, T-cell acute lymphoblastic leukemia, bladder cancer	metabolic defects, DiGeorge or Velocardiofacial syndrome, cardiovascular defects, Weaver syndrome
ASH1	sinonasal neuroendocrine tumors, lung cancer, prostate cancer	impaired ovule and anther development
H3K9 methyltransferases	**Related cancer**	**Related developmental defects**
SUV39h1	lung cancer, breast cancer, bladder cancer, acute myeloid leukemia, glioma, prostate cancer, hepatocellular carcinoma	fetal lung defects, defects in terminal differentiation of the intestine, exocrine pancreas and retina
SUV39h2	glioma, hepatocellular carcinoma, prostate cancer, lung cancer	fetal lung defects
G9a	leukemia, breast cancer, head and neck squamous cell carcinoma, oral squamous cell carcinoma, endometrial cancer, lung cancer, hepatocellular carcinoma, glioma	congenital and metabolic defects, growth retardation of embryos, calvaria defects, postnatal lethality, atrioventricular septal defects, retina defects
EZH2	prostate cancer, colorectal cancer, breast cancer, hepatocellular carcinoma, lung cancer, T-cell acute lymphoblastic leukemia, bladder cancer	metabolic defects, DiGeorge or Velocardiofacial syndrome, cardiovascular defects, Weaver syndrome
GLP	breast cancer, hepatocellular carcinoma, pancreatic ductal adenocarcinoma, leukemia, prostate cancer	growth retardation of embryos, ossification defects of calvaria, postnatal lethality, atrioventricular septal defects
RIZ	lung cancer, breast cancer, neuroblastoma, prostate cancer, leukemia, gastric and colorectal cancer	N/A
SETDB1	lung cancer, hepatocellular carcinoma, breast cancer, prostate cancer, glioma	congenital and metabolic defects, brain defects and early lethality
ASH1	sinonasal neuroendocrine tumors, lung cancer, prostate cancer	impaired ovule and anther development
H3K27 methyltransferases	**Related cancer**	**Related developmental defects**
EZH1	leukemia, myeloproliferative neoplasms, breast cancer	impaired differentiation of skeletal muscle cells
EZH2	prostate cancer, colorectal cancer, breast cancer, hepatocellular carcinoma, lung cancer, T-cell acute lymphoblastic leukemia, bladder cancer	metabolic defects, DiGeorge or Velocardiofacial syndrome, cardiovascular defects, Weaver syndrome
NSD2	acute lymphoblastic leukemia, malignant lymphoproliferative diseases, prostate cancer	Wolf-Hirschhorn syndrome
G9a	leukemia, breast cancer, head and neck squamous cell carcinoma, oral squamous cell carcinoma, endometrial cancer, lung cancer, hepatocellular carcinoma, glioma	congenital and metabolic defects, growth retardation of embryos, calvaria defects, postnatal lethality, atrioventricular septal defects, retina defects
H3K36 methyltransferases	**Related cancer**	**Related developmental defects**
NSD1	breast cancer, lung cancer, prostate cancers, acute myeloid leukemia, neuroblastoma and glioma	Sotos or Weaver syndromes
NSD2	acute lymphoblastic leukemia, malignant lymphoproliferative diseases, prostate cancer	Wolf-Hirschhorn syndrome
NSD3	breast cancer and acute myeloid leukemia	N/A
ASH1	sinonasal neuroendocrine tumors, lung cancer, prostate cancer	impaired ovule and anther development

SetD2	renal clear cell carcinoma, lymphoblastic leukemia, breast cancer, prostate cancer, lung cancer, glioma, thymic carcinoma, acute myeloid leukemia	severe vascular defects, embryonic lethality, Sotos or Weaver syndromes
H3K79 methyltransferases	Related cancer	Related developmental defects
DOT1L	acute myeloid leukemia, breast cancer, gastric cancer, colorectal cancer, prostate cancer	impaired oocytes meiosis, growth impairment, angiogenesis defects in the yolk sac, cardiac dilation
H4K20 methyltransferases	Related cancer	Related developmental defects
SUV4-20H1	lung cancer	N/A
SUV4-20H2	lung cancer, breast cancer, hepatocarcinogenesis	N/A
NSD1	breast cancer, lung cancer, prostate cancers, acute myeloid leukemia, neuroblastoma and glioma	Sotos or Weaver syndromes
ASH1	sinonasal neuroendocrine tumors, lung cancer, prostate cancer	impaired ovule and anther development
SET9	multiple myeloma, prostate cancer	N/A
SET8/PR-SET7	lung cancer, hepatocellular carcinoma, non-Hodgkin's lymphomas, pancreatic cancer, cervical cancer, prostate cancer, breast cancer	early embryonic lethality

eight types of HMTs regulating H3K36 methylation levels in mammals, including nuclear receptor SET domain-containing 1 (NSD1), nuclear receptor SET domain-containing 2 (NSD2), SET domain containing 2 (SetD2), and other methyltransferases (i.e., nuclear receptor SET domain-containing 3 (NSD3), mesoderm-expressed 4 (MES-4), absent small and homeotic disks protein 1-like protein (ASH1L), SET domain and mariner transposase fusion (SETMAR), SET and MYND domain-containing 2 (SMYD2) and SET domain containing 3 (SETD3) [70]. All H3K36-specific methyltransferases contain highly conserved SET domains, but vary with patterns of H3K36 methylation, which mainly include mono-, di- and tri-methylation. In yeast, however, H3K36 can be mono-, di- and tri-methylated by Set2 simultaneously; in higher eukaryotic species, these patterns of methylation require coordination and division of mono-, di-methyltransferases and SET2-related tri-methyltransferases [71]. H3K36-specific methyltransferases possess a variety of domains which interacted with chromatin, i.e., PWWP domain interacted with methylated H3K36, and PHD fingers interacted with other methylated histone residues [72]. Novelly, for most species there is a domain interacted with RNA polymerase II (RNAPII) at the C-terminus of Set2, termed RNAP II subunit B1 (RPB1) [73].

Nuclear receptor SET domain-containing 1 (NSD1): The initial discovery of nuclear receptor SET domain-containing 1 (NSD1), also known as androgen receptor activator protein (KMT3B), is because it can bind to nuclear steroid receptors, which, together with NSD2 and NSD3, belongs to the NSD protein family. After that, it is found that NSD1 can methylate H3K6 and H4K20 by its own SET domain. However, besides histones, it can methylate non-histones as well, such as p65 subunit of nuclear factor kappa B (NF-κB) [74]. To date, it has been found that the substrate of NSD1 is mainly unmethylated H3K36 or H3K36me1, which mainly methylate H3K36 in a mono- or di-methylated manner to produce H3K36me1 or H3K36me2 [75].

Nuclear receptor SET domain-containing 2 (NSD2): Nuclear Receptor SET Domain-Containing 2 (NSD2) is also known as Wolf-Hirschhorn syndrome candidate 1/Multiple Myeloma SET domain (WHSC1/MMSET). Currently, the methylated site of NSD2 is still controversial. There are two major viewpoints. Some researchers believed that NSD2 could trimethylate H3K4, H3K27, H3K36 and H4K20 to produce H3K4me3, H3K27me3, H3K36me3 and H4K20me3 [76, 77]; while some believed that NSD2 could dimethylate H4K20 and H3K36 to produce H4K20me2 and H3K36me2 [78]. However, a study of the protein spatial structure by Kuo et al. found that neither full-length NSD2 nor NSD-SET domain methylated H4K20, while research on protein functions found that NSD2 could mono- and di-methylate H3K36 to produce H3K3me and H3K36me2 [79]. It was also found that methylation of NSD2 depends on the substrate specificity. For example, NSD2 dimethylate H3K36 and H4K20 if the substrate is nucleosome; whereas, NSD2 methylate H4K44 if the substrate is histone octamer [80]. Hence, different sites and patterns of methylation of NSD2 determine the diversity of NSD2 regulating the gene transcription. That is to say,methylation of lysine residues can either activate or repress the gene transcription, showing that NSD2 represses the gene transcription by methylation of H3K27, H3K36 and H4K20 while methylation of H3K4 and H3K36 promotes the gene transcription [81]. To sum up, further research will be required to find out the biological functions of NSD2 and its exact regulation mechanism.

SET domain containing 2 (SetD2): Human-derived histone methyltransferase SetD2, also known as huntingtin-interacting protein B (HYPB), is closely related to Huntington's disease (HD). Located at 3p21.31, it is a protein with a molecular weight of approximately ~230 kDa. Its open reading frame (ORF) is ~6,186 bp, encoding 2,061 amino acid residues [82]. SetD2 also contains a vital active methyltransferase domain, the SET domain; beside it is the pre- and post-SET domain, respectively. The three domains are termed the associate with SET (AWS)-SET-Post SET domain, which is cysteine-rich and specifically methylates H3K36. Behind this domain is a cysteine- and proline-rich domain, termed the low charged region (LCR) domain, which activates the gene transcription and is highly conserved in vertebrates. There is a WW domain binding to the LCR domain, with two conservative tryptophan residues, which is currently accepted that its functions may be related to the regulation of the protein-protein interaction and that it exerts its effects by binding to the proline-rich region or

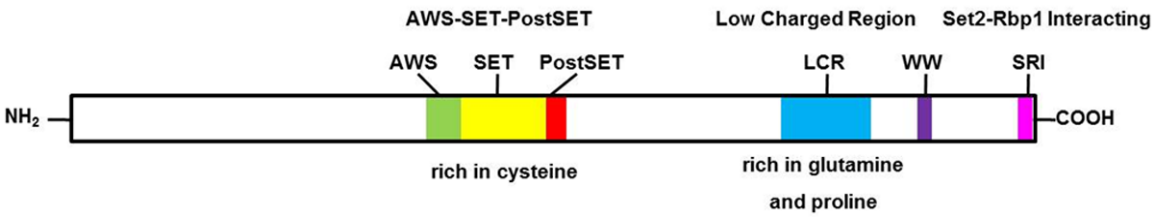

Figure 1. Schematic representation of SetD2 structure.

SH3-binding motif. There is a 142 amino acid-long Set2-Rbp1 interacting (SRI) domain close to the C-terminus of SetD2, which interacts with phosphorylated RNA polymerase II (RNAPII) but not non-phosphorylated RNAPII [83-85] (**Figure 1**). Studies have found that SetD2 is related to the heterogeneous nuclear ribonucleo protein L (hnRNPL) and that partial knockout of hnRNPL results in a marked decrement in H3K36me3 level while levels of H3K36me1 and H3K36me2 are not affected, suggesting that SetD2 can specifically catalyze H3K36 and modify the H3K36me3 level [86].

Specificity of H3K36 methylation

Structural specificity of H3K36-specific methyltransferase also determines its specific patterns of methylation, including mono-, di- and tri-methylation. However, so far, the specificity or difference of modification patterns of H3K36-specific methyltransferase is related to many factors in studies themselves, including assays of substrate specificity (peptide fragments, histones and nucleosomes), enzyme sources (the full-length and SET domain of the enzyme), and condition differences of the assay itself (antibody specificity and mass spectrometry). To date, 8 substrates of H3K36-specific methyltransferase and their patterns of methylation have been confirmed by means of experiments for loss-of-function and/or nucleosome-related substrates [70, 87, 88]. However, as for SETD3, its action characteristics are demonstrated by peptide fragments and core histones, their specific active sites and patterns of methylation should be further verified [89].

Roles of H3K36 methylation in regulation of gene expression

H3K36 methylation and transcriptional regulation: So far, many studies have confirmed in many ways that H3K36 methylation is inseparable from transcriptional activation [90-93]. In general, H3K36 methylation commonly occurs at the promoter and 3-terminus of the gene, from mono- to tri-methylation [81]. It has been found that H3K36 methylation is mostly present in the coding region of the transcriptional activator, which mainly affects the elongation of transcription. During transcription elongation, when phosphorylation occurs at Serine 2 of RNAPII C-terminal domain, the Paf1 elongation complex promotes Set2 to 5-ORF so that H3K36 is methylated. Two subunits of the Rpd3S histone deacetylase complex, Rco1 and Eaf3, can specifically recognize H3K36me. Once identified, Rpd3S can deacetylate the RNAPII-affected histone so as to prevent the latent transcription [94-96]. Moreover, regulation of H3K36 methylation levels by NSD1 and NSD3 is related to the transcription initiation. NSD1 binds to the upstream of the BMP4 promoter through RNAPII recruitment, regulating levels of H3K36me, H3K36me2 and H3K36me3 in organism [97]; NSD3 binds to LSD2 and BRD4 complexes or locate at the promoters or in interior regions of above complexes in order to facilitate H3K36 methylation and thus regulate the transcription initiation and elongation of genes [98].

H3K36 methylation and DNA replication/mismatch repair: DNA replication principally occurs during the S-phase. Initiation is defined by the origin recognition complex (ORC), which can recruit many factors before promoting DNA polymerase, such as CDC6, minichromosome maintenance proteins (MCMs) and CDC45. Studies of yeast conclude that H3K36 methylation plays an initiating role in DNA replication and that deletion of Set2 leads to delayed CDC45 recruitment [99]. In addition, H3K36 methylation is also closely associated with the checkpoint of DNA replication [100]. However, the exact molecular mechanisms by which H3K36 methylation regulates DNA replication during the S-phase have not been established. DNA mismatch repair can ensure high-fidelity-replication by correcting the mismatch gener-

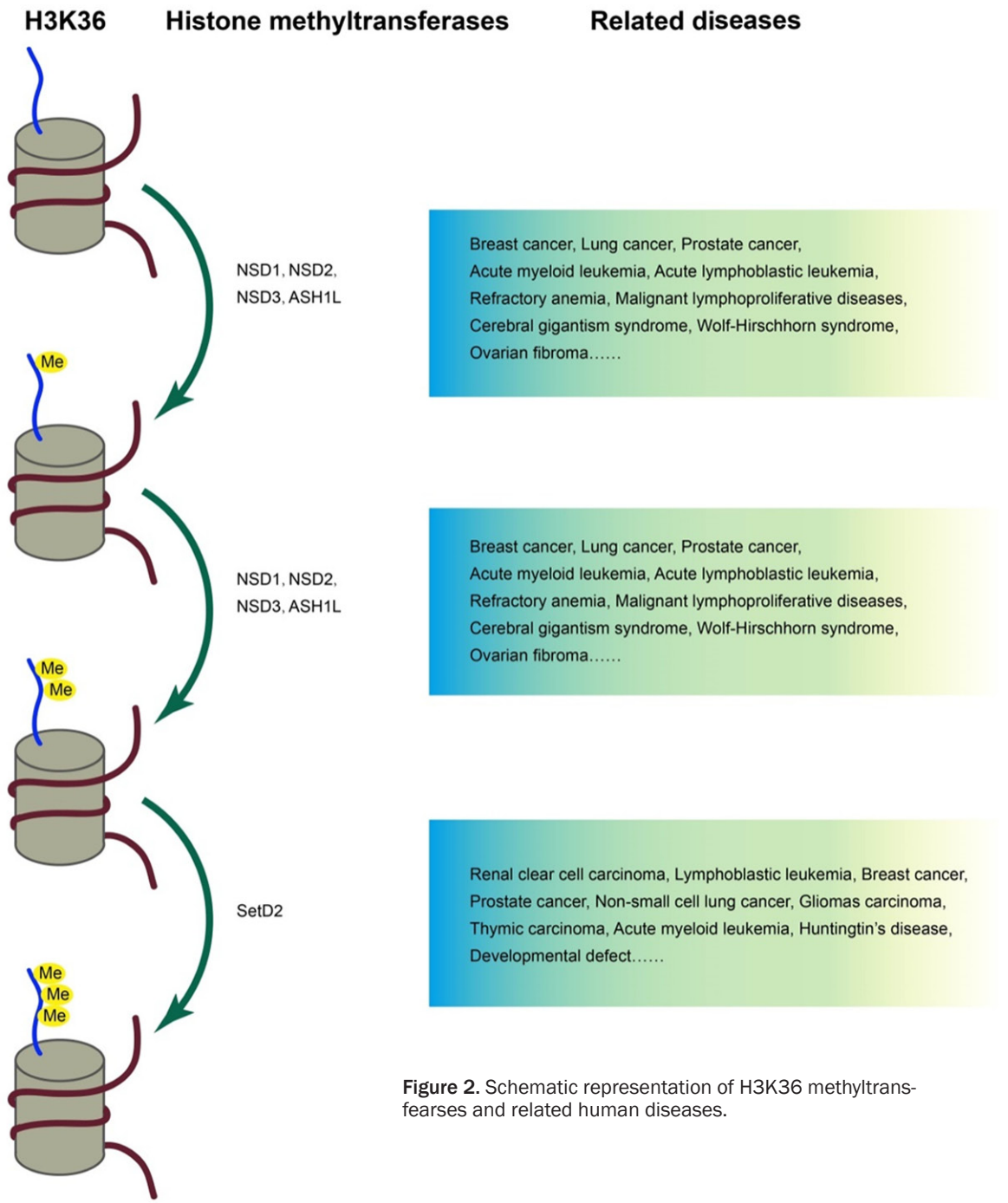

Figure 2. Schematic representation of H3K36 methyltransfearses and related human diseases.

ated during DNA replication. A study by Li F et al. reported that recruitment of in vivo mismatch recognition protein hMutSα (hMSH2-hMSH6) to chromatin needed H3K36me3, which could directly bind to the PWWP domain of hMSH6 to recruit hMutSα to chromatin [101]. Thus, a massive gathering of H3K36me3 during the G1 and S phases is to ensure hMutSα recruitment to chromatin before the mismatch occurs during DNA replication. The incidence of mutations will rise sharply when SetD2 is deleted. It follows that normal H3K36 methylationis of importance in DNA replication and mismatch repair.

Correlation between H3K36 methylation and human diseases

At present, research on H3K36 methylation has found that abnormities of patterns or levels

of H3K36 methylation in higher eukaryotes may result in tumor genesis and progression, developmental defects, cerebral gigantism syndrome, ovarian fibroma, Wolf-Hirschhorn syndrome, etc [102-107] (**Figure 2**).

H3K36 methylation and tumor genesis/progression: To date, many studies have found that the members of the NSD family function as tumor suppressors of many cancers. For example, NSD1 is closely related to breast, lung and prostate cancers, acute myeloid leukemia and refractory anemia [108-111]; NSD2 is closely related to acute lymphoblastic leukemia, malignant lymphoproliferative diseases and prostate cancer [112, 113]; whereas NSD3 is closely related to breast cancer and acute myeloid leukemia [114, 115]. Recently, it was reported in Blood, a peer-reviewed medical journal, that co-expression of NUP98/NSD1 and FLT3/ITD was closely related to poor prognosis in acute myeloid leukemia [116]. Berdasco M et al. found inactivation of NSD1 in neuroblastoma and glioma that leads to abnormal H3K36 methylation levels and thus overgrowth and proliferation of tumor cells, which might be one of the prognostic markers for treating these two diseases [117]. Zhao Q et al. identified genomic alterations in the breast cancer cell line HCC1954 using high-throughput transcriptome sequencing and concluded that rearrangements and mutations occurred in the gene of NSD1, suggesting that NSD1 mutations are likely to play a key role in genesis of breast cancer [118]. Yang P et al. found that NSD2 was critical for cytokine-induced recruitment of NF-κB and acetyltransferase p300 and histone acetylation, and more importantly, they also found that NSD2 was overexpressed in prostate cancer tissues and its overexpression correlated with the activation of NF-κB signal pathway. Furthermore, they also indicated that NSD2 expression was induced by tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-60), playing a crucial role in tumor growth [119]. These results suggested that NSD2 played critical roles in cancer cell proliferation and tumor growth and progression.

Furthermore, current studies have found that H3K36 trimethyltransferase SetD2 mutates and is expressed aberrantly in renal clear cell carcinoma, lymphoblastic leukemia, breast and prostate cancers, gliomas and thymic carcinoma. To date, studies have suggested that SetD2 is a tumor suppressor gene [120-126]. SetD2 was initially found to mutate in patients with renal clear cell carcinoma and show a marked decrease in mRNA level so as to be identified as a novel tumor suppressor [120]. A most recent study found detrimental mutations in SetD2 in tumor tissues of patients with non-small cell lung cancer (NSCLC), speculating that SetD2 is promising to be one of the clinical diagnostic or prognostic markers for NSCLC in the future [127]. Moreover, it was reported that frameshift or nonsense mutation in many epigenetic regulators, including SetD2, was noted in patients with acute myeloid leukemia, with an incidence of 12%, suggesting epigenetic regulators will be one of the important targets for treating this disease [128].

H3K36 methylation and developmental defects: Apart from tumor genesis and progression, it has been widely reported that abnormity of H3K36 methylation is also related to developmental defects. Research found that: SetD2-deficient embryos failed to undergo normal implantation at the blastocyst stage while some lineage specific factors, including Eomes, Elf5 and Sox2, were distinctly reduced in SetD2-deficient embryos and that several imprinted genes (Mest, Peg3, Snrpn and Meg3) were aberrantly expressed, speculating that H3K36 trimethylation regulated by histone methyltransferase SetD2 plays an important role in maintaining normal embryo implantation [129]. In addition, Chen Zhu (an academician of the Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, China) and colleagues found that the H3K36me3 level was reduced in fibroblasts of SetD2-deficient embryos but there was no distinct change in levels of H3K36me and H3K36me2, and that all homozygous embryos of SetD2-knockout mice were lethal at E11-E11.5. Postmortem and pathological staining showed that embryonic growth retardation, incomplete chorio-allantoic fusion and araphia were noted in SetD2-deficient mice. Most notably, vascular developmental defects were noted in SetD2-deficient embryos during vascular development, including abnormal vasodilatation, morphological abnormalities of mesodermal epithelial cells, and increased spacing between epithelial cells. Microarray assay for SetD2-deficient embryos indicated that 10 angiogenesis-related secreted factors (Ang, Angptl3, Angptl6, CTGF, Cyr61,

Igf1, Pdgfc, Plg, Serpinf1 and VEGFb) and 8 membrane proteins (Cav1, Flt1, Gja4, Lama1, Lama4, Rhob, Sema3c and Serpine3) were significantly expressed aberrantly [130]. It can be concluded that the mechanism by which SetD2 deficiency results in embryonic lethality may be related to anomalous blood supply due to embryonic abnormal angiogenesis.

H3K36 methylation and other diseases: Apart from tumor genesis and developmental defects, more and more studies using gene sequencing have found that NSD1 mutates in patients with cerebral gigantism syndrome and ovarian fibroma, which may be a promising new diagnostic marker in the future [131]. Furthermore, it has been found that NSD2-deficient mice may develop Wolf-Hirschhorn syndrome manifesting as growth retardation and congenital vascular malformation. In addition, Wolf-Hirschhorn syndrome is significantly more severe in NSD2- and-Nkx2.5-knockout heterozygous mice than in NSD2-knockout mice, speculating that Wolf-Hirschhorn syndrome is regulated by the interaction between NSD2 and transcription factor Nkx2.5 [132]. Nevertheless, SetD2, also known as huntingtin-interacting protein B, was initially discovered in Huntington's disease, a neurodegenerative disorder. SetD2 is identified as a member of the huntingtin-interacting protein family because there is a WW motif in it.

Conclusions

Histone methylation is one of the most important fields in epigenetics. Therefore, further investigation of the regulatory effects and mechanisms of histone methylation on physiological functions (gene transcription and regulation, biological inheritance, etc.), and perfection of roles of histone methylation in human tumorigenesis, growth/development and immunoregulation by bioinformatics techniques will provide important clues and targets for diagnosis, prevention and treatment of human diseases. For example, it is very prospective for developing new drugs to investigate active sites or substrates of HMTs, their characteristics of domains, and specific recognition of their proteins or polypeptides.

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Disclosure of conflict of interest

The authors have declared that no conflict of interest exists.

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