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REVIEW ARTICLE

Biological function and regulation of histone 4 lysine 20 methylation in DNA damage response

Sara Moghaddam Kohi[1] · Tingting Feng[1] · Yuan Tian[1,2] · Wei-Guo Zhu[1,2]

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Abstract

Cells are often under attack from various DNA-damaging agents. Accurate repair is required to protect cells from the genome instability induced by DNA lesions. DNA damage response (DDR) signaling involves sensitizing, transmitting, and repairing different types of damage within chromatin complexes. Chromatin is a highly ordered complex packed with repeating units of nucleosomes and linker DNA sequences. Chromatin structure, gene transcription, and various biological processes are regulated by histone post-translational modifications (PTMs), including acetylation, methylation, phosphorylation, and ubiquitylation. Of these, the involvement of lysine methylation, regulated by numerous lysine methyltransferases and demethylases, in the DDR has been extensively explored. In particular, histone 4 lysine 20 methylation is one of the most essential histone PTMs for biological processes and ensures genome integrity. In this review, we summarize the dynamics and modulations of histone lysine methylation during the DDR. We also comprehensively describe the functions, mechanisms, and regulation of H4K20 methylation and its modifying enzymes in response to DNA damage.

Keywords DNA damage response · Histone modifications · Lysine methylation · H4K20 methylation

Introduction

DNA in eukaryotic cells is continually being damaged in an abundance of ways. This damage usually includes DNA single-strand breaks (SSBs), DNA double-strand breaks (DSBs), adducts, intrastrand and interstrand cross-links, and insertion/deletion mismatches. Among them, DSBs are considered the most cytotoxic type of damage because they induce genomic instability. Ionizing radiation (IR), ultraviolet light, and various chemical agents represent commonly exogenous sources of DNA damage. Deregulated DNA replication can also lead to DNA damage, and the disruption of replication forks has been shown to cause severe lesions, including DSBs (Jackson & Bartek, 2009; Lord & Ashworth, 2012). To preserve genomic integrity, mammalian cells repair damaged DNA via at least nine distinct pathways (Ceccaldi et al., 2016; Clauson et al., 2013; Kennedy & D'Andrea, 2006; Kramara et al., 2018). For example, IR-induced DSBs are mainly repaired by non-homologous end-joining (NHEJ) and homologous recombination (HR). Each repair pathway is detected and activated by different repair factors to form a precise network that ensures genome stability.

The nucleosome is the basic structural unit consisting of two copies of the histone proteins, H4, H3, H2A, and H2B, and is wrapped in 147 bp of DNA nucleotides. They are further packaged into a 30-nm fiber to form the chromatosome core particle consisting of a linker histone (H1) bound to the nucleosome with 10 bp of DNA. Histone tails are susceptible to a variety of covalent post-translational modifications (PTMs), including methylation, acetylation, ubiquitination, and phosphorylation. The PTMs of the histone tails tightly control many biological processes by regulating chromatin structure and function (Van & Santos, 2018). The second most common histone modification linked to the DNA

Sara Moghaddam Kohi and Tingting Feng contributed equally to this work.

✉ Yuan Tian
tianyuan1020@szu.edu.cn

✉ Wei-Guo Zhu
zhuweiguo@szu.edu.cn

[1] Guangdong Key Laboratory of Genome Instability and Human Disease Prevention, Department of Biochemistry and Molecular Biology, School of Medicine, Shenzhen University, Shenzhen 518055, China

[2] Shenzhen Bay Laboratory, School of Medicine, Shenzhen University, Shenzhen 518055, China

damage response (DDR) is histone methylation, which is under the control of histone methyltransferase (HMT) and the histone demethylases (HDMs) (Chen & Zhu, 2016). Both the lysine and arginine residues of histones can be methylated, and histone methylation presents as three forms: mono-(me1), di-(me2), and rei-(me3). It has been demonstrated that histone methylation, particularly lysine methylation, plays a crucial role in the response to DNA damage (Uckelmann & Sixma, 2017; J. Zhang et al., 2021). Methylations of lysine are modulated by lysine methyltransferases (KMTs) and lysine demethylases (KDMs). While the role of histone acetylation in the DDR has been extensively studied (Li & Zhu, 2014; Li et al., 2020), the investigation of histone methylation is still at a relatively early stage. Therefore, elucidating the mechanisms and functions of histone lysine methylation will be helpful for developing novel therapeutic strategies for DNA-damage-related diseases, including aging and cancer.

The common lysine methylation sites are residues 4, 9, 27, 36, and 79 of histone H3 and lysine 16 and 20 of histone H4. Genome-wide localization analyses indicate that each residue and degree-specific methylation has a distinct distribution pattern, indicating their unique functions during diverse cellular processes. Lysine 20 is a major methylated lysine residue on the histone H4 tail. Each degree of methylation at this residue has distinct regulation properties and shares similar functions during the DDR. For example, H4K20me1 regulates the cell cycle through condensin II, H4K20me2 negatively correlates with H4K16ac, and H4K20me3 is highly enriched in heterochromatin. Moreover, all three forms of H4K20 methylation are reported to contribute to p53-binding protein 1 (53BP1) recruitment (Tan et al., 2011). A better understanding of the mechanisms that regulate H4K20 methylation will enable us to gain deeper insights into how different degrees of methylation are controlled and what cellular machinery requires this modification. In this review, we summarize the critical roles of histone lysine methylation, especially H4K20 methylation, and the relevant modulating enzymes in the DDR.

Histone lysine methylation

Histone methylation has been recognized as being a PTM since 1964 (Murray, 1964). Since this time, methylation has been identified on various lysine residues on histone H3 and H4 tails, including H3K4me1/2, H3K9me2/3, H3K27me2/3, H3K36me1/2/3, H3K79me1/2/3, H4K16me1, and H4K20me1/2/3 (Husmann & Gozani, 2019). Other basic residues, e.g., H1.4 and H2AX, have been shown to be methylated in the nucleosome (Black et al., 2012) (Fig. 1). Histone methylation plays an essential role in the DDR, chromatin structure, gene transcription, and cell cycle regulation. To date, many histone methylation sites have been shown to be related to the DDR in different ways. Histone methylation can provide high-affinity binding sites or platforms for repair factor recruitment (Chen & Zhu, 2016). In addition, DNA-histone interactions in the chromatin structure are mediated by the attraction between the negatively charged DNA backbone and positively charged lysine and arginine residues of the histones. Although histone methylation cannot directly change the charge of histones, it is involved in crosstalk with

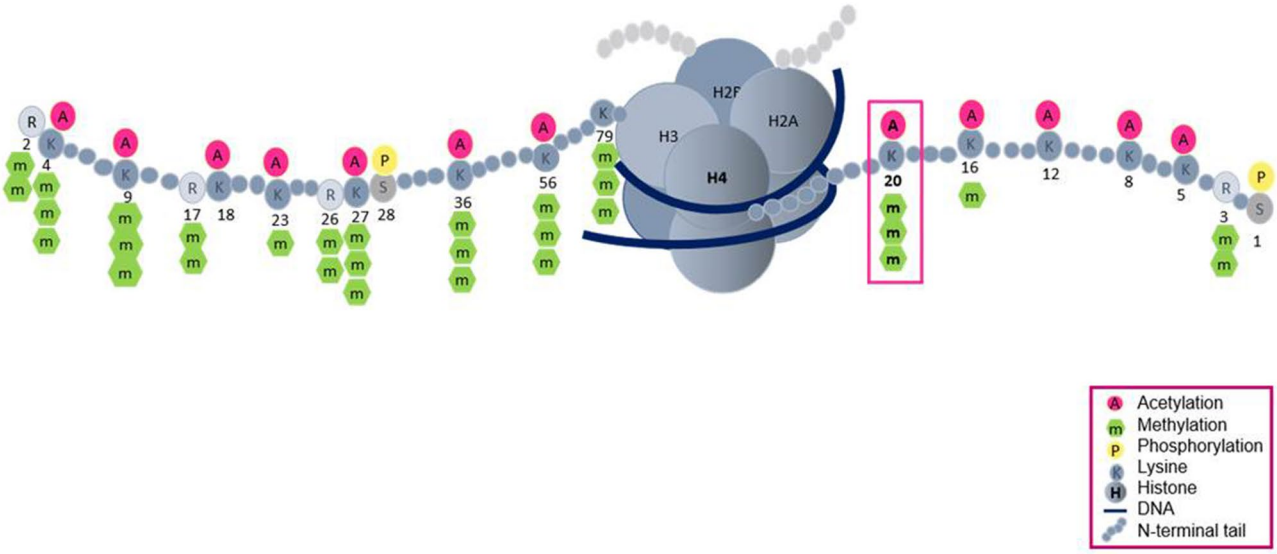

Fig. 1 Overview of histone modifications. Representative modification sites are shown. Numbers indicate lysine or arginine residues on histones H3 and H4

other histone modifications, such as acetylation, to regulate the compaction of chromatin (Cao et al., Cao, Shen, et al., 2016, Cao, Wei, et al., 2016). To date, although almost all identified histone methylation sites reportedly participate in the DDR, deeper investigation is required into whether and how crosstalk among different sites affects DNA repair.

H3K4 is a major methylation site of histones and is often associated with transcriptionally active genes (Santos-Rosa et al., 2002). At sites of DNA damage, H3K4me3 regulates NHEJ efficiency and S-phase transition in yeast cells (Faucher & Wellinger, 2010). The methylation of H3K9 is tightly linked to stable inheritance of the heterochromatic state (Bannister et al., 2001; Rea et al., 2000). H3K9me3 interacts with the chromo domain of *Drosophila* heterochromatin protein 1 (HP1) to regulate chromatin compaction during the DDR (Jacobs & Khorasanizadeh, 2002). Loss of H3K9me3 at DSBs leads to defective repair and increased radiosensitivity (Ayrapetov et al., 2014). H3K14 methylation, the latest methylation site discovered on H3, negatively correlates with H3K14 acetylation to repress host gene expression (Rolando et al., 2013). Recently, H3K14me3 is reported to respond to replication stress by enhancing ataxia-telangiectasia-mutated-and-Rad3-related kinase (ATR) activation (Zhu et al., 2021). H3K27 methylation is an established gene-silencing marker (Cao et al., 2002; Margueron & Reinberg, 2011). H3K27me2/3 facilitates NHEJ efficiency by triggering the chromatin accumulation of Fanconi anemia complementation group D2, a central player in the cell's choice of DNA repair pathway (Zhang et al., 2018). H3K36 methylation is associated with the elongating form of Pol II (Wagner & Carpenter, 2012). H3K36me2 recruits and stabilizes DNA repair components, including Nijmegen breakage syndrome protein 1 and Ku70, at DSBs (Fnu et al., 2011). H3K36me3 promotes HR repair by facilitating DNA end resection (Pfister et al., 2014). H3K79 methylation participates in active transcription and the DDR (Huyen et al., 2004; Mehta et al., 2016). H3K79me3 binds to 53BP1 to regulate the higher-order chromatin structure during the DDR (Huyen et al., 2004). H4K16me1 was originally suggested following a mass spectrometry study a decade ago, its biological function in the DDR has only recently been clarified (Lu et al., 2019; Tan et al., 2011). H4K16me1 can cooperate with H4K20me2 to facilitate 53BP1 recruitment and NHEJ mediated DNA repair (Lu et al., 2019). H4K20 methylation is one of the most thoroughly studied histone modifications: it has diverse functions and is regulated by various histone modifiers (Jorgensen et al., 2013). The role H4K20 methylation in DDR will be discussed in detail in this review. The first mammalian H1 methylation site identified was H1.4K26. The methylation of H1.4K26 provides a recognition surface for the chromatin-binding of HP1 and lethal 3 malignant brain tumor 1 (L3MBTL1) (Trojer et al., 2009; Walport et al., 2018). Although most common methylation sites are reported to be involved in DNA repair efficiency, the detailed mechanisms underlying how specific site contributes to DDR require further investigations. For example, although H3K4me3 are shown to be important for responses to DNA damaging agents, understanding the contribution of H3K4 methylation and its methyltransferases in repair factors recruitment needs further studies. In addition, several other identified methylation sites exist, but their biological modulation, especially about DDR, remains unclear. For example, little is known about the methylation of H4 K5, K8, or K12 catalyzed by yeast Set5, except for its role in cell growth and stress responses (Green et al., 2012).

Histone lysine methyltransferases and the DNA damage response

HMTs are enzymes that transfer the methyl groups from S-adenoidal-L-methionine (SAM) to histone proteins (Fig. 2). HMTs are divided into three groups, SET-domain-containing proteins, DOT1-like proteins, and protein arginine N-methyltransferases (PRMTs). The first two families are lysine KMTs, whereas the PRMT family has been shown to methylate arginine. In mammalian cells, PRMTs consist of PRMT1 to PRMT9, whereas KMTs encompass KMT1 to KMT9 (Greer & Shi, 2012). The roles of KMTs vary with their histone substrates or interacting partners, and many KMTs are thought to participate in the DDR (Biggar & Li, 2015) (Table 1).

KMT1A (SUV39H1) is a methyltransferase of H3K9 methylation and is linked to the organization of higher-order chromatin and the recruitment of HP1 to DSBs (Ayrapetov et al., 2014; Rea et al., 2000; Tu et al., 2020). KMT1B (SUV39H2) is a methyltransferase of H2AXK134me2 (Sone et al., 2014), which regulates γ-H2AX levels during the DDR (Sone et al., 2014). KMT1C (G9a, EHMT2) methylates H3K9me1/2 and H1.4K26me1/2 to provide a recognition surface for the chromatin-binding of replication protein A (RPA), HP1, and L3MBTL1 (Trojer et al., 2009; Yang et al., 2017). KMT1D (GLP, EHMT1), the methyltransferase of H3K9me1/2 and H4K16me1, facilitates 53BP1 recruitment in the NHEJ pathway (Lu et al., 2019; Takahashi et al., 2012). KMT1E (SETDB1) methylates H3K9me3, which promotes 53BP1 reposition at damaged sites (Alagoz et al., 2015). The methyltransferase KMT3A (SETD2) catalyzes H3K14me3 and H3K36me3 to facilitate RPA complex loading onto chromatin and HR repair, respectively (Pfister et al., 2014; Zhu et al., 2021). KMT4 (DOT1L) methylates H4K79me1/2/3 to recruit 53BP1 to DSBs (Huyen et al., 2004). KMT5A (PR-Set7, SET8, SETD8) is responsible for H4K20me1, which is involved in DNA damage accumulation, 53BP1 recruitment, S-phase checkpoint, and ATR dependent cell cycle arrest (Beck et al.,

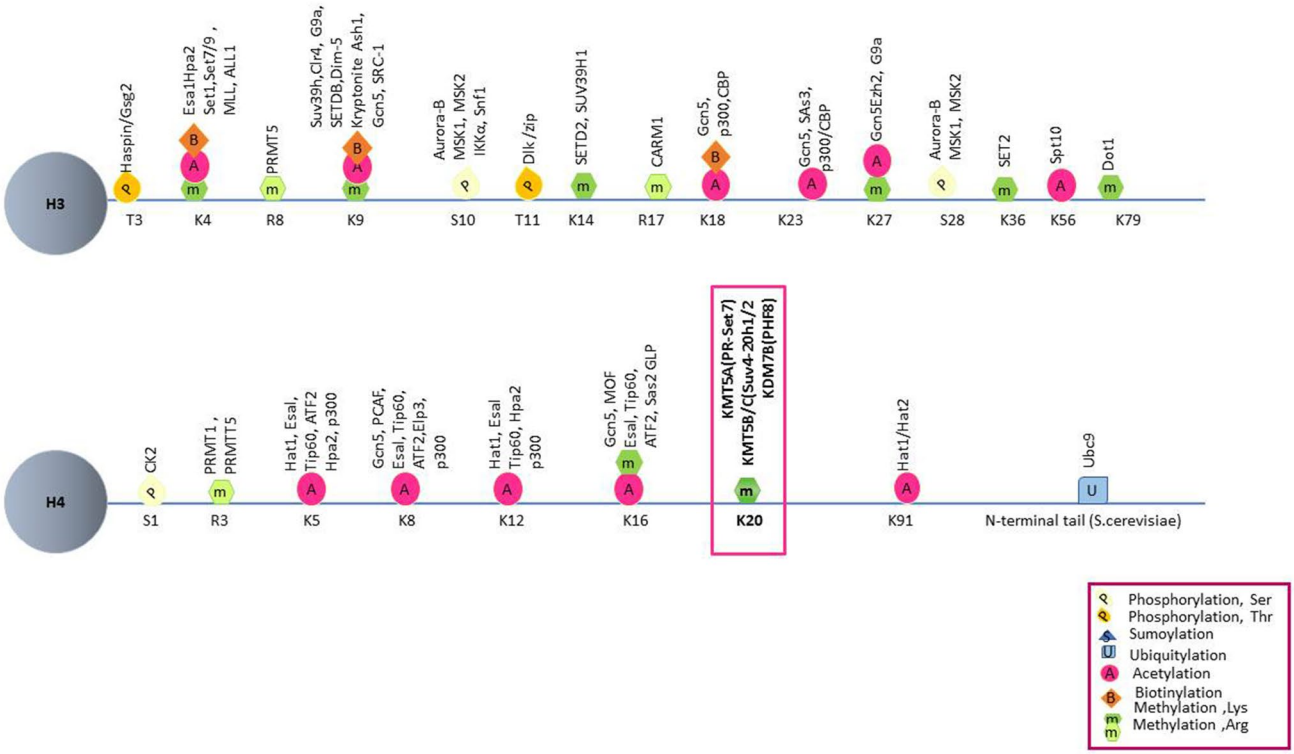

Fig. 2 Histone modifications and modifiers. Each residue with known modifiers on histones H3 and H4 are shown for each specific site

Table 1 Histone lysine-specific methyltransferases and DNA damage repair

Name	Synonym	Histone	Mechanism	References
KMT1A	SUV39H1	H3K9me3	ATM activation	Ayrapetov et al., (2014)
KMT1B	SUV39H2	H3K9me3 H2AXK134me2	γ-H2AX formation	Alagoz et al., (2015), Peters et al., (2001), Sone et al., (2014)
KMT1C	G9a EHMT2	H1.4K26me1/2 H3K9me1/2	Recruitment of HP1 and L3MBTL1	Trojer et al., (2009), Yang et al., (2017)
KMT1D	GLP EHMT1	H3K9me1/2 H4K16me1	53BP1 recruitment	Lu et al., (2019), Takahashi et al., (2012)
KMT1E	SETDB1	H3K9me3	53BP1 recruitment	Alagoz et al., (2015)
KMT3A	SETD2	H3K14me3 H3K36me3	RPA recruitment	Pfister et al., (2014), Zhu et al., (2021)
KMT3G	MMSET	H4K20me2/3	53BP1 recruitment	Pei et al., (2011)
KMT4	DOT1L	H3K79me1/2/3	53BP1 recruitment	Farooq et al., (2016), Huyen et al., (2004)
KMT5A	PR-Set7 SET8 SETD8	H4K20me1	53BP1 recruitment	Dulev et al. (2014), Li et al., (2016), Oda et al., (2010) Schotta et al., (2008)
KMT5B	SUV4-20H1	H4K20me2	Replication origins activation	Bromberg et al., (2017), Long et al., (2020), Schotta et al., (2008)
KMT5C	SUV4-20H2	H4K20me3	Repetitive elements silencing	Bromberg et al., (2017), Kapoor-Vazirani et al., (2011), Schotta et al., (2008)
KMT6A	EZH2	H3K27me2/3	Transcription silencing	Karakashev et al., (2020), Kuser-Abali et al., (2018)
KMT7	SETD7 SET7/9	H3K4me3	P53 activity, heterochromatin relaxation	Liu et al., (2011; Wang et al., (2013)
KMT8A	PRDM2 RIZ1	H3K9me2	BRCA1 recruitment	Khurana et al.,(2014)
SETMA	Metnase	H3K4me H3K36me2	Opening chromatin, DNA end-joining	Lee et al., (2005)

2012; Li et al., 2016; Tuzon et al., 2014). KMT5B (SUV4-20H1) catalyzes H4K20me2 to regulate the licensing and activation of early replication origins and maintains replication timing (Long et al., 2020). KMT5C (SUV4-20H2) catalyzes H4K20me3, allowing it to function in repetitive element silencing (Fodor et al., 2010), and the elimination of KMT5B/C proteins causes defected S-phase entry, whereas loss of KMT5A leads to fatal consequences (Schotta et al., 2004). KMT6A (EZH2) is responsible for H3K27me3 and accumulates at actively transcribes gene promoters during DDR (Karakashev et al., 2020; Kuser-Abali et al., 2018). KMT7 (SET7/9) demethylates P53 and SUV39H1 to facilitate an efficient DDR (Liu et al., 2011; Wang et al., 2013), KMT8A (PRDM2) promotes recruitment of BReast-CAncer susceptibility gene 1 (BRCA1), but not 53BP1, to contribute to chromatin condensation (Khurana et al., 2014). SETMA (Metnase) methylates H3K4 and H3K36, which are associated with chromatin opening (Lee et al., 2005). Although many studies confirmed the recruitment of different KTMs, their specific methylation site are not increased. For example, KMT6 accumulates at DSB site, while the increase of H3K27me3 is not detected during DNA damage repair (Zhang et al., 2021). Other substrates, including histones and non-histones, might be involved in this progress.

Histone demethylases and the DNA damage response

HDMs, which are responsible for methyl group removal, are divided into histone arginine demethylases and KDMs (Chang et al., 2019; Dimitrova et al. 2015; Shi et al., 2003, 2004). KDMs are further divided into two groups: the first is the mono-amine oxidases, which use flavin adenine dinucleotide (FAD) as a cofactor to oxidize methyl groups and hydrolyze them into formaldehyde, and the second is the Jumonji-C (JmjC) class, which uses Fe(II) and 2-oxoglutarate (2-OG or α-ketoglutarate) as cofactors to hydroxylate the methyl groups via a free-radical mechanism (Jambhekar et al. 2017). Many KDMs are reported to participate in the DDR (Table 2).

KDM1A (LSD1) specifically demethylates H3K4 methylation, which is linked to active transcription (Shi et al., 2004); KDM1A lies downstream of RING finger protein 168 (RNF168) but upstream of 53BP1 and BRCA1 in the DDR (Mosammaparast et al., 2013). KDM2A (JHDM1A, FBXL11), the demethylase for H3K36me3, acts as a substrate of *Ataxia telangiectasia* mutated kinase (ATM) to modulate the recruitment of the meiotic recombination 11 (MRE11) complex to DNA-damage sites (Cao et al., Cao, Shen, et al., 2016, Cao, Wei, et al., 2016). KDM2B (JHDM1B, FBXL10) is responsible for the timely dissociation of proliferating cell nuclear antigen (PCNA) from chromatin, allowing for efficient DNA replication (Kang et al., 2020). Four proteins belong to the KDM4, also known as JMJD2, encompasses four subfamilies, including KDM4A (JMJD2A), KDM4B (JMJD2B), KDM4C (JMJD2C), and KDM4D (JMJD2D), all of which have a JmjC domain. KDM4 demethylates H3K9me2/3, H3K36me2/3, and H1.4K26 to regulate gene expression and chromatin structure (Lee et al., 2020). The degradation of KDM4A and KDM4B restores the formation of 53BP1 foci in RING finger protein 8 (RNF8)- and RNF168-deficient cells (Mallette et al., 2012), and KDM4D knockdown disrupts the DNA-damage-induced association between ATM, RAD51, 53BP1, and chromatin (Khoury-Haddad et al., 2014). KDM5A-dependent H3K4me3 demethylation is required for Zinc finger and MYND (Myeloid, Nervy, and DEAF-1) domain containing 8 (ZMYND8)–Nucleosome remodeling and histone deacetylation (NuRD) recruitment to DNA damage sites (Gong et al., 2017). KDM5B contributes to the recruitment of Ku70 and BRCA1 to facilitate efficient DSB repair (Li et al., 2014). The KDM7 subfamily consists of KDM7A (KIAA1718), KDM7B (plant homeodomain

Table 2 Histone lysine-specific demethylases and DNA damage repair

Family	Subfamily	Alias	Substrate	Mechanism	References
KDM1	KDM1A	LSD1	H3K4me1/2	H2AX ubiquitylation,	Fang et al., (2010)
KDM2	KDM2A KDM2B	JHDM1A FBXL11 JHDM1B FBXL10	H3K36me3 H3K36me2 H3K4me1	ATM substrate, PCNA dissociation	Cao et al. (Cao, Shen, et al., 2016; Cao, Wei, et al., 2016), Kang et al., (2020)
KDM4	KDM4A KDM4B KDM4C KDM4D	JMJD2A JMJD2B JMJD2C JMJD2D	H3K9me2/3 H3K36me2/3 H1.4K26me2/3	53BP1 recruitment, Recruitment of ATM, Rad51 and 53BP1	Khoury-Haddad et al., (2014), Mallette et al., (2012)
KDM5	KDM5A KDM5B	JARID1A JARID1B	H3K4me3 H3K4me3	Recruitment of ZMYND8–NuRD Recruitment of Ku70 and BRCA1	Gong et al., (2017), Liu et al., (2011)
KDM7	KDM7B KDM7C	PHF8 PHF2	H4K20me1, H3K9me2	ATR activation, Recruitment of 53BP1 and BRCA1	Alonso-de Vega et al., (2020), Ma et al., (2021)

finger-containing protein 8 [PHF8]), and KDM7C (PHF2). KDM7C has recently been found to control the formation of 53BP1 and BRCA1 (Alonso-de Vega et al., 2020). Although many KDMs are reported to accumulate at DSB sites, our knowledge of most KDMs is largely lacking. The functions of two demethylases those are responsible for different forms of the same methylation site could be opposite. For example, deficiency of KDM1A, the demethylase of H3K4me2, increased the HR repair efficiency, whereas loss of KDM5A/B, the demethylases of H3K4me2/3, impaired HR (Li et al., 2014; Mosammaparast et al., 2013). This contradiction might indicate that additional non-histone sustrates are involved.

Histone H4 lysine 20 methylation

H4K20 was one of the earliest described histone modifications was discovered in 1969 and forms the majority of lysine residue methylation on the H4 N-terminal tail (Fang et al., 2002; Schotta et al., 2004). Studies have shown that H4K20 must first be mono-methylated then di- and trimethylated (Weirich et al., 2016; Wu et al., 2013). Several different methyltransferases and demethylases have been identified that regulate H4K20 methylation in mammals (Beck et al., 2012; Oda et al., 2010) (Fig. 3). KMT5A was previously recognized as the only mono-methyltransferase, whereas H4K20me2 and H4K20me3 are catalyzed by KMT5B/KMT5C (Nishioka et al., 2002; Schotta et al., 2008). KDM7B demethylates H4K20me1, while the demethylase for H4K2/3 is still largely unclear (Qi et al., 2010). Of note, KDM7B may not be the sole histone demethylase for H4K20me1, as KDM7B is not present in yeast (Klose et al., 2006), while H4K20me1 is (Edwards et al., 2011; Sanders et al., 2004). Consistent with this notion, using high-content cell-based screening of 2500 nuclear proteins, hHR23A was identified as a demethylase for H4K20me1/2/3 (X. Cao et al., 2020). In addition, DPY-21 was recently also found to be an H4K20me2 demethylase (Brejc et al., 2017). Since the expression and activity of KMT5A are regulated by the cell cycle, the methylation state of H4K20 exhibits dynamic change (Liu et al., 2010) (Fig. 4). H4K20me1 is in decline during the G1 phase, leading to the accumulation of the unmodified form of H4K20 at the beginning of the S phase. H4K20me1 accumulates at the G2/M phase and then is gradually converted to H4K20me2/3 (Oda et al.,

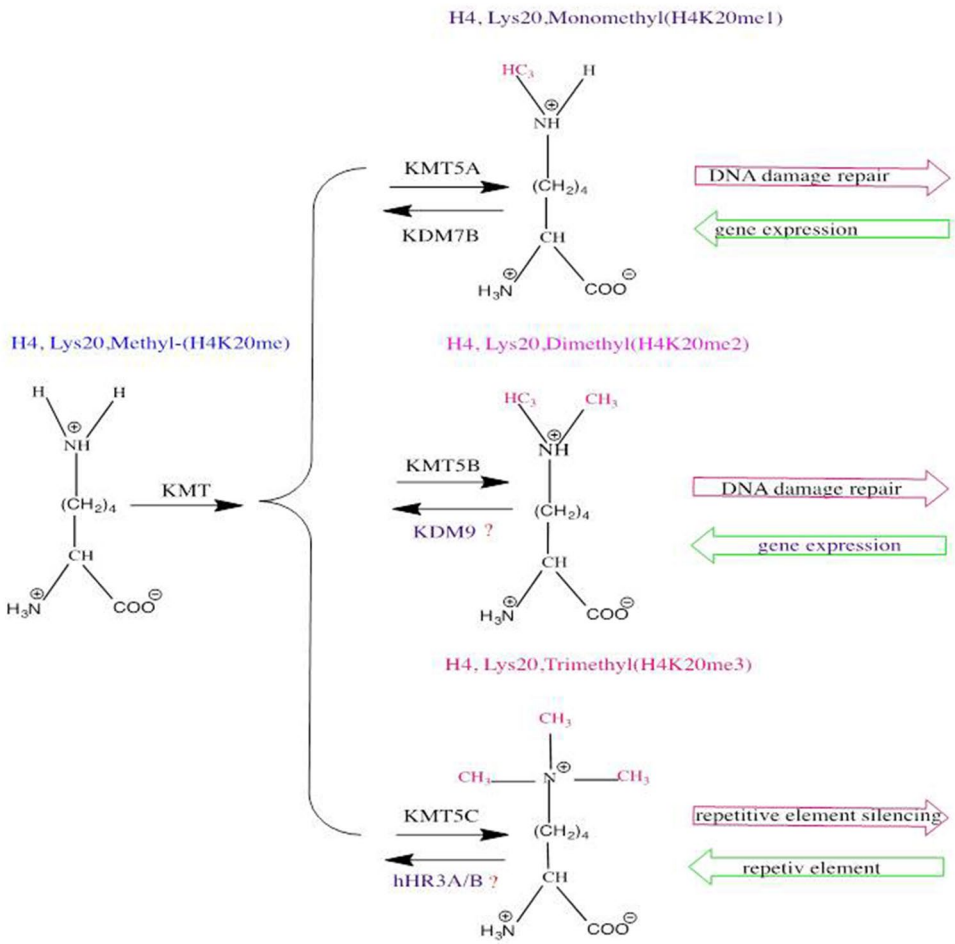

Fig. 3 Schematic diagram of H4K20 methylation. The different methylation states of H4K20 with the various mammalian enzymes that perform these post-translational modifications are shown

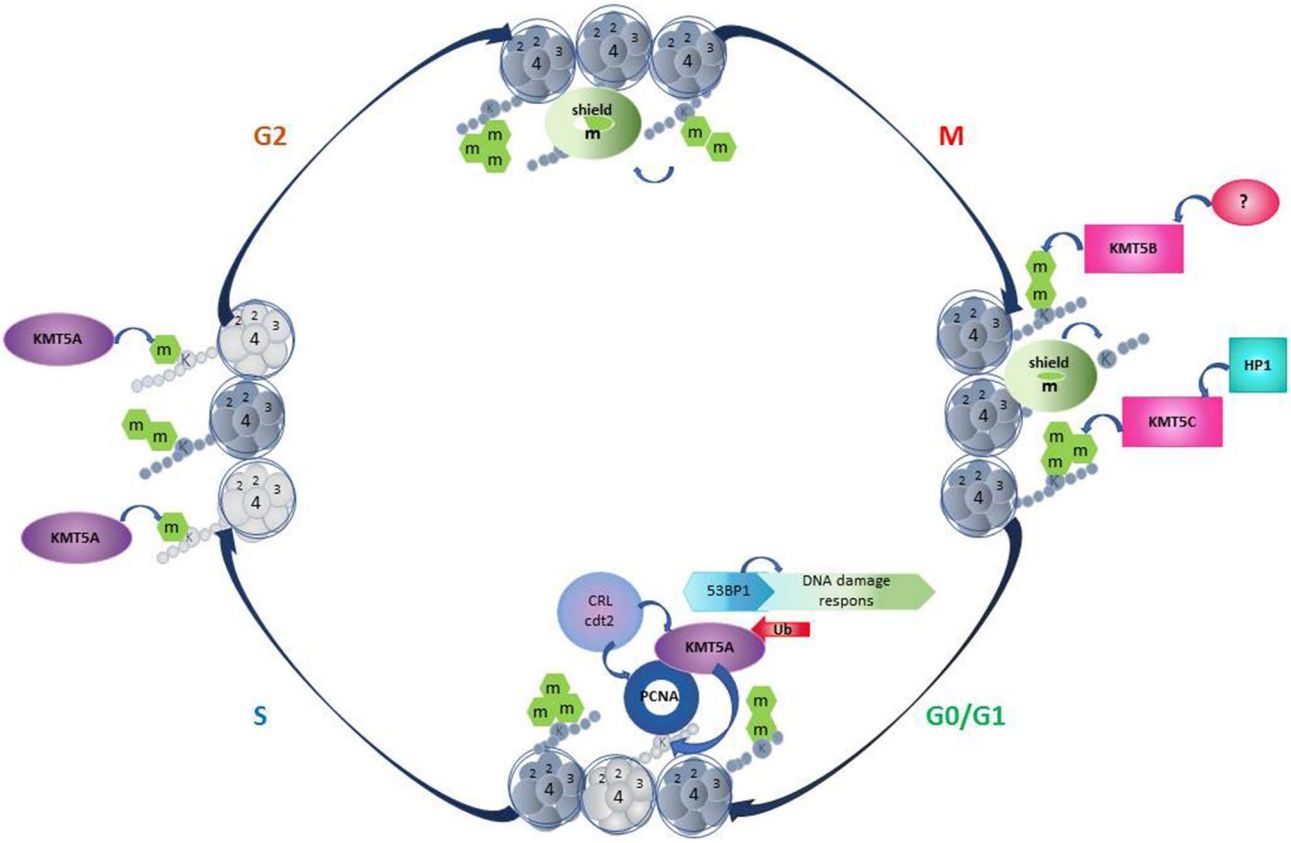

Fig. 4 Diagram of H4K20 methylation during cell cycle regulation. The dynamics of H4K20me1/2/3 and established enzymes during cell cycle progression are depicted

2009; Pesavento et al., 2008). H4K20 methylation plays an essential role in chromatin compaction, genome integrity, DNA damage repair, and DNA replication (Jorgensen et al., 2013). H4K20me1 and H4K20me2 are often associated with DNA damage repair and DNA replication after exposure to DNA damaging agents, whereas H4K20me3 is associated with the silencing of the heterochromatic state (Jorgensen et al., 2013).

Histone H4 lysine 20 mono-methylation

The fundamental role of H4K20me1 in DNA repair comes from analyses of the first identified mono-methyltransferase, KTM5A (Lu et al., 2021). Deletion of KTM5A results in lethality in mice because of the huge numbers of DSBs (Oda et al., 2009). In mammalian cells, RNA interference of KTM5A expression leads to increased γH2AX foci, recruitment of DNA repair proteins, and activation of DNA damage checkpoint (Paulsen et al., 2009; Sakaguchi & Steward, 2007). The significant role of KTM5A in DNA damage may partially lie in its regulation of the cell cycle, as it was shown that defects in KTM5A and H4K20me1 demethylation result in a delay in G1-S transition (W. Liu et al., 2010). In addition, loss of KTM5A causes DNA damage, specifically during DNA replication, which is abrogated by the co-depletion of RAD51, a critical HR repair factor. H4K20me1 functions in DNA damage in direct and indirect ways. On one hand, KMT5A-mediated H4K20me1 directly regulates chromatin structure, without other cofactors, to contribute to DNA repair. On the other hand, H4K20me1 serves as a platform for cooperation with its binders, including 53BP1, L3MBTL1, condensin II, and PCNA.

BRCA1 and 53BP1 play essential roles in DNA repair with HR and NHEJ, respectively (Shibata, 2017). 53BP1 is the DNA damage checkpoint protein required for an efficient NHEJ pathway (FitzGerald et al., 2009), and in the absence of 53BP1, BRCA1 promotes repair through the HR pathway (Shibata, 2017). RNF8 and RNF168 cause the ubiquitination of H2AK15 via its ubiquitination dependent recruitment (UDR) motif; then H2AK15ub is recognized by 53BP1 and facilitates 53BP1 foci formation in the NHEJ pathway (Fradet-Turcotte et al., 2013) (Fig. 5).

H4K20me1 is linked to 53BP1 in the DDR, an association that was first identified in *Schizosaccharomyces pombe*. Crb9, the homolog of 53BP1, binds to Set9-catalyzed H4K20me1 for its recruitment to DNA damage sites

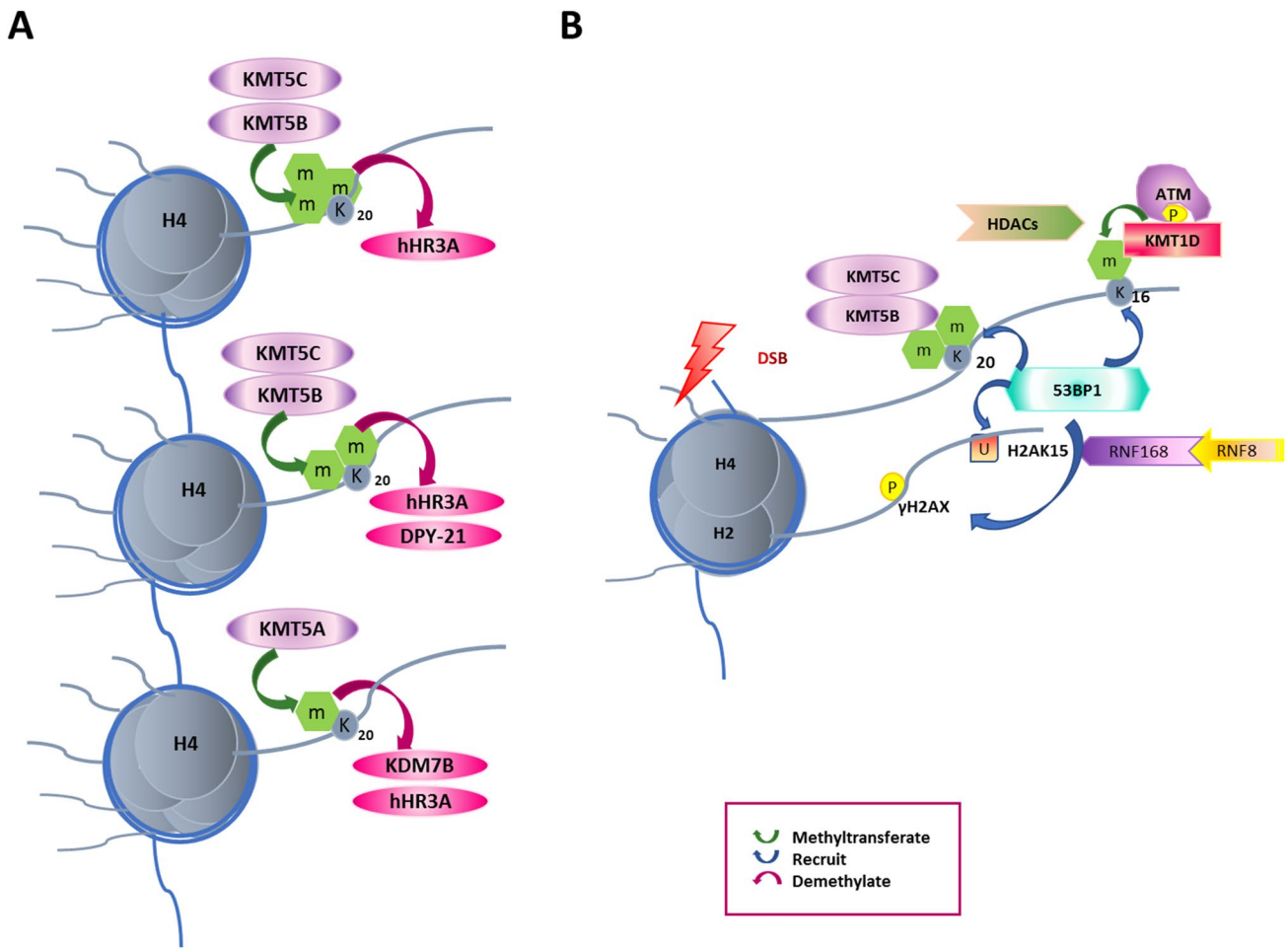

Fig. 5 53BP1 is highly correlated with H4K20 methylation during DDR. **A** Essential methyltransferases of H4K20me/1/2/3 are known as KMT5A/B/C. KDM4A and KDM7B regulate H4K20 di/tri-demethylation. The crosstalk between H4K20 and H4K16 is also indicated. **B** 53BP1 binds to H4K20me/1/2/3 to facilitate DDR through the NHEJ pathway. After DSBs, ATM is auto-phosphorylated and further phosphorylates H2AX. RNF8/RNF168 ubiquitinates H2AK15 and disturbs the association of 53BP1 and H4K20 methylation

(Sanders et al., 2004), an interaction that is also present in mammalian cells (Botuyan et al., 2006). A further study found that the tandem Tudor domain of 53BP1 is conserved and required for its recruitment. Knockdown of KMT5A inhibits 53BP1 recruitment to DNA damage sites and blocks checkpoint signaling, validating the idea that H4K20me1 contributes to 53BP1 recruitment (Oda et al., 2010). Interestingly, several studies demonstrated that Cullin ring-finger ubiquitin ligase 4 (CRL4, Cdt2) degrades chromatin-bound KMT5A via the PCNA-interacting motif (PIP-box). However, there is no increase in H4K20 methylation or recruitment of 53BP1 before KMT5A degradation (Abbas et al., 2010; Centore et al., 2010; Jorgensen et al., 2007). In addition, 53BP1 transfers to damaged sites even in the absence of KTM5A, indicating that there are other proteins and histone modifications involved in the 53BP1 recruitment platform and that KMT5A has other functions in addition to 53BP1 recruitment (Jorgensen et al., 2007; Tardat et al., 2007). In support of this notion, the ectopic expression of a non-degradable form of KMT5A led to chromatin condensation, activation of the DDR, and cell cycle progression failure (Beck et al., 2012).

Another factor that is recruited by H4K20me1 is L3MBTL1 (Trojer et al., 2007), which directly binds components of the replication machinery, potentially linking H4K20me1 with genome instability (Gurvich et al., 2010). Condensin II was also discovered to recognize H4K20me1 through its N-CAPD3 and N-CAPG2 subunits via two Huntingtin, elongation factor 3, protein phosphatase 2A, and lipid kinase TOR domains, and it is involved in mitotic progression (Liu et al., 2010).

Histone H4 lysine20 di/tri-methylation

H4K20me2 is an extremely abundant marker exhibited by more than 80% of nucleosomes, whereas H4K20me1 and

H4K20me3 are present at comparably low levels. KMT5B and KMT5C are responsible for most of the di- and trimethylation of H4K20, respectively (Schotta et al., 2004). KMT5B and KMT5C require SAM as a co-factor in the transfer of a methyl group to proteins, lipids, or nucleic acid substrates (Schotta et al., 2004; Southall et al., 2014; Wu et al., 2013). The KMT5B/C enzymes are characteristics of the DDR; they maintain telomere length and regulate heterochromatin compaction, and therefore are linked to genome instability (Schotta et al., 2008). In human cells, an inhibitor of KMT5B/C (A-196) has been shown to significantly impact genome integrity (Bromberg et al., 2017).

H4K20me2 has been observed to further accumulate at DNA-damage sites and to play a role in DNA-damage repair mainly by serving as a 53BP1-binding platform (Pei et al., 2011). In agreement with this, KMT5A was reportedly insufficient for 53BP1 recruitment. The de novo H4K20me1 facilitates KTM5B/C recruitment and catalyzes H4K20me2/3 to regulate 53BP1 binding (Tuzon et al., 2014). Actually, peptide-affinity studies suggested that 53BP1 has a higher affinity for H4K20me2 peptide than for H4K20me1 peptide (Schotta et al., 2004). KTM5B/C double-knockout cells showed significantly delayed 53BP1 foci formation (Schotta et al., 2008). In addition to KTM5B/C, multiple myeloma SET domain-containing protein (MMSET), the primary methyltransferase for H3K36me, has also been identified as a methyltransferase for H4K20me2/3, but not H4K20me1. The downregulation of MMSET significantly decreases the accumulation of 53BP1 (Pei et al., 2011). Besides methyltransferases, several other proteins containing a Tudor domain bind to H4K20me2 to regulate 53BP1 recruitment in response to DNA damage. KDM4A competes with 53BP1 for binding to H4K20me2 and facilitates 53BP1 movement to sites of damage; KDM4A is degraded in an RNF8- and RNF168-dependent manner after DNA damage (Mallette et al., 2012). In contrast to human L3MBTL1, which mainly binds to H4K20me1, dl(3)MBT binds to all three methylated H4K20 peptides in *Drosophila* cells, with the highest affinity being for H4K20me1/2 (Scharf et al., 2009). The ATPase activity of valosin-containing protein promotes the release of L3MBTL1 from chromatin; L3MBTL1 binds to H4K20me2, thereby facilitating 53BP1 recruitment (Acs et al., 2011). Other histone modifications can occupy 53BP1 to disrupt its binding with H4K20me2 and thus direct DNA repair. H3K79me2 is primarily responsible for 53BP1 binding in budding yeast (Chen & Zhu, 2016). In human cells, although both methylations H3K79 and H4K20 are able to bind to 53BP1, H4K20 methylation binds more tightly. Additionally, TIP60-catalyzed H4K16ac impacts the ability of 53BP1 to bind neighboring H4K20me2 (Panier & Boulton, 2014; Tang et al., 2013). Similarly, 53BP1 recognizes mononucleosomes containing both H4K20me2 and H2AK15ub as a dimer using its methyl-lysine-binding Tudor domain and UDR motif, respectively (Fradet-Turcotte et al., 2013). Interestingly, the ubiquitylation of H2AK15 by RNF168 can be blocked by Tat-interactive protein 60-kDa (Tip60)-catalyzed acetylation of H2AK15 in cis form (Jacquet et al., 2016); therefore, TIP60 regulates 53BP1-dependent repair through the competitive bivalent modification of chromatin.

H4K20me3 is enriched in constitutive heterochromatin and regulates the stability of telomere and heterochromatin structures (Jorgensen et al., 2013). Because the specific partner proteins of H4K20me3 are unknown, the detailed mechanisms of how H4K20me3 regulates heterochromatin and DNA damage are not understood.

Histone H4 lysine20 demethylation

KDM7B is the first reported demethylase of H4K20me1 that is also linked to the enzymes related to cell cycle progression and proliferation (Liu et al., 2010) (Yatim et al., 2012). The stabilization of histone demethylase KDM7B by ubiquitin specific protease 7 is augmented during DNA damage (Wang et al., 2016). Replication stress results in KDM7B phosphorylation and dissociation from topoisomerase IIβ-binding protein 1 (TOPBP1). Consequently, hypomethylated TOPBP1 facilitates RADiation-sensitive 9-binding to chromatin to fully activate ATR and thus safeguard the genome and protect cells against replication stress (Feng et al., 2020; Ma et al., 2021). Although many studies have observed the role of KDM7B in transcription silencing, further studies are needed to elaborate on the role of KDM7B in the DDR.

Perspectives and concluding remarks

In contrast to DNA methylation, which only exists in higher eukaryotes, histone methylation is conserved in organisms such as *Drosophila melanogaster* and *C. elegans*, in which DNA methylation is absent. In addition, histone methylation is a reversible event that is modified by methyltransferases and demethylases. Therefore, dynamic histone methylation is indispensable for maintaining normal biological function. During the past two decades, there has been several progress are reported to be regulated by H4K20 methylation. On one hand, H4K20 methylation is able to directly affect chromatin compaction. On the other hand, different degrees of H4K20me serve as a binding platform for a variety of effectors, and therefore, the modification is associated with diverse functions. The identification of novel proteins that either regulate H4K20 modulators or recognize different degrees of H4K20 methylation will enrich our knowledge of the roles of H4K20 methylation.

Methylation can occur at multiple different sites on the same histone, and one site can incur different modifications. Different combinations of histone modifications can alter

the recognition and binding of DDR factors. For example, H4K16ac adjacent to H4K20me2 affects 53BP1 binding through the disruption of H4K16 and Tudor domain interactions (Lu et al., 2019). Because histone methylation marks do not appear in isolation, it is important to determine the collaborative and antagonistic relationships among different histone marks.

The importance of histone methylation is also being demonstrated by emerging evidence that links histone methylation to disease and ageing. At present, a select group of demethylase inhibitors is being evaluated in clinical trials (Wen et al., 2019). One of the most studied inhibitors is tranylcypromine, which efficiently inhibits KDM1A by forming a covalent adduct with the FAD cofactor, and two clinical trials using tranylcypromine to cure acute myeloid leukemia and myelodysplastic syndrome are underway (Jambhekar et al., 2017). The removal of H4K20 methyltransferases results in lethality, and loss of H4K20me3 is a hallmark of many human cancers. However, there are still no specific inhibitors of H4K20me demethylases. Developing highly efficient inhibitors is of significance to transfer our understanding of methylation from the bench to the bedside.

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Declarations

Conflict of interest Author Wei-Guo Zhu is Editor-in-Chief of Genome Instability & Disease.

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