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Review

Review

Biological function and regulation of histone and non-histone lysine methylation in response to DNA damage

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Abstract

DNA damage response (DDR) signaling network is initiated to protect cells from various exogenous and endogenous damage resources. Timely and accurate regulation of DDR proteins is required for distinct DNA damage repair pathways. Post-translational modifications of histone and non-histone proteins play a vital role in the DDR factor foci formation and signaling pathway. Phosphorylation, ubiquitylation, SUMOylation, neddylation, poly(ADP-ribosyl)ation, acetylation, and methylation are all involved in the spatial–temporal regulation of DDR, among which phosphorylation and ubiquitylation are well studied. Studies in the past decade also revealed extensive roles of lysine methylation in response to DNA damage. Lysine methylation is finely regulated by plenty of lysine methyltransferases, lysine demethylases, and can be recognized by proteins with chromodomain, plant homeodomain, Tudor domain, malignant brain tumor domain, or proline–tryptophan–tryptophan–proline domain. In this review, we outline the dynamics and regulation of histone lysine methylation at canonical (H3K4, H3K9, H3K27, H3K36, H3K79, and H4K20) and non-canonical sites after DNA damage, and discuss their context-specific functions in DDR protein recruitment or extraction, chromatin environment establishment, and transcriptional regulation. We also present the emerging advances of lysine methylation in non-histone proteins during DDR.

Key words: DNA damage response, lysine methylation, histone methylation

Introduction

Cells are under attack all the time from various exogenous or endogenous DNA damage stimulations. Commonly employed exogenous resources include physical ionizing radiation (IR), ultraviolet (UV) light, and various chemical agents, such as alkylating agent methyl methanesulfonate (MMS), cross-linking agent cisplatin, topoisomerase inhibitors doxorubicin (adriamycin), and etoposide (VP-16). The types of DNA lesion induced by specific treatment are diverse, thus cause various cellular impacts and are repaired by distinct pathways [1,2]. For example, cyclobutane-pyrimidine dimers and 6–4 photoproducts induced by UV light are mainly repaired by photoreactivation, base excision repair, nucleotide excision repair (NER), and mismatch repair (MMR), while IR-induced single-strand break (SSB) and double-strand break (DSB) are repaired by single-strand break repair, non-homologous end-joining (NHEJ), and homologous recombination (HR), respectively [3–7]. Upon DNA damage, cells initiate a rapid DNA damage response (DDR) signaling network that allows DNA lesion sensing

and signaling (including DNA damage checkpoint) and triggers a wide variety of processes, including chromatin remodeling, cell cycle arrest, RNA processing, DNA repair, autophagy, apoptosis, and senescence [1,8–10]. These events collaboratively establish favorable environments for DNA repair, regulate the occurring biological processes, and activate apoptosis when needed to prevent the DNA damage-induced genome instability.

It is now widely accepted that protein post-translational modifications (PTMs) play prominent roles in DDR factors foci formation and signaling [9,11]. Beyond the most well-studied phosphorylation and ubiquitination, other PTMs also play emerging roles in various DDRs, such as methylation, acetylation, poly (ADP-ribos)ylation (PARylation), sumoylation, and neddylation [12]. Eukaryotic genome is organized as chromatin which consists of histones and DNA. Histone modifications play multiple roles in DDR signaling and DNA damage repair [12–14]. Methylations on lysine produce different forms (mono-, di-, or trimethylation) and are modified by lysine methyltransferases and lysine demethylases targeting both histone and non-histone proteins. Recent studies show that increasing lysine methylation and modifying enzymes are involved in DDR, and lysine methylation is becoming an important signaling mechanism. Besides phosphorylation, the role of histone acetylation in DDR regulation has also been extensively studied. In addition, many histone deacetylase inhibitors (HDACi) are proved to be very effective on cancer cell lines. Several of them have been approved by Food and Drug Administration and are being used in clinics [15]. However, histone methylation is still at an early stage in DNA damage research, and this is why it is emerging to focus on histone methylation. Here, we focus on reviewing the dynamics and regulation of lysine methylation upon DNA damage, their diverse functions in DDR signaling and DNA damage repair, with primarily strengthening on histone methylation.

Histone Methylation

The main widely studied histone lysine methylation sites include H3K4, H3K9, H3K27, H3K36, H3K79, and H4K20. Besides their canonical roles in transcriptional activation/elongation and suppression, they have also been implicated in DNA replication, chromatin remodeling, as well as DDR and repair [16]. These lysine residues mainly localize on the N-terminal tail of histone H3 or H4, with the exception of H3K79 [17]. Histone methylation mainly functions through recruitment of non-histone proteins, because the methyl group is relatively small and does not alter the basic charge of amino-acid residue, unlike phosphorylation and acetylation [16]. The histone lysine sites and the modifying enzymes involved in DDR are summarized in **Fig. 1**.

H3K4 methylation

H3K4 methylation by Set1 in yeast and the homologous mixed-lineage leukemia complex in mammalian cells have been linked to transcriptional active genes, and are indirectly regulated by a conserved mechanism through E2 ubiquitin-conjugating enzyme and E3 ubiquitin ligase complex Rad6/Bre1-dependent H2B monoubiquitination [18,19]. Consistent with the role of H2BK123 ubiquitination by Rad6/Bre1 complex in DNA damage checkpoint [20] and the identification of H3K4 methylation by PRDM9 (PR domain 9) as a mark of meiotic DSB formation and recombination [21], Set1 as well as H3K4me3 accumulates at newly created DSBs induced by homothallic switching endonuclease (HO) in budding yeast [22] or by I-SceI endonuclease in DR-GFP HeLa cells, which is dependent on H2B ubiquitination by E3 ubiquitin protein ligase RNF20 (ring finger protein 20) [23], the mammalian homology of Bre1 in yeast. This recruitment of Set1 is dependent on remodels the structure of chromatin (RSC) complex, a chromatin remodeler, and cells lacking Set1 and H3K4 methylation display a significant defect in DSB

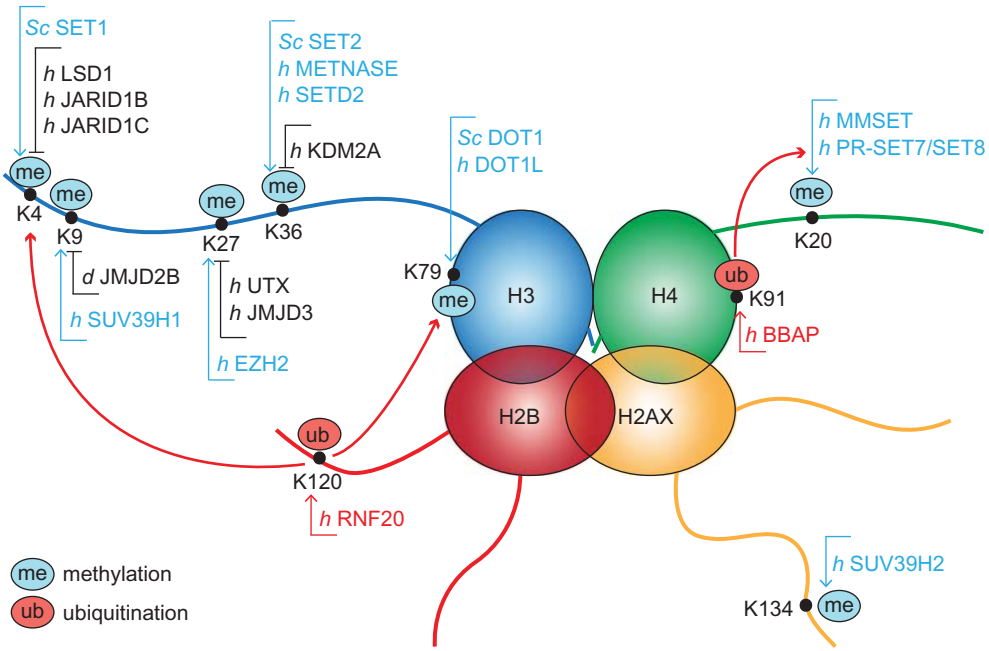

Figure 1. Positions and modifiers of histone lysine methylation in DDR Numbers indicate the lysine residues for methylation or ubiquitination. Blue arrows indicate the methyltransferases responsible for the corresponding methylation, red arrows indicate the cross-talk between ubiquitination and methylation, and black rests indicate the demethylases of each lysine methylation. Italics indicate the organisms in which the enzymes are found: Sc, *Saccharomyces cerevisiae*; h, *Homo sapiens*; and d, *Drosophila melanogaster*.

repair by the NHEJ pathway and difficulty to pass through S-phase under replication stress [22], which is similar to RSC-deficient cells [24,25]. However, global increase of H3K4me3 in chromatin is not detected following DNA-damaging agent phleomycin or radiomimetic drug neocarzinostatin (NCS) treatment [22,26]. The failure to detect an increase in global H3K4 methylation after various DNA damage treatments could be explained by relatively high basal level of H3K4 methylation and the insensitivity to small differences of immunoblotting analysis. The induction of H3K4 methylation [23] and the failure of observation for H3K4me2 [26] at DSB sites raised the possibility that H3K4me3 is mostly contributed to the DDR.

Given that transcription is largely repressed at local DSB sites [27] and H3K4 methylation does not change globally, the inducible H3K4 methylation is not considered to be linked to transcriptional activation. Intrinsically, chromatin remodeling is required for both efficient transcription and DNA repair. The chromatin remodeler RSC-dependent H3K4me3 by Set1 is speculated to be important for relative chromatin opening [22]. During transcription, the chromatin remodeling ATPase Isw1p in yeast and hSNF2H in human are recruited by H3K4me2/3 [28]. Interestingly, SNF2H is also enriched at the damage sites [23], and this suggests that H3K4me3 facilitates the recruitment of SNF2H. However, inhibitor of growth (ING) family proteins specifically recognize H3K4me3 via their plant homeodomains (PHD) and are involved in the control of cell growth, chromatin remodeling, DNA repair, and apoptosis [29]. For example, H3K4me3 binding is necessary for ING1 to stimulate DNA repair after UV irradiation and promote DNA damage-induced apoptosis [30] (**Fig. 2A**). These studies indicate that induced

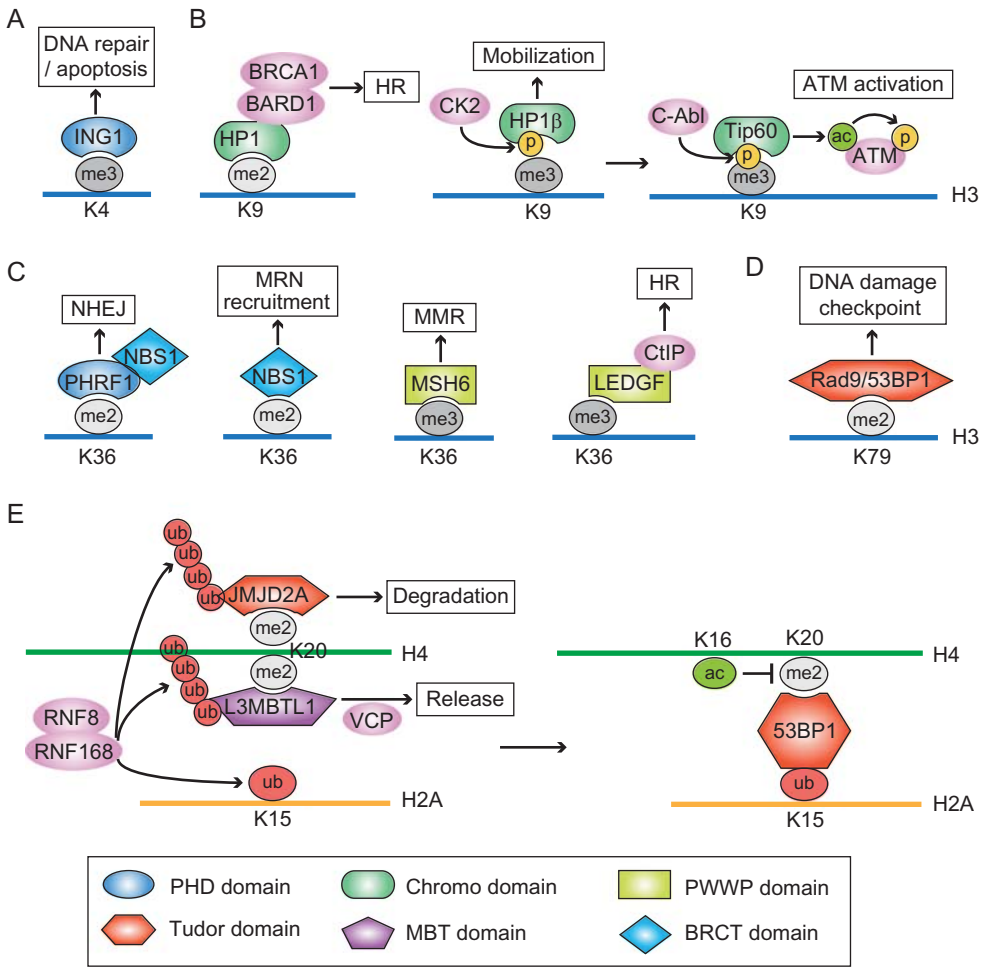

Figure 2. Dynamics and functions of histone lysine methylation readers in DDR (A) ING1 recognizes H3K4 methylation via PHD domain for DNA repair or apoptosis after UV treatment. (B) CK2-dependent Thr51 phosphorylation promotes HP1β mobilization from H3K9me3, then C-Abl-mediated Tyr44 phosphorylation of Tip60 is recruited for ATM acetylation and activation, as well as H4K16 acetylation. H3K9me2 promotes BARD1/BRCA1 complex recruitment and homologous repair through interaction with HP1. (C) H3K36me3 participates in various DNA repair pathways. H3K36me3 is recognized by PWWP domain-containing protein LEDGF, which is required for CtIP recruitment and homologous repair and MSH6, which mediates MMR. H3K36me2 is also recognized by PHD domain of PHRF1, which binds to NBS1 and promotes NHEJ repair. H3K36me2 can also directly bind to non-canonical BRCT2 domain of NBS1 for MRN complex recruitment. (D) H3K79me2 recruits Rad9 for DNA damage checkpoint in *S. cerevisiae*. H3K79me2 is also required for 53BP1 recruitment in mammalian G1/G2 cells when H4K20me2 levels are low. (E) RNF8- and RNF168-dependent polyubiquitination promotes degradation of JMJD2A and VCP-mediated release of L3MBTL1 from chromatin and exposes H4K20me2. RNF168 also catalyzes H2A Lys15 ubiquitination, which promotes 53BP1 recruitment with exposed H4K20me2. Reader domains: PHD, plant homeodomain; PWWP, proline-tryptophan-tryptophan-proline domain; MBT, malignant brain tumor domain; BRCT, BRCA1 C-terminus domain. Readers: ING1, inhibitor of growth family, Member 1; LEDGF, lens epithelium-derived growth factor; MSH6, MutS homolog 6; PHRF1, PHD and ring finger domains 1; 53BP1, p53-binding protein 1; HP1, heterochromatin protein 1; JMJD2A, Jumonji domain-containing 2 A; L3MBTL1, Lethal(3)malignant brain tumor-like protein 1.

H3K4me3 mainly serves as a docking site for related 'reader' proteins participated in DDR.

However, several studies later revealed that H3K4me2/3 could also be down-regulated at DNA damage sites. H3K4me2 is reduced at DNA damage sites induced by UV laser microirradiation and I-PpoI endonuclease in an LSD1 (lysine-specific demethylase 1 A)-dependent manner [31]. This H3K4me2 demethylation primarily occurs in late S/G2 cells, when LSD1 protein levels are higher. The fact that LSD1 promotes tumor suppressor 53BP1 (p53-binding protein 1) recruitment in late S/G2 cells by promoting H2A/H2A.X ubiquitination downstream of RNF168 (ring finger protein 168) recruitment suggests that H3K4me2 might promote HR, as revealed by modestly increased HR in LSD1-deficient cells. H3K4me3 is also inconsistently decreased upon I-SceI transfection in DR-GFP U2OS cells in a KDM5B-dependent manner, which requires PARP1 [poly (ADP-ribose) polymerase 1]-mediated PARylation and histone variant macroH2A1.1 [32]. The decrease of H3K4me3 is required for efficient recruitment of Ku70 and BRCA1 (breast cancer 1), essential factors for NHEJ and HR, respectively. Although the reduction of H3K4 methylation at DSB sites might promote HR [31], the direct role of H3K4 methylation remains to be investigated *in vivo*, despite *in vitro* peptide pull-down analysis showed that BRCA1 bound to H3-unmethylated peptides much more efficiently than that in H3K4me3 peptides [32].

The results above suggest that the spatio-temporal regulation and function of H3K4 methylation might be in a context-specific manner. However, the global levels of H3K4me2 and H3K4me3 are generally constant, but the DNA damage site-specific alterations are seemly controversial, perhaps due to the lack of time-course kinetic analysis in living cells. For example, LSD1 is recruited to microirradiation sites within 10 min, and then reduced significantly 60 min after treatment [31]. This indicates that the recruitment of LSD1 is rapid, but the retention is relatively transient. More elaborative observations are needed to elucidate the functions of H3K4 methylation in DDR.

H3K9 methylation

H3K9 methylation by G9a and SUV39H1 has been shown to correlate with transcriptional repression. H3K9me3 is also crucial for pericentric heterochromatin binding by HP1 (heterochromatin protein 1) specifically dependent on SUV39H1 [33]. Chromodomain-containing HP1 proteins are important H3K9 methylation-binding partners and are crucial for heterochromatin assembly and maintenance, gene expression regulation, and DNA replication [34,35]. HP1β is phosphorylated on Thr51 by CK2 (casein kinase 2) and released from chromatin within 5 min after IR by disrupting the interaction between its chromodomain and H3K9 methylation [36]. This mobilization of HP1β is independent on DNA damage-induced H3K9me3, as neither the distribution nor abundance of H3K9me3 is altered [36–38]. The exposed H3K9me3 facilitates Mre11/Rad50/NBS1 (MRN) complex-dependent Tip60 recruitment via chromodomain and activates its acetyltransferase activity [39], which is required for ATM (ataxia–telangiectasia-mutated) protein kinase acetylation and activation [40,41] (**Fig. 2B**). In contrast to HP1β, Tyr44 phosphorylation in chromodomain of Tip60 by the proto-oncogene c-Abl upon IR promotes Tip60 binding to H3K9me3 [42]. These studies present sequential mobilization of HP1β and replacement of Tip60 via H3K9me3 after IR treatment.

Although HP1β mobilizes from H3K9me3 upon DNA damage, three isoforms of HP1, HP1α, HP1β, and HP1γ, are all recruited to UV- and IR-induced DNA damage and oxidative lesions in human cells [37,43]. However, these recruitments are dependent on the chromo shadow domain of HP1, rather than the chromodomain, indicating its independence of H3K9me3. HP1α is recruited to laser-induced damage sites dependent on the interaction with PxVxL domain of p150CAF-1, the largest subunit of chromatin assembly factor 1 (CAF-1) [38]. HP1α accumulation is early downstream of MDC1 (mediator of DNA damage checkpoint 1), rapidly disappears within 30 min as well as HP1β and HP1γ, and is required for 53BP1, BRCA1, and DNA recombinase RAD51 accumulation at damage sites. The *de novo* accumulation of HP1 is not inconsistent with increased HP1β mobilization, because reaccumulation of HP1β was observed gradually over 90 min [36]. These results above suggested that the exposed H3K9me3 with HP1β mobilization is protected by Tip60, and HP1 proteins are involved in novel functions with DDR factors dependent on chromo shadow domain.

Interestingly, the local H3K9me3 near DSBs can be intrinsically dynamic at euchromatin, as H3K9me3 primarily localizes within heterochromatin regions without DNA damage. A complex containing Kap-1, HP1, and the H3K9 methyltransferase SUV39H1 moves onto the chromatin at DSBs and methylates H3K9 in a PARP1-dependent manner, and the H3K9 trimethylation further facilitates the recruitment of Kap-1/HP1/SUV39H1 [44]. This positive feedback allows spreading of Kap-1/HP1/SUV39H1 and H3K9me3 for tens of kilobases away from DSB sites. Importantly, the subsequent activated ATM removes SUV39H1 complex through Kap-1phosphorylation by ATM [45,46] and functions as a negative feedback loop. Consistently, the levels of H3K9me3 are increased adjacent to DSB sites induced by p84-zinc finger nuclease (p84-ZFN) in intron 1 of the PPP1R12C gene within opening chromatin domains which lacks H3K9me2/3. The transient increase of local H3K9me3 is dependent on SUV39H1, and is proposed to be important for rapid formation of repressive chromatin and recruitment of Tip60 especially in euchromatin domains. The studies above collaboratively indicate that H3K9me3 are exposed at heterochromatin while transiently increased at euchromatin, for the establishment of local chromatin environment and the recruitment of DDR factors.

Increase of H3K9me2 is also observed at I-SceI induced DSB sites [47,48], but the function of induced H3K9me2 is largely unexplored. Recently, BARD1 (BRCA1-associated RING domain protein 1) was found to bind to H3K9me2 through the interaction between its conserved PxVxL motif in BRCT domain and HP1 chromo shadow domain [49] (**Fig. 2B**). Although the H3K9-specific inhibitor UNC0638 disrupts the retention of BRCA1/BARD1, the function of H3K9me2 remains to be further investigated, as previous study showed that HP1 recruitment is dependent on chromo shadow domain, but not on H3K9 methylation-binding chromodomain [37].

Global decrease of H3K9 methylation has also been reported to be responsible for global chromatin relaxation after DNA damage [50]. A rapid global decrease in both H3K9me2 and H3K9me3 was observed at very early time after exposure to γ-radiation in U2OS cells [51]. H3K9me2 decreases from 15 min and is recovered within 60 min, while H3K9me3 is transiently suppressed from 5 to 15 min. Loss of H3K9me2 at laser micro-irradiation-induced damage sites was also detected in U2OS cells expressing low level GFP-KDM4B. The decrease is probably dependent on KDM4B/D, but not on KDM4A/C, which is recruited to laser microirradiation-induced DNA damage sites very rapidly by PARP1, rather than ATM [51,52]. The loss of H3K9me2/3 is suggested to contribute to local chromatin relaxation. SUV39H1 is responsible for the establishment and maintenance of heterochromatin [53]. We revealed that

H3K9me3 at centromeric satellite loci is down-regulated by the suppression of SUV39H1 activity, which is methylated at lysines 105 and 123 by SET7/9 upon DNA damage [54]. This significant decrease of H3K9me3 results in elevated expression of α-satellite2, and heterochromatin over-relaxation [54]. Moreover, expression of KDM4B and suppression of SUV39H1 by p53 also contribute to global decrease of H3K9me3 and heterochromatin relaxation after UV or IR in *Drosophila* and HCT116 cells [55,56]. Collectively, decrease of H3K9 methylation is important for global chromatin relaxation at early and late time points after DNA damage.

Interestingly, DNA damage-induced H3K9 methylation changes are also associated with DNA demethylation. For example, depsipeptide, an HDACi, was also shown to be a potent DNA damage inducer [57]. After treatment with depsipeptide at very low doses, expression of SUV39H1 and G9a was significantly decreased, by which histone H3K9me2 and H3K9me3 were significantly decreased globally [58]. Consequently, HP1 and DNA methyltransferase 1 (DNMT1) were decreased at chromatin, which eventually leads to DNA methylation deficiency in several human cancer cells. These examples reveal that DNA damage may also erase DNA methylation pattern by a different pathway.

H3K27 methylation

In addition to H3K9 methylation, H3K27 methylation has also been linked to silencing phenomena including PcG-mediated gene silencing, X chromosome inactivation, and gene imprinting [59–61]. Polycomb repressive complex 2 (PRC2) methylates H3K27me2 and H3K27me3 via its catalytic subunits EZH1 and EZH2 [62,63]. Enrichment of EZH2 is demonstrated to be near the break site induced by I-SceI endonuclease in the promoter of the E-cadherin CpG island, which is frequently and aberrantly DNA-hypermethylated in epithelial cells or DNA lesions by UV laser microirradiation, but not at ionizing radiation-induced foci (IRIF) [48,64,65]. Enrichment of sirtuin 1 (SIRT1), DNMT1, DNMT3B, hypoacetyl H4K16, H3K9me2/3, and H3K27me3 appears as well, indicating their potential role in local gene silencing. The recruitment of EZH2 is dependent on PARP, rather than ATM or γ-H2AX [64]. Enrichment of H3K27me3 at UV laser microirradiated damage tracks was observed by Chou *et al.* [64] in mouse embryonic fibroblast (MEF) cells, but not observed by Campbell *et al.* [66] in U2OS cells, which is consistent with an earlier study [66]. H3K27me3 can recruit chromodomain-containing polycomb protein [67]. The elevated H3K27me3 possibly contributes to PARP-dependent transcriptional repression by removing nascent DNA and elongating RNA polymerase II from sites adjacent to DNA breaks, since MEL-18/PCGF2 and BMI1/PCGF4 subunits of PRC1 complex are also recruited to sites of damage [64,68,69]. Although how EZH2 binds to chromatin and methylates H3K27me3 is controversial, our recent study indicated that EZH2 is recruited by MTA2, a submember of NuRD complex, in which MTA exhibits histone-binding activity [70]. The interaction between NuRD and PRC2 complex via MTA2 and EZH2 is also context dependent. ChIP sequencing data analysis showed that both EZH2 and MTA2 are well co-occupied in the promoters of DDR genes and autophagy-related genes, indicating that EZH2 and MTA2 may be involved in DNA damage and repair.

H3K27me3 also plays roles in transcriptional regulation of specific genes after DNA damage treatments. For example, there is enrichment of EZH2 and H3K27me3 at the promoters of high-expression genes upon oxidative damage with H_2O_2 for 30 min, with the reduction of H3K4me3 and H4K16ac at the same regions [71]. In addition, EZH2-mediated H3K27me3 suppresses the expression of FBXO32 which binds to and promotes the degradation of p21 in a p53-dependent manner after treatment with DNA-damaging agents adriamycin and etoposide, thus regulating DNA damage checkpoints [72]. Moreover, H3K27 demethylases UTX and JMJD3 (Jumonji domain-containing protein 3) are recruited to promote the expression of specific genes, including Ku80, in a p53-dependent manner upon exposure to IR [73,74]. These studies above collectively indicate that H3K27me3 functions in local and gene-specific transcriptional repression upon DNA damage.

H3K36 methylation

Besides its association with transcriptional activation, H3K36 methylation has also been linked to multiple biological processes, including transcriptional suppression, DNA methylation, alternative splicing, DNA replication, as well as DDR and repair [75]. H3K36me2 is rapidly induced globally and locally after IR or etoposide treatment in a very rapid manner [47,76]. The increase of H3K36me2 at I-SceI-induced DSB site is mediated by recruiting Metnase (also SETMAR) and is required for NHEJ repair [47], which is in agreement with the role of Metnase in NHEJ [77,78]. The recruitment of Metnase relies on phosphorylation of Ser495 by Chk1, but not by ATM [79]. We also found that dissociation of H3K36me2-specific demethylase JHDM1a/KDM2A with chromatin is also critical for this process, and this dissociation between KDM2A and chromatin is a result of ATM-dependent phosphorylation on Thr632 [76]. PHRF1 (PHD and ring finger domains 1) recognizes H3K36me2 via its PHD domain, meanwhile binds to NBS1 through the SDTE motif [80] (**Fig. 2C**). More importantly and interestingly, H3K36me2 can improve the association of MRN complex with chromatin through direct interaction with the BRCT2 domain of NBS1 [76] (**Fig. 2C**). In brief, H3K36me2 is an early marker in response to DNA damage.

H3K36me3 is also involved in various DNA repair pathways including MMR, NHEJ, and HR, as well as in DNA repair pathway choice. H3K36me3 is required for the recruitment of mismatch recognition protein hMutSα (hMSH2–hMSH6) through interactions with the PWWP domain of hMSH6 in G1 and early S-phase cells, when H3K36me3 is abundant [81] (**Fig. 2C**). SETD2 (SET domain-containing 2), the only reported methyltransferase responsible for H3K36me3 [82], is required for MMR. Overexpression of KDM4A/B/C disrupts MSH6 foci formation by demethylating H3K36me3 [83]. Recently, several excellent studies also revealed the function of H3K36me3 in RAD51 recruitment in active transcription-associated HR [84–86]. With a stable human cell line expressing AsiSI-restricting enzyme which recognizes an 8-bp sequence fused to a modified estrogen receptor (ER) ligand-binding domain and ChIP-seq, it is identified that DSBs in actively transcribed genes are prone to be repaired by HR, marked by the recruitment of HR protein RAD51 [84]. RAD51 recruitment depends on the interaction between H3K36me3 and the PWWP domain of LEDGF (lens epithelium-derived growth factor; also known as p75), the latter can promote CtIP (also known as RBBP8) recruitment, thus facilitating end resection for RAD51 loading [87] (**Fig. 2C**). H3K36me3, a transcription elongation-associated marker [88], is required because HR is severely impeded after depletion of SETD2. Interestingly, no increase of H3K36me3 levels is detected at DSB sites following I-SceI induction [85], which is consistent with other observations

either globally [47,86] or locally [84,89], indicating that pre-existed H3K36me3 is involved in this process. However, a study in yeast revealed that global levels of H3K36me3 and H3K36me2 are altered after phleomycin treatment. H3K36me3 increases within 1 h and then returns to basal levels after 4 h, when H3K36me2 is increased [90]. The study in yeast suggested that the role of H3K36me3 in DDR is not conserved during evolution. Given the cell-cycle-dependent manner, H3K36me3 is also important for DNA repair pathway choice. H3K36me3 promotes NHEJ in G1 by means of reducing chromatin accessibility and resection, whereas histone acetyltransferase GCN5-dependent H3K36ac promotes HR in S/G2 via increasing chromatin accessibility and resection in fission yeast [91]. These studies suggest that H3K36 methylation plays multiple roles during DDR in a context-dependent manner.

H3K79 methylation

H3K79 methylation is the well-studied histone methylation which localizes within the globular domain of the nucleosome. All three forms of H3K79 methylation are modified by Dot1 in yeast or Dot1L in mammal, which is also regulated by H2B ubiquitination, like H3K4 methylation [19]. In addition to its role in active transcription and transcriptional elongation, H3K79 methylation also participates in DDR.

H3K79 methylation is mainly involved in Rad9-associated DSB damage response in budding yeast [92] (**Fig. 2D**). 53BP1 orthologue Rad9 is an important DNA damage checkpoint regulator [93]. Deletion mutants of Dot1, the only methyltransferase identified for H3K79 methylation, are G1 and intra-S-phase checkpoint defective after IR by preventing activation and recruitment of Rad9, but remain competent for G2/M checkpoint [94]. Methylation of H3K4 by Set1 and methylation of H3K79 by Dot1 require Rad6/Bre1-H2B monoubiquitination pathway [19], and consistent with this, mutations in this pathway display similar checkpoint defects with Dot1 deletion [20,94,95], but not Set1 deletion. Although not required for G2/M cell cycle checkpoint, Dot1 and H3K79 methylation promote recruitment of hypophosphorylated Rad9 to DSBs and efficient DNA repair in G2 cells [96]. It is proposed that H3K79me2 promotes 53BP1 binding through interaction with its Tudor domain in human cells, and Rad9, the yeast orthologue of 53BP1, also possesses Tudor domain [97]. However, H3K79 methylation increases neither globally by western blot analysis nor locally by ChIP assay at the HO cleavage sites [94]. The hypothesis that exposed H3K79 methylation promotes Rad9 recruitment through chromatin relaxation indirectly seems not to be the case, as mutants lacking chromatin remodelers SWR or INO80 remain checkpoint competent [98]. However, the role of H3K79me2 is not clearly known in the recruitment of Rad9 in budding yeast after DNA damage, since H3K79me2 is also cell cycle dependent, low in G1 and high in G2/M, although H3K79me3 is generally consistent [99]. Interestingly, H3K79 methylation and recruited Rad9 also inhibit end resection and control ssDNA accumulation at DSBs and uncapping telomeres [100]. Anyway, Dot1-mediated H3K79 methylation plays dual roles in the activation of G1/S checkpoint and DNA repair at late G2 phase in Rad9-mediated DDR.

H3K79 methylation has also been found to play roles in repairing other types of DNA lesions in *S. cerevisiae*. An epistasis analysis between Dot1 and various UV repair genes indicates that H3K79 methylation plays multiple roles in NER, post-replication and recombination repair, as well as in Rad9-mediated checkpoint function after exposure to UV [101]. During replication, H3K79 methylation-mediated Rad9 recruitment contributes to sister chromatid recombination repair by regulating cohesion binding to damage sites [102]. H3K79me3 largely contributes to UV-induced sister chromatid exchange [103]. In addition, Dot1-dependent H3K79 methylation modulates the resistance to the alkylating agent MMS in translesion synthesis regulation, thereby maintains genome integrity [104–106]. Moreover, Dot1 and H3K79 methylation are required for global genomic repair in both nucleosomal core DNA and internucleosomal linker DNA, but not in transcription coupled repair [107].

However, earlier studies revealed the competence of H3K79me2 in 53BP1 binding [97], but the later studies proved that H4K20me2 is primarily responsible for this process in mammalian cells.

H4K20 methylation

H4K20 methylation is critically important for genomic integrity upon DNA damage. In mammalian cells, H4K20me1 is maintained by methyltransferase PR-Set7/Set8/KMT5A and demethylase PHF8/KDM7B, and H4K20me2/3 is methylated by Suv4–20h1/2. All three types are involved in DDR [89]. Several excellent reviews have highlighted the function of H4K20 methylation [108–111], but the field is still rapidly ongoing.

H4K20 methylation is mainly involved in the recruitment at DSB sites of 53BP1, which plays multiple roles in the recruitment of DSB-responsive proteins, checkpoint signaling, DSB repair pathway choice, and synapsis of distal DNA ends during NHEJ [111,112]. The tandem Tudor domain of 53BP1, which can bind to histone methylation, is required for its recruitment [97]. Initially, H4K20 methylation by Set9 in fission yeast was found to be required for Crb2 localization to DNA damage sites [113,114]. Although *in vitro* peptide pull-down analysis showed that H3K79 methylation, especially H3K79me2, is also competent for 53BP1 binding [97], later isothermal titration calorimetry results revealed that 53BP1 and Crb2 interact with H4K20me2 specifically [115]. The K_D of 53BP1 for H4K20me2-containing peptide is 19.7 μM, in contrast, the K_D of 53BP1 for H4K20me1 peptide is 52.9 μM, and the K_D of 53BP1 for non- or tri-methylated peptide is about 1 mM. Meanwhile, the affinity between 53BP1 and H3K79me2 peptide is very low (K_D, ~2 mM) and no interaction could be detected for non-, mono-, and tri-methylated H3K79 peptides. Moreover, down-regulation of Dot1 has no effect on IRIF formation of 53BP1 [115]. This suggests a conserved requirement of H4K20me2 for 53BP1 localization at DSB sites.

How this interaction functions upon DNA damage is perplexing for a long time because the total level of H4K20me2 is altered neither in fission yeast [113] nor in human cells [115]. A 'buried-exposed-recognized' model [113] is proposed. H4K20me2 is an extremely abundant marker that is present in >80% nucleosomes in both mammal and *Drosophila* [116–118]. A group of proteins can bind to H4K20me2 with tandem Tudor domain similar to 53BP1 [108], such as L3MBTL1 [lethal (3) malignant brain tumor-like protein 1] [119–121] and JMJD2A/KDM4A [122,123]. This suggests that the abundant H4K20me2 is occupied by specific readers in undamaged cells and is exposed upon DNA damage. Indeed, L3BMTL1 is released from UV-A laser-induced damage sites in U2OS cells by recruiting AAA-ATPase valosin-containing protein (VCP; also known as p97) and cofactor NPL4 (nuclear protein localization protein 4), which are recruited by RNF8/168-mediated ubiquitin chains [124] (**Fig. 2E**). Similarly, JMJD2A/KDM4A, as well as JMJD2B/KDM4B, is ubiquitinated by RNF8 and RNF168, and degraded by proteasome following doxorubicin, IR, or UV

treatment [125] (**Fig. 2E**). H4K20me2 has significantly higher affinity for JMJD2A (K_D, 2.0 μM) and JMJD2B (K_D, 27.7 μM) than L3MBTL1 (K_D, 211 μM) and 53BP1 (K_D, 50.8 μM). This argues that JMJD2A/B degradation is more important for the exposure of H4K20me2 and recruitment of 53BP1, although the K_D of H4K20me2 for 53BP1 is different in two studies [115,125].

Alternatively, H4K20me2 is induced locally at DSB sites, and total increase cannot be easily observed due to its high basal level. In agreement with this, H4K20me1/2/3 all significantly increase at I-SceI-induced DSB sites in DR-GFP HeLa cells, as does H4K20me2 at IR-induced damage sites [89]. This increase of H4K20me2/3 is dependent on local accumulation of MMSET (multiple myeloma SET; also known as WHSC1, NSD2), but not Suv4-20h1 [89,126]. The recruitment of MMSET requires the interaction between BRCT domain of MDC1 and MMSET Ser102 phosphorylation by ATM [89]. However, MMSET primarily targets H3K36 and H4K44 [127–129]. The specificity of MMSET for H4K20 upon DNA damage needs further elucidation.

Then, is the pre-existence of H4K20me2 critical for 53BP1 recruitment? The formation of 53BP1 foci is impaired after bleomycin treatment in HeLa cells with Suv4–20h1 knockdown [116]. However, in Suv4–20h1-double-null MEFs, 53BP1 foci formation is delayed only in the early time of IR and recovered after 10 min [118] or not impaired [130]. Meanwhile, knockdown or knockout of PR-Set7/Set8, an H4K20me1 methyltransferase, severely impairs 53BP1 foci formation upon treatment with IR [115,130,131]. These results collectively indicate that the pre-existence of H4K20me1 by PR-Set7 is required for 53BP1 recruitment primarily *in vivo*. In agreement with this, PR-Set7 is recruited to localized irradiation (405 nm UV laser) sites by proliferating cell nuclear antigen (PCNA) or to DSBs induced by AsiSI/I-SceI endonuclease in U2OS cells, which is dependent on Ku70, after which H4K20 is *de novo* methylated and 53BP1 is recruited [131–133]. Interestingly, it was reported that the rapid recruitment of PR-Set7 is not sufficient for 53BP1 recruitment. The *de novo* H4K20me1 then facilitates Suv4–20 methyltransferases recruitment and the generation of H4K20me2 for 53BP1 binding [133]. However, the PR-Set7 at damage sites is unstable and degraded by PCNA-coupled CRL4Cdt2 E3 ligase complex. The PR-Set7 recruitment is also regulated by BBAP-dependent H4K91 monoubiquitination [134] and Sirt2-dependent deacetylation of PR-Set7 at K90 [135], but negatively regulated by HDAC [131]. The dynamics of H4K20 methylation at DSB sites and the enzymes required remain to be further investigated.

Another interesting issue is the requirement of H3K79 and H4K20 methylation for 53BP1 recruitment in different organisms. As described above, H3K79me2 is primarily responsible for 53BP1 orthologue Rad9 recruitment in budding yeast. However, in fission yeast, the recruitment of 53BP1 orthologue Crb2 is dependent on H4K20 methylation by Set9 [113,114]. In budding yeast, H4K20 methylation and methyltransferases are difficult to detect, while in fission yeast, H3K79 methylation is not readily detectable [136]. In human cells, although both H3K79 methylation and H4K20 methylation are able to bind to 53BP1, H4K20 methylation binds more tightly [97,115]. This is probably a result of evolutional selection. It is partially supported by the requirement of H3K79me2 in IR-induced 53BP1 foci formation in G1/G2 U2OS cells [137], when H4K20me2 levels are low [138]. The interaction between a conserved Bat3 ubiquitin-like motif and a conserved Dot1L ubiquitin-interacting motif is required for efficient H3K79me2. Nonetheless, the functional relationship between H4K20 and H3K79 methylation in DDR needs further exploration.

Other histone methylations

In addition, many other lysine residues have also been identified to be methylated by mass spectrometry and quantitative proteomic analysis [139,140]. Although the function of these new histone methylations is largely unknown, they may play important roles in various chromatin functions, including DDR and repair. For example, H2AX K134 near Ser139 can be dimethylated by SUV39H2, and this methylation is critical for the production of γH2AX [141]. H3K23me3 by Ezl3p regulates the localization of meiosis-induced DSBs to protect heterochromatin [142]. H3K56me1 by G9a/KMT1C serves as a docking site for PCNA in DNA replication [143], but its role in DDR has not been reported, although H3K56ac is involved in both DNA replication and damage response. H3K64me3 is a novel heterochromatin mark which depends on Suv39 [144]. H1.4K26 methylation by EZH2 or G9a and H1.2K187 methylation by G9a/GLP are also identified in mammalian cells to be linked to transcriptional repression [121,145,146]. It is interesting to further investigate the roles of these newly characterized histone methylations in DDR.

Non-histone Protein Methylation

Lysine methylation of non-histone proteins is also involved in various cellular functions such as chromatin remodeling, gene transcription, protein synthesis, signal transduction, DNA repair, and in the development and progression of various diseases including cancers [147,148]. An increasing number of non-histone substrates are being identified by various lysine methyltransferases [54,147,149–151]. Lysine methylation of non-histone proteins may affect their subcellular localization, chromatin binding, enzymatic activity, protein stability, other modifications, or protein–protein interaction [148]. Although the role of arginine methylation of non-histone proteins in DDR has been discussed [152], lysine methylation is not well documented. Lysine methylation sites of non-histone proteins and their regulators involved in DDR are summarized in **Fig. 3**.

The most widely studied lysine methylation substrate beyond histones is p53 [153–155], a critical regulator at the cross-road of various cellular functions including DDR. Among the identified lysine methylation residues of p53, K370me1 by SMYD2, K373me2 by G9a/GLP, and K382me1 by SET8 are linked to the suppression of p53 [156–158]. Although monomethylation of K370 and K382 suppresses p53 activity, dimethylation promotes p53 function through facilitating the association with Tudor domain of 53BP1 [159,160]. Binding to these dimethylation marks with Tudor domain, PHF20 (PHD finger protein 20) homodimer can also stabilize and activate p53 through inhibiting MDM2 activity, an E3 ubiquitin ligase of p53 [161]. K370me2 of p53 is negatively regulated by LSD1 [159], but methyltransferases for K370me2 and K382me2 of p53 are not known. However, K372me1 by SET7/9 promotes subsequent acetylation by Tip60, as well as p53 activation and stability in human [151,162–164], but it is not conserved for mouse, in which Set7/9 is dispensable for p53-mediated DDR [165]. K372me1 also suppresses K370me1 through disturbing the interaction between SMYD2 and p53 [156], indicating the existence of cross-talk between different modifications sites, which needs further investigation.

Apart from p53, methylation or demethylation of many non-histone proteins are involved in DDR. E2F transcription factor 1 (E2F1) plays an important role in DNA damage-induced cell death. In p53-deficient cells, E2F1-K185 methylation by SET9 prevents E2F1 accumulation and apoptotic function during DNA damage

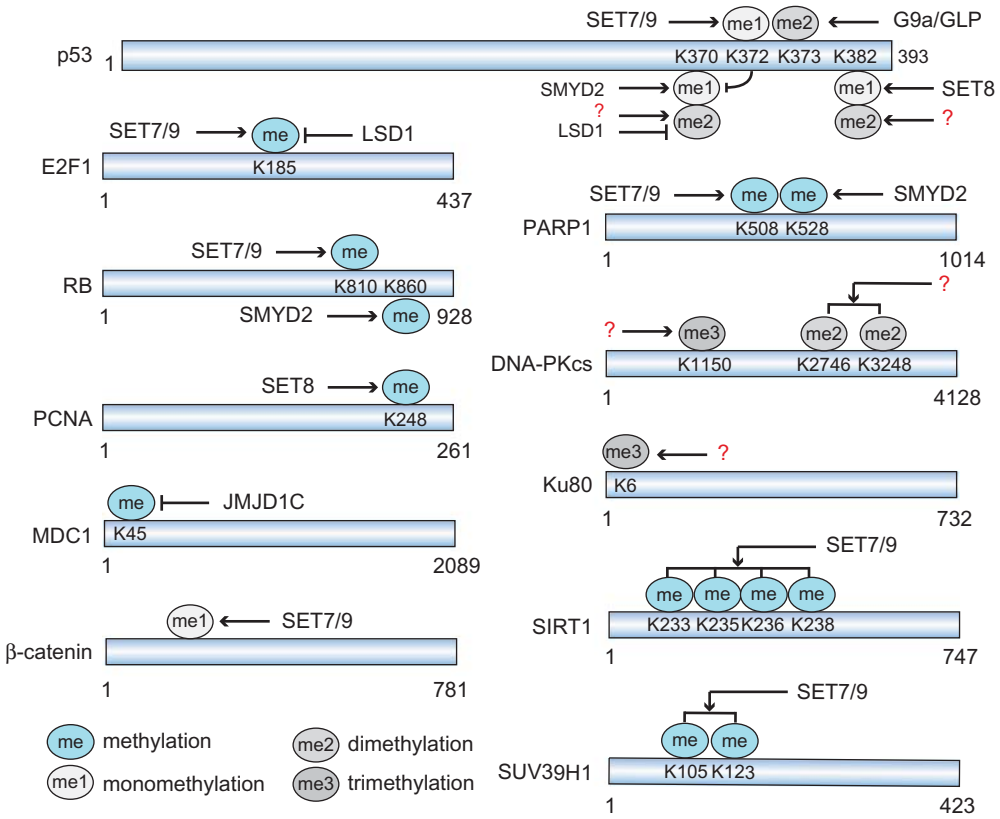

Figure 3. Identified non-histone lysine methylation in DDR Numbers indicate the lysine residues for methylation. Black arrows indicate the methyltransferases responsible for the corresponding methylation and black rests indicate the demethylases of each lysine methylation. A red '?' indicates an unidentified methyltransferase.

[166]. This modification can be reversed by LSD1. K860 methylation of tumor suppressor retinoblastoma (RB) by SMYD2 provides a docking site for L3MBTL1, while K810 methylation by SET7/9 binds to the Tudor domain of 53BP1 and influences the γH2AX signal [167,168]. In addition to direct regulation of p53, SET7/9 also methylates SIRT1 at multiple lysine residues and disrupts the association with p53 during DDR [151]. In addition, SET7/9-mediated SUV39H1-K105 and -K123 methylation decreases its methyltransferase activity, thus leading to chromatin over-relaxation and genome instability after DNA damage treatment [54]. Moreover, β-catenin, a key mediator of the wingless-integration site (Wnt)/β-catenin signaling pathway, is monomethylated by SET7/9 at lysine 180 in response to H_2O_2-induced oxidative stress, which influences the stability of β-Catenin and regulates cancer cell proliferation [169]. Furthermore, PARylation by PARP1 appears very early and rapidly after DNA damage and is crucial for the recruitment of many DDR factors. Both K528 methylation by SMYD2 and K508 methylation by SET7/9 of PARP1 enhance its recruitment and PAR formation after IR [170,171]. Recently, a systematic approach identified K1150me3, K2746me2, K3248me2 of DNA-PKcs and K6me3 of Ku80, which are read by HP1β and involved in the localization of early DDR factors [172]. Other fascinating studies reveal that demethylation of MDC1 at K45 by JMJD1C facilitates RNF8-dependent polyubiquitination and RAP80-BRCA1 recruitment, thereby promoting NHEJ repair [173,174]. The restriction of RAD51 foci formation by JMJD1C suggests that this demethylation plays a role in DNA damage repair pathway choice. Mutation of PCNA at SET8-mediated Lys248 methylation site is more susceptible to DNA damage, indicating its involvement in DDR [175]. It is sure that more non-histone substrates and more lysine sites of methylation will be discovered in DDR.

Perspective and Concluding Remarks

As described above, the response of histone methylation after DNA damage is relatively controversial. These changes can be globally or locally, early or late, rapid or long, as summarized in **Tables 1** and **2**.

This is probably due to the intrinsic rapid dynamics of histone methylation, distinct DNA damage induction and analyzing methods, or context-specific DDR. Generally, DDR is quite rapid. For example, γH2AX, the most widely used DNA damage marker, appears rapid in seconds after exposure to IR, and reaches maximum amounts within 10 min [176]. SSB-related PARylation reaches maximal accumulation within 1–2 min at the damaged sites induced by UV laser microirradiation [177]. As listed in **Table 1**, IR (including X-ray and γ-ray) and various chemical agents (including phleomycin, bleomycin, IR-mimetic NCS, MMS, etoposide, and adriamycin) are often used for global analysis with immunoblotting or imaging, while local analysis is achieved by imaging and UV laser microirradiation, or ChIP-qPCR and endonuclease (including I-SceI, HO, I-PpoI, AsiSI, and p84-ZFN). Although increase of histone methylation is often observed at damaged sites, western blot analysis reveals no significant changes [22,44,89] due to method obstacle. Diverse types of DNA damage induced by different damage resources need distinct damage signaling and repair pathways [9]. More importantly, DDR is also a context-specific process. For example, H3K9me3 is always not altered globally and locally after

Table 1. Global dynamics of histone methylation in DDR

Position	Modification	Modifier	Change	Treatment	System	Reference
H3K4	H3K4me3	Set1p	No	Phleomycin	Budding yeast	[22]
			No	X-ray, release 5–180 min	MEF	[36]
	H3K4me2		No	NCS, 1 h	U2OS	[26]
H3K9	H3K9me3		No	X-ray, release 5–180 min; bleomycin, 0.5–2 h	MEF; HeLa	[36,39]
	H3K9me2/3	JMJD2B	Decrease	γ-radiation, release 5–60 min; UV	U2OS; *Drosophila*	[51,55]
H3K36	H3K36me2	Metnase, KDM2A	Increase	IR, etoposide, adriamycin, 5-aza-CdR	Many cell lines	[47,76]
	H3K36me3		No	IR, etoposide, NCS, phleomycin	Many cell lines	[47,86]
	H3K36me2/3	Set2	Increase	Phleomycin	Budding yeast	[90]
H4K20	H4K20me1/2/3		No	IR, UV, camptothecin (CPT)	Fission yeast; human cell	[113,115]
	H4K20me2	MMSET	Increase	IR at high dose (160 Gy)	U2OS	[89]

Table 2. Local dynamics of histone methylation in DDR

Position	Modification	Modifier	Change	Treatment	System	Reference
H3K4	H3K4me3	Set1p	Increase	HO	Budding yeast	[22]
		JARID1B/KDM5B	Decrease	I-SceI	DR-GFP U2OS	[32]
	H3K4me2		No	UV laser	U2OS	[26]
		LSD1	Decrease	UV laser; I-PpoI	Late S/G2 U2OS	[31]
	H3K4me2/3		Increase	I-SceI and DNA-PKcs inhibitor	DR-GFP HeLa	[23]
H3K9	H3K9me3		No	UV	Human fibroblasts; 3T3	[37,38]
		SUV39H1	Increase	p84-ZFN	PPP1R12C intron 1 in euchromatin	[44]
	H3K9me2		Increase	I-SceI	HT1904 cell system	[47]
			No	UV laser	U2OS	[31]
	H3K9me2/3		Increase	I-SceI	Hypermethylated E-cadherin promoter	[48]
H3K27	H3K27me3	EZH2	Increase	I-SceI; UV laser	Hypermethylated E-cadherin promoter; MEF	[48,64]
			No	UV laser	U2OS	[65,66]
H3K36	H3K36me2	Metnase, KDM2A	Increase	I-SceI	HT1094	[47]
	H3K36me3		No	I-SceI; AsiSI	DR-GFP HeLa; DIvA	[84,89]
	H3K36me2/3	set2	Increase	gal-induced DSB	Budding yeast	[90]
H3K79	H3K79me3		Increase	UV laser	U2OS	[26]
H4K20	H4K20me1/2/3	MMSET	Increase	I-SceI	DR-GFP HeLa	[89]
	H4K20me1	PR-SET7/8	Increase	AsiSI	U2OS	[131]

DNA damage because of its pre-existence, but when lacking at euchromatin it needs to be induced [44]. A recent study even shows the dynamics of H3K9me2/3 in cell-type specific manner [178]. These collectively demand for more appropriate strategies for histone methylation studies in DDR.

There are three forms for histone lysine methylation and not all three forms at each site are investigated after DNA damage treatment. Histone methylation is one of the most characterized modifications perhaps due to the specificity of modifying enzymes. Different methylation forms may have distinct distribution along the chromatin. For example, although transcriptional suppression-related H3K9me2/3 and H3K27me2/3 are higher at silent genes, H3K9me1 and H3K27me1 are higher at active promoters, similar to H4K20me1 [179]. Specific type of histone methylation may also function differently when localized at different functional regions. For example, H3K36 methylation in the coding region promotes transcription but suppresses transcription when in the promoter, while H3K9 methylation in the promoter suppresses transcription but activates transcription when in the coding region [16,180]. It is interesting to explore how these modifications function in DDR coupling with their localization. Moreover, histone monomethylation has not been largely investigated in DDR, except for H4K20me1 which has been paid increasing attention [109].

Because the overall charge of lysine residue is not altered, methylation functions as a docking site for non-histone proteins. Lysine methylation can be recognized by chromodomain, Tudor domain, malignant brain tumor domain, PWWP domain, PHD fingers, and WD40/β propeller [15]. An increasing list of histone methylation readers have been identified [181]. These readers could be involved in DDR either through recruitment to damage sites or release from chromatin. For example, H3K36me3-binding protein MSH6 is recruited through PWWP domain in MMR [81], while H4K20me2-binding proteins L3MBTL1 and JMJD2A are released from chromatin upon DNA damage for 53BP1 recruitment [124,125]. It is believed that more DDR factors will be identified through interaction with histone methylation.

Both KMT and KDM for H3K4 or H3K9 methylation are recruited to damage sites. It is possible that they accumulate sequentially or function in distinct DNA damage signaling and repair pathways. More importantly, it might be suggested that additional non-histone substrates are involved. For example, H3K9-specific demethylase JMJD1C is recruited to DNA damage site for the demethylation of MDC1, RNF8-dependent MDC1 polyubiquitination and RAP80-BRCA1 recruitment [173]. Lysine methylation on non-histone proteins has emerged as an important regulator in cellular signaling and in the development and progression of various

diseases including cancers [147,148]. Special attention should be paid to possible non-histone targets for KMTs and KDMs at DNA damage sites.

Cross-talk between PTMs is employed to facilitate the spatio-temporal regulation of DDR signaling [182]. Cross-talk between histone modifications during DDR has been summarized [183,184]. Histone modifications can be activated or suppressed by each other sequentially, or they can function in specific DDR factor recruitment collaboratively. For example, H4K16ac adjacent to H4K20me2 affects 53BP1 binding through disruption of a salt bridge between H4K16 and Glu1551 in the 53BP1 Tudor domain [185,186] (**Fig. 2**E), while H2A/H2AX ubiquitination at K15 and H4K20me2 are both required for 53BP1 recruitment [111,112] (**Fig. 2E**). H3K4 and H3K79 methylation are regulated by H2B ubiquitination and are both involved in DDR [23,26]. It is interesting to elucidate whether they function in DDR together in a conserved mechanism.

Although PTMs in DDR signaling pathways have been widely explored in the past decades, methylation of histone and non-histone proteins is an emerging field. Lysine methylation has been widely involved in DNA damage-associated DDR protein recruitment or extraction, global and local chromatin environment establishment, local transcriptional suppression, and gene-specific suppression or activation. It is evident that more histone lysine sites and new non-histone substrates will be found to be associated with DDR. In this case, each type of lysine methylation (mono-, di-, or tri-) should be considered because of their potential distinct functions. Although KMTs and KDMs are relatively specific, different substrates need to be distinguished, especially between histone and non-histone proteins. Moreover, DDR is regulated spatially and temporally by various PTMs. High-resolution spatio-temporal information of lysine methylation at sites of DNA damage demands more sensitive detection methods. Meanwhile, the combinational roles of different PTMs at DNA damage sites raise a great challenge for the elucidation of 'methyl-cross-talk' network. Clearly, the dynamics, regulation, and functions of lysine methylation during DDR are complicated. However, new technical methods and research strategies will help us to elucidate the complex network successfully, thus to develop more efficient cancer therapeutics.

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