



Emerging posttranslational modifications and their roles in DNA damage response

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Abstract

Posttranslational modifications (PTMs), occurring on various histones and nonhistone proteins, greatly enrich the diversity of the proteome, thereby profoundly affecting protein structures and biological functions. Histones are particularly important components of genomic chromatin and their modifications represent a critical event in the control of DNA damage response (DDR) induced by endogenous or exogenous insults. Extensive studies have revealed the roles of classical PTMs including phosphorylation, acetylation and ubiquitination, in modulating chromatin dynamics through the recruitment of chromatin remodeling complex and repair machinery during DDR process, thus successfully maintaining genome stability and preventing the cells from adverse fates such as apoptosis or malignant transformation. In recent years, several novel PTMs, such as ufmylation, crotonylation, succinylation and lactylation, have been discovered on both histones and nonhistone proteins. Their potential roles and regulatory mechanisms during DDR process have indeed emerged, but are still far from completely understood. This review primarily focuses on the regulation of novel PTMs in DDR, and further discusses the repair networks of cell in response to DNA damage and the interplay between diverse modifications in DNA damage response, which aims to expand the understanding of PTMs involved in DDR regulation and provides potential insights into disease intervention.

Keywords Posttranslational modification · Histone · Nonhistone · DNA damage response

Introduction

As the essential carrier for storing genetic information in eukaryotes, DNA undergoes numerous damage events at any time. Endogenous stimuli (oxidation, hydrolysis, misalignment, etc.) or exogenous stimuli (UV/ionizing radiation, chemical damage, etc.) can lead to various types of DNA damage, including base damage or mispairing, single-strand breaks (SSBs) or double-strand breaks (DSBs), and covalent crosslinking of DNA chains (Jackson & Bartek, 2009). DSBs are the most severe form of damage. If DNA damage is not promptly corrected, it can result in gene mutations or even genomic variations, thereby threatening the normal function of cells and tissues. To maintain the integrity and

stability of the genome and ensure normal physiological activities, cells have evolved a sophisticated regulatory network known as the DNA damage response (DDR) to counter various threats of DNA damage (Groelly et al., 2023; Jackson & Bartek, 2009). This network encompasses a series of processes, such as the recognition of DNA damage and the initiation of DNA damage repair programs.

However, specific signaling pathways and damage repair mechanisms could be initiated for different types of DNA damage, of which homologous recombination (HR) and nonhomologous end joining (NHEJ) are the two main repair modes in response to DSBs (Lieber et al., 2003; San Filippo et al., 2008). HR uses the homologous sequence of uninjured sister chromatids as its repair template to complete DSB repair under the action of the homologous repair complex composed of repair-related proteins. The repair process mainly occurs in the S and G2 phases of the cell cycle. BRCA1 and BRCA2 proteins encoded by the breast cancer susceptibility *BRCA* are key proteins in the complex signaling pathway of HR. The functional inactivation of these proteins could lead to HR repair defects, thereby resulting in genomic instability. Clinical studies

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have shown that mutations related to the HR pathway may be the leading cause of various cancers (Krejci et al., 2012; San Filippo et al., 2008). Unlike the HR repair mode, NHEJ is independent of homologous DNA sequences and has a faster repair process, accounting for approximately 75% of repair events (Her & Bunting, 2018; Mao et al., 2008). In the process of NHEJ repair, Ku70/80 first recognizes and binds to the DSB site to form the NHEJ initiation complex with DNA protein kinase and at the same time recruits repair proteins, DNA ligase and other components to directly connect and repair at the end of DSBs (Lieber, 2010). Increasing evidence indicates that abnormal regulation of DNA damage repair networks contributes to a variety of diseases, including aging (Hoeijmakers, 2009; Ribezzo et al., 2016), developmental defects (Hakem, 2008), and cancer progression (Hoeijmakers, 2009; Huang & Zhou, 2021).

Proteins are the main undertakers of life activities, and their posttranslational modifications (PTMs) have a significant impact on their structure and functions. PTMs typically exist in proteins with prominent structure and function, such as histones, membrane proteins, and secreted proteins, which have a certain impact on the occurrence of almost all biological events, including gene expression regulation, DNA damage repair, protein–protein interaction, cell cycle, intracellular signal transduction, and intercellular communication. However, histones, as the main protein components of chromatin, play a vital role in regulating the structure and function of chromatin conformational transformation. Studies have found that the different states of chromatin are closely related to the epigenetic modifications of histones. The diversified modification types established by various modifying enzymes and demodifying enzymes on histones constitute the “histone code”, and the dynamic regulation relies on the synergistic effect of various modifying enzymes (“writers”) and demodifying enzymes (“erasers”) on histones. Nevertheless, the occurrence of these processes disturbs the binding between histones and DNA, thus affecting the transcriptional status of the local genome, gene expression, DNA replication and cell fate determination. The discovery and functional regulation of a variety of classical PTMs, including phosphorylation, ubiquitination, acetylation, etc., have been thoroughly studied. It has been proven that PTMs are indispensable components of the complex and refined DDR regulatory network and are mainly involved in numerous aspects of the DDR by regulating downstream effector protein binding or recruiting DNA damage repair proteins. However, with the further development and application of new types of mass spectrometry, various novel PTMs have also been found in recent years, such as ufmylation, crotonylation, succinylation and lactylation, which is of great significance for us to further enrich the DDR and repair mechanism network (Fig. 1).

Ufmylation

Mechanism of ufmylation

Ufmylation is a new type of ubiquitin-like modification that is similar to the tertiary enzyme-linked catalytic reaction composed of ubiquitin-activating enzyme E1, ubiquitin-conjugating enzyme E2 and ubiquitin-ligase E3. Ubiquitin-fold modifier 1 (UFM1), a novel small molecule ubiquitin-like protein, was first discovered in 293 T cells (Komatsu et al., 2004). Its precursor pro-UFM1 form is synthesized and then cleaved at its C-terminus by UFM1-specific proteases UFSP1 and UFSP2, thereby exposing the conserved glycine residue required for binding to form the activated UFM1-G83 form (Ha et al., 2008; Kang et al., 2007). Subsequently, mature UFM1 is activated by the specific E1-like enzyme ubiquitin-like modifier activating enzyme 5 (UBA5) in an ATP-dependent manner and transferred to the E2-like enzyme ubiquitin-fold modifier-conjugating enzyme 1 (UFC1), together with the E3-like ligase UFM1-specific ligase 1 (UFL1), to recognize the target protein for ufmylation. DDRGK1, encoded by *C20orf116*, was the first identified UFM1 target protein (Tatsumi et al., 2010). In addition, the target proteins modified by UFM1 can also be cleaved by UFSP1 and UFSP2, and UFM1 re-enters the cycle to participate in ufmylation. A large number of studies have revealed that ufmylation is widespread in organisms and regulates a variety of biological functions, such as the cell cycle (Gak et al., 2020), endoplasmic reticulum stress (Gerakis et al., 2019; Ishimura et al., 2022; Xie et al., 2019) and cell apoptosis (Lemaire et al., 2011; Zhang et al., 2021).

Regulation of ufmylation in DDR

Based on the bioUFM1 method, 82 substrate proteins modified by ufmylation, including DSB repair proteins such as MRE11A and PARP1, were isolated and identified by mass spectrometry in human cells (Pirone et al., 2017). In addition, there was colocalization of the ufmylation factor UFL1 and the DSB marker γ H2AX under UV-induced DNA damage, suggesting that ufmylation was involved in the DDR. Ufmylation of MRE11 at the K282 site, mediated by E3-like ligase UFL1, is necessary for undisturbed MRN (MRE11/RAD50/NBS1) complex formation, DSB-induced ATM activation, HR-mediated repair, and genome integrity. Moreover, the deletion of ufmylation caused by this site mutation exhibited the same phenotype as the pathogenic mutation MRE11 (G285C) found in endometrioid carcinoma, indicating that ufmylation of MRE11 is a potential therapeutic target (Wang et al., 2019). As an

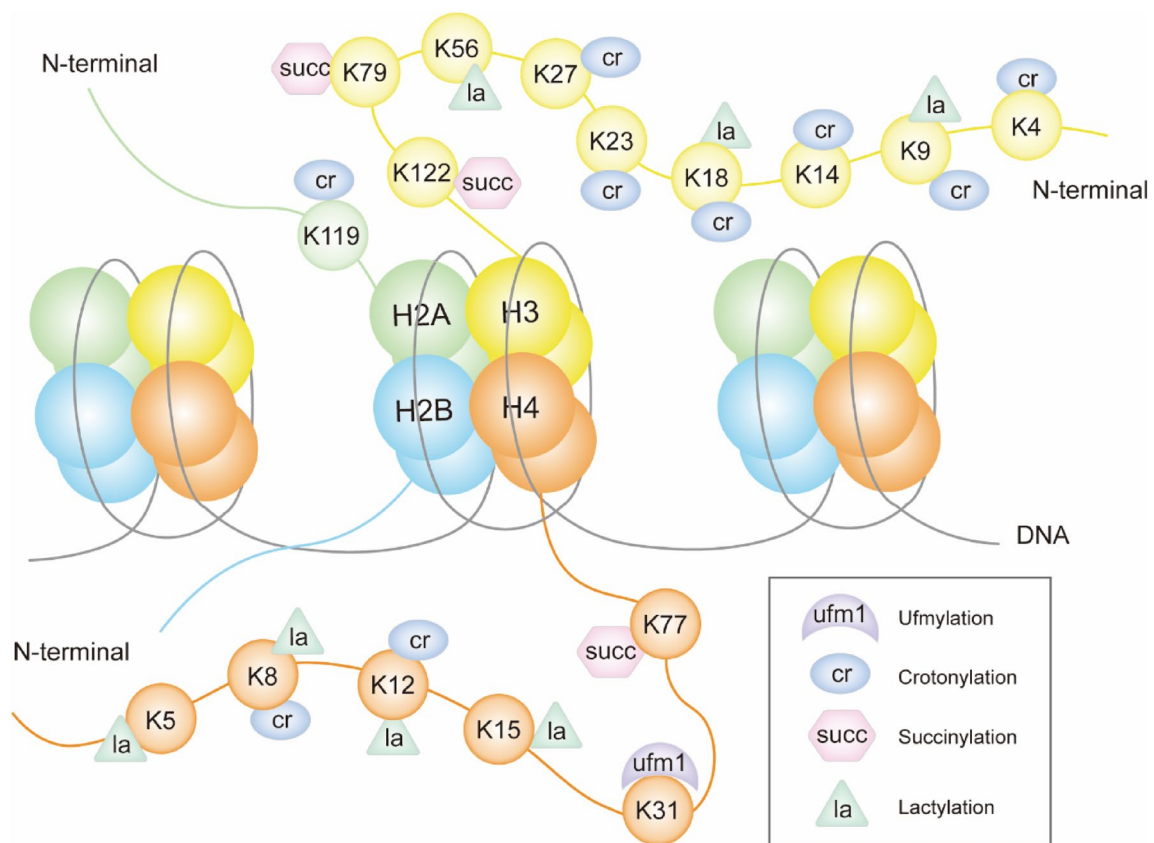


Fig. 1 Summary of novel histone posttranscriptional modifications (PTMs) at lysine sites, including ufmylation, crotonylation, succinylation and lactylation

important upstream kinase in the DDR, ATM is rapidly activated during DNA damage, thereby phosphorylating downstream target proteins to initiate DDR-related signaling pathways. Growing studies have indicated that the E3-like ligase UFL1 is closely related to the activation of ATM kinase (Qin et al., 2019, 2020; Wang et al., 2019). UFL1 can be recruited by the MRN complex to DSBs, and the STK38 kinase with the ufm1 binding domain serves as a reader for histone H4 ufmylation, recognizing and mediating mono-ufmylation of histone H4 at the K31 site. Enhanced recruitment of the methyltransferase SUV39H1 leads to H3K9 methylation and the activation of Tip60, thereby promoting the activation of ATM to maintain genome integrity (Qin et al., 2020). However, ATM activation is negatively regulated by the UFM1-specific peptidase UFSP2, which binds to the MRN complex in the absence of DSBs, and radiation-induced ATM phosphorylation of UFSP2 results in the dissociation of UFSP2 from the MRN complex. Phosphatase WIP1 can remove the phosphorylation of UFSP2, thereby recruiting UFSP2 to deubiquitinate histone H4 and inhibit ATM activation (Qin et al., 2022). Therefore, the balance between UFL1- and

UFSP2-mediated ufmylation plays a significant role in ATM activation and DDR in response to DSBs.

Crotonylation

Mechanism of crotonylation

With the update of mass spectrometry, crotonylation, a novel evolutionally conserved epigenetic modification, was identified by affinity enrichment using the pan anti-Kcr antibody and HPLC–MS/MS, and 28 and 24 histone lysine crotonylation sites have been identified in HeLa cells and mouse MEF cells, respectively (Tan et al., 2011). This novel PTM utilizes crotonyl-CoA as the donor and is regulated by a range of enzymes including crotonyltransferases (“writers”) and decrotonylases (“easers”), as well as by other functionally related proteins (e.g., the “readers” proteins), to maintain crotonylation balance and actions, which mainly exist in transcriptionally active promoter and enhancer regions and are closely related to gene transcription regulation (Tan et al., 2011). In addition, histone and nonhistone lysine

crotonylation is also involved in biological processes such as spermatogenesis, the cell cycle, and protein stability and localization, thus affecting the progression of various diseases.

Crotonyltransferase

As a classic histone acetyltransferase, studies have found that p300 also has crotonyltransferase activity to catalyze the crotonylation of Lys18 on histone H3 (H3K18cr), which can stimulate gene transcription more strongly than histone acetylation. Moreover, the level of histone crotonylation is regulated by the concentration of intracellular crotonyl-CoA, indicating that cell metabolism can affect gene regulation by intervening in histone acylation (Sabari et al., 2015). However, the transcriptional corepressor CDYL acts as a crotonyl-CoA hydratase to convert crotonyl-CoA to β -hydroxybutyryl-CoA, which, in turn, negatively regulates histone crotonylation by decreasing the level of the substrate crotonyl-CoA, thereby affecting gene transcription and spermatogenesis (Liu et al., 2017a). In addition to p300/CBP, MOF also exhibits crotonyltransferase activity, which catalyzes the multisite crotonylation of K4, K9, K18, and K23 on histone H3 and K8 and K12 on histone H4 (Liu et al., 2017b). Chromatin binding protein molecules (“readers”) are also important members of the epigenetic regulation of histones, and the evolutionarily conserved YEATS domain with stronger binding activity than bound acetylated histone lysine is defined as the crotonylation reader family. AF9 colocalizes with histone H3 modified by crotonylation, and its YEATS domain recognizes histone crotonylation by having an open end “aromatic sandwich” pocket, thereby positively activating gene transcription (Li et al., 2016a). The Taf14 YEATS domain in yeast acts as a reader for crotonyllysine and binds to histone H3 with crotonylation at Lys9 (H3K9cr) (Andrews et al., 2016). In addition, the GCN5 complex can mediate the crotonylation-dependent transcription of histones H3 and H4 (Kollenstart et al., 2019), and the acetyltransferase PCAF also exhibits crotonyltransferase activity (Xu et al., 2017).

Decrotonylation

In terms of decrotonylation, Madsen reported that the deacetylase HDAC3 has decrotonylation activity based on substrate analysis of zinc-dependent lysine deacylase and forms the HDAC3-NCOR1 complex with nuclear receptor core inhibitor 1 (NCOR1) to mediate decrotonylation in vitro (Madsen & Olsen, 2012). Due to the discovery of class I deacetylases HDACs with decrotonylation activity and as the main histone decrotonylation enzymes, there is also homeostasis in histone decrotonylation. Consistent with the positive regulation of gene transcription by p300 and

AF9 YEATS involved in histone crotonylation, removal of histone crotonylation by HDACs inhibits gene transcription and affects self-renewal of mouse embryonic stem cells (Wei et al., 2017). In addition, HDAC1 can also form a ternary complex with CoREST1 and LSD1 to hydrolyze histone H3K18cr crotonylation-modified peptide substrates. Knockout of HDAC1/2 in ES cells significantly reduced the decrotonylation activity by 85%, thereby improving the overall crotonylation level of histones and simultaneously affecting the acetylation level of histones, indicating that HDAC1/2 and its complexes play a significant regulatory role in regulating histone decrotonylation and coordinating two types of histone modifications at specific sites (Kelly et al., 2018). Sirtuins (SIRT1–SIRT7) are members of the NAD⁺-dependent class III deacetylase family. Studies have found that SIRT1 and SIRT2 can also act as decrotonylases, effectively removing crotonylation on the H3K9 peptide modified by histone crotonylation (Feldman et al., 2013). CLASPI, a protein identification method based on cross-linked assisted and stable isotopically labeled cell culture amino acid (SILAC), screened and identified histone H3K4Cr-labeled proteins. The results showed that SIRT1, SIRT2, and SIRT3 can catalyze the hydrolysis of histone crotonylation-modified peptides and global decrotonylation of all core histones in vitro, with the strongest activity on H3K4cr and H3K27cr. Endogenous SIRT3 can serve as an “eraser” for crotonylation to regulate the homeostasis of crotonylation on histones and gene expression in chromatin regions (Bao et al., 2014) (Fig. 2).

Regulation of histone crotonylation in DDR

In addition to affecting transcriptional regulation, reproductive development, stem cell differentiation and other biological functions, histone crotonylation is also involved in the DDR. At the site of DSBs induced by the endonuclease AsiSI, CDYL1 is recruited and produces a transcription-dependent marker of histone lysine crotonylation and a local reduction in H3K9cr modification levels, which leads to the blocking of the transcription extension factor ENL and promotes transcriptional silencing. However, inhibition of CDYL1 hydratase activity blocked the reduction in H3K9cr modification and alleviated DSB-induced transcriptional silencing, but the efficiency of HR repair remained unchanged, indicating that CDYL1 participated in DSB-induced histone crotonylation and interfered with gene silencing and DSB repair independently (Abu-Zhayia et al., 2022). U2OS cells were labeled with the DNA double-strand break marker γ H2AX protein by laser microirradiation, and the level of in situ total crotonylation was significantly decreased. Moreover, different types of DNA damage can lead to a reduction in crotonylation at histone H3K9, but the complete recovery of crotonylation levels at this site can be

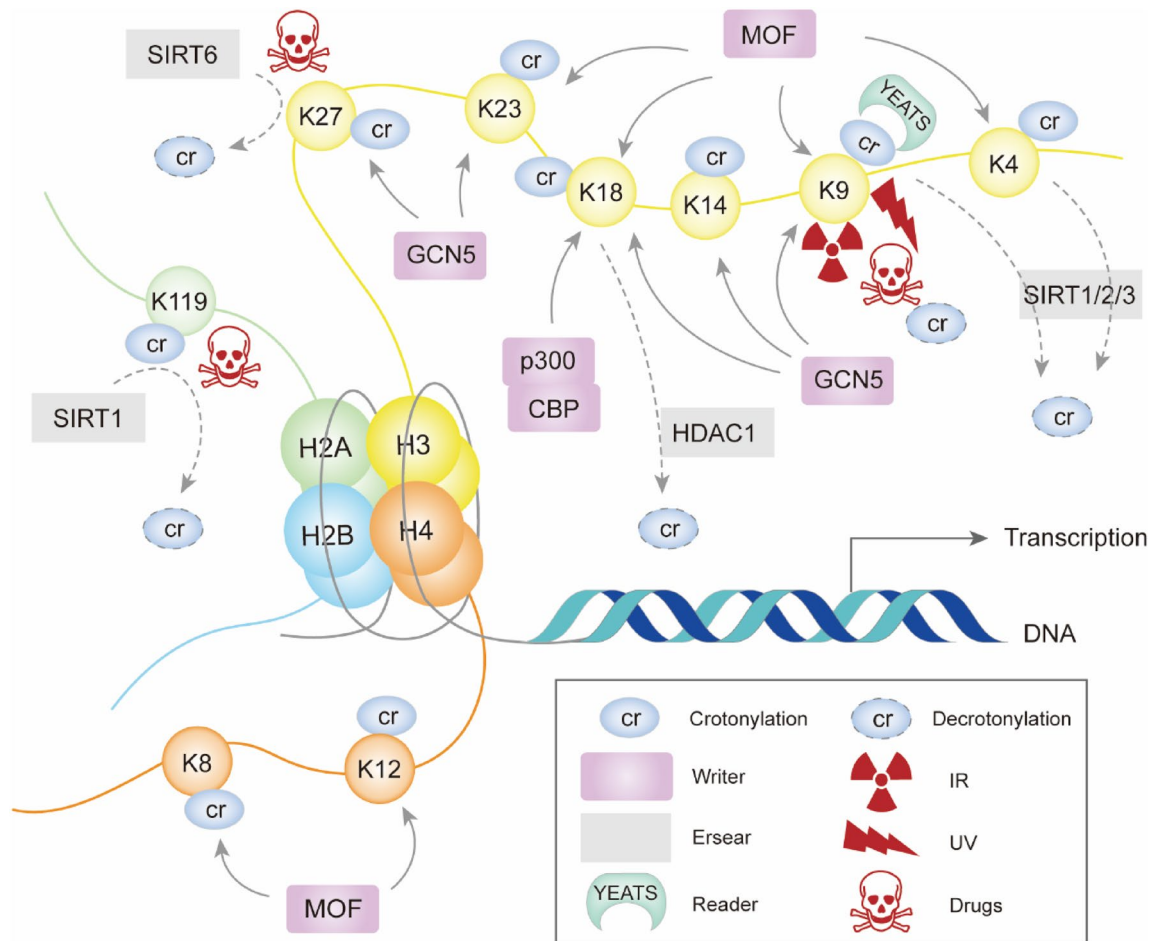


Fig. 2 Schematic of site-specific histone crotonylation catalyzed by crotonyltransferases (“writers”) and decrotonylases (“erasers”). H3K9, H3K27 and H2AK119 are involved in DDR

observed after treatment with ionizing radiation (IR) and the DNA damage drug VP16, while UV radiation cannot return to normal levels, showing that the recovery of H3K9cr after DNA damage is influenced by the type of DNA damage. The decrotonylation activity of HDACs can significantly promote the reduction of crotonylation levels induced by DNA damage, which can regulate histone lysine crotonylation at the DNA break site, resulting in gene silencing (Abu-Zhayia et al., 2019). Treatment with DNA damage drug topoisomerase inhibitors can also reduce crotonylation at histone H2AK119, and its effect on this modification is related to replication stress itself or replication stress-induced DSBs. SIRT1 can mediate decrotonylation at this site, and SIRT1 knockout cells are more sensitive to the DNA damage drug doxorubicin. A large number of DSBs have been observed in these cells, indicating that SIRT1 plays an important role in preventing DNA breaks caused by replication stress (Hao et al., 2022). In addition, the DNA damage drugs CPT and VP16 can also mediate the reduction in crotonylation levels at histone H3K27 in a concentration-dependent manner

in HCT-116 cells. SIRT6, as a DSB sensor, participates in decrotonylation and regulates intracellular H3K27cr levels. Combined with bioinformatics analysis, it was found that genes whose promoters were occupied by histone crotonylation in colon cancer patients were related to the degree of DNA damage. In patients with low DNA damage activity, genes occupied by H3K27cr in the promoter were enriched in multiple DNA damage-related pathways (27/78), indicating that DNA damage in colon cancer is negatively correlated with H3K27cr modification (Liao et al., 2022).

Regulation of nonhistone crotonylation in DDR

Xu et al. first reported the presence of crotonylation on non-histone proteins in 2017, and 2696 crotonylation sites were identified on 1024 proteins in H1299 cells. Most of these modified proteins are located in the cytoplasm (40%), 27% in the nucleus, and 13% in mitochondria, participating in a variety of important cellular pathways and performing different biological functions (Xu et al., 2017). In addition,

acetyltransferases CBP, PCAF, and MOF, as well as deacetylases HDAC1 and HDAC3, can also participate in the regulation of nonhistone crotonylation as crotonyltransferases and decrotonylases (Xu et al., 2017). Replication protein A (RPA), as a single-strand DNA binding protein in eukaryotic cells, plays an important role in DNA metabolism processes such as replication, repair, and homologous recombination (Maréchal & Zou, 2015). Among them, RPA1 can interact with the MRN complex to participate in the HR process. It was found that CDYL was involved in the negative regulation of histone crotonylation and affected the modification process of nonhistone RPA1, and knockout of *CDYL* in HeLa cells led to a significant increase in crotonylation levels at the K88, K379 and K595 sites of RPA1. The crotonylation of RPA1 promotes its binding to ssDNA and recruits it to interact with HR factors at DNA damage sites, thereby promoting the regulation of HR repair processes during DSBs. In addition, crotonylation of RPA1 is equally important for cell survival and anti-apoptosis in response to DNA damage, such as CPT (Yu et al., 2020), which indicates that nonhistone crotonylation plays a crucial role in the DDR. However, whether a large number of crotonylated nonhistone proteins identified by mass spectrometry are involved in the DDR and its mechanism need to be further investigated.

Succinylation

Mechanism of succinylation

Succinylation is a dynamically regulated PTM widely found in eukaryotes and prokaryotes. It is a type of acid acylation modification that can change the charge of lysine from +1 to –1 under physiological conditions, and studies have found that the succinylation site highly coexists with the acetylation site. Due to the occurrence of lysine succinylation, succinyl groups (-CO-CH₂-CH₂-COOH) can be covalently bound to lysine residues, and larger modification groups are introduced than acetylation, methylation and other modifications, which will cause significant structural changes and lead to more vital changes in protein structure and functions (Zhang et al., 2011). Lysine succinylation was mainly localized in mitochondria, followed by the cytoplasm and nucleus. Current studies have shown that succinylation of histones and nonhistone proteins can regulate gene expression, interfere with mitochondrial function and metabolism, and affect tumor occurrence and progression.

Desuccinylation

In contrast to the mechanism of succinylation, desuccinylation mediates the erasability of the modification. By knocking out SIRT5 in mice, an increase in the succinylation level

at K1291 of CPS1, a known target protein, was observed, but the level of acetylation at this site and the degree of succinylation at Lys44 and Lys287 were unchanged. Moreover, enzyme kinetics confirmed that the degree of desuccinylation of SIRT5 was much higher than its deacetylation level (29- to > 1000-fold), indicating that SIRT5 with weak deacetylation activity could effectively remove succinylation in vivo and in vitro (Du et al., 2011). Superoxide dismutase (SOD) (Lin et al., 2013), glutaminase (GLS) (Lukey et al., 2020) and pyruvate kinase M2 (PKM2) (Xiangyun et al., 2017) can be catalyzed, thus inhibiting enzyme activity. In addition, studies have found that another NAD⁺-dependent histone deacetylase, SIRT7, can also catalyze the occurrence of desuccinylation (Li et al., 2016b).

Nonenzyme-mediated succinylation

At present, it is believed that there are two main mechanisms of succinylation, including nonenzyme-mediated chemical reactions and enzyme-catalyzed reactions. Succinyl-CoA is an important metabolic intermediate in various metabolic pathways, such as the TCA cycle, porphyrin synthesis and catabolic metabolism of fatty acids. The succinyl-CoA ligase SCL produces succinic acid, and succinyl-CoA is also a significant coregulator of succinylation. Succinyl-CoA induced an increase in succinylation at defined succinylation sites on BSA and ovalbumin in a concentration-dependent manner in vitro (Weinert et al., 2013), and the physiological pH and acyl-CoA concentrations detected in the mitochondrial matrix were sufficient to cause concentration- and time-dependent but nonenzyme-dependent succinylation of mitochondrial and nonmitochondrial proteins (Wagner & Payne, 2013). It was suggested that protein succinylation in mitochondria may be a chemical event promoted by alkaline pH and the high concentration of reactive acyl-CoA present in the mitochondrial matrix (Wagner & Payne, 2013). Succinylation can occur through a nonenzyme-mediated chemical reaction mechanism dependent on the succinyl-CoA concentration (Weinert et al., 2013).

Enzyme-catalyzed succinylation

As with other modification types, succinylation also involves enzyme-catalyzed modification reactions. Lysine acetyltransferase 2A (KAT2A, also known as GCN5) also has succinyltransferase activity, and structural analysis revealed that succinyl CoA on succinyl CoA can protrude to the very end of KAT2A flexible ring 3 and interact specifically with Y645 in ring 3, so succinyl-CoA has stronger binding than acetyl-CoA with KATA2. At the same time, the α -ketoglutarate dehydrogenase (α -KGDH) complex located in the nucleus can bind to the gene promoter region KAT2A to compensate for the low

concentration of succinyl CoA in the nucleus and promote the succinylation modification at the histone H3K79 site. By blocking the incorporation of the α -KGDH complex into the nucleus or mutating KATA2 (Y645A) to weaken its binding and catalytic activity, the succinylation modification level of the H3K79 site was significantly reduced, thus inhibiting gene transcription and tumor cell proliferation and tumor progression. It has been suggested that the coupling of nuclear α -KGDH and KAT2A plays an important role in catalyzing the succinylation of H3K79 (Wang et al., 2017). Carnitine palmitoyl transferase 1A (CPT1A) can also use succinyl-CoA to exert lysine succinyltransferase activity *in vivo* and *in vitro*. Based on SILAC quantitative succinylated proteomic analysis, 171 lysine sites on 101 proteins were identified to catalyze succinylation. The CPT1A mutation (G710E) can inactivate carnitine palmitoyl transferase activity but has no effect on lysine succinyltransferase activity. It can still catalyze enolase 1 succinylation modification, reduce enolase activity and promote cell proliferation under glutamine deprivation. In addition, CPT1A cannot

catalyze KAT2A-mediated succinylation modification of BSA protein, indicating that CPT1A is substrate selective and can catalyze substrate succinylation modification in a manner independent of its classical carnitine palmitoyl transferase activity, affecting cell function (Kurmi et al., 2018). In addition, the GAS41 YEATS domain was found to act as a “reader” for succinylated histone H3 at site K122 in a pH-dependent interaction. The affinity of the GAS41 Yeats domain increased significantly when the pH value changed from 7.4 to 6.0. The specific binding mechanism found that GAS41 uses the characteristic salt bridge established by the protonated His39 residue in the pocket structure to recognize and participate in the regulation of the succinylation of histone H3K122 (Wang et al., 2018). However, by knocking down histone acetyltransferase *in vivo*, it was found that knocking down p300 and CBP led to a significant reduction in the level of succinylation of histone H3K122, and the same result was detected with curcumin, a p300/CBP inhibitor. In addition, *in vitro* succinyltransferase catalytic experiments further verified that p300 can increase the succinylation

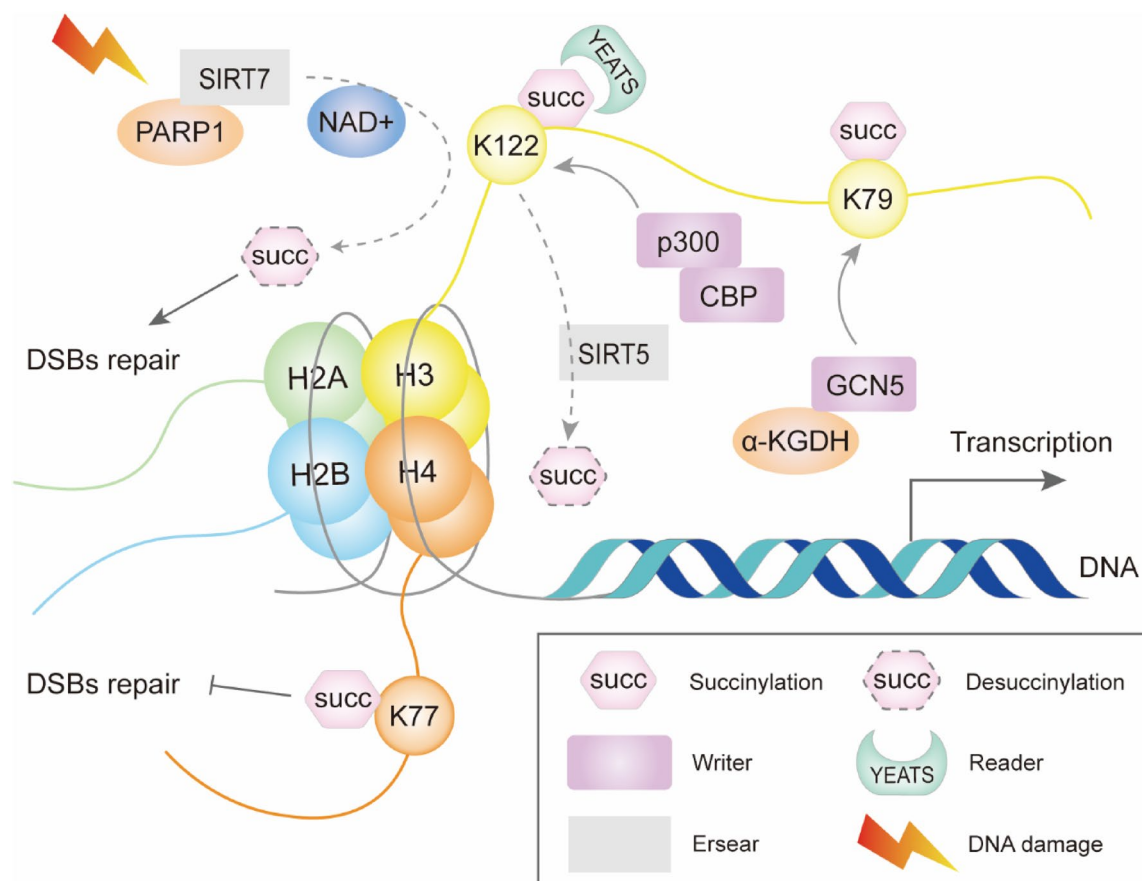


Fig. 3 Schematic of site-specific histone succinylation catalyzed by succinyltransferases (“writers”) and desuccinylases (“erasers”). H3K122 and H4K77 are involved in the DDR, and SIRT7 mediates

the desuccinylation of H3K122 in a PARP1-dependent manner in response to DNA damage, thereby promoting efficient DSB repair

of histone H3 in a concentration-dependent manner, and the modification can be removed by SIRT5, indicating that p300/CBP and SIRT5 participate in the succinylation of histone H3K122. Succinylation of H3K122 leads to nucleosomal instability that detaches histone octamers from DNA, thereby increasing access to DNA and stimulating gene transcription (Zorro Shahidian et al., 2021) (Fig. 3).

Regulation of histone succinylation in DDR

Among the various biological processes regulated by succinylation, the epigenetic modification of histones by succinylation also plays an important role in the DDR and maintenance of genome integrity. The NAD⁺-dependent Class III histone deacetylase SIRT7 was found to have enzyme-catalyzed activity-dependent histone desuccinylation activity, which also requires NAD⁺ as a coregulator. In the early stage of DNA damage, SIRT7 is rapidly and briefly recruited to DSBs in a PARP1-dependent manner, catalyzing the desuccinylation of histone H3K122, thus promoting chromatin condensation and effective DSB repair. However, knockdown of SIRT7 weakens chromatin compaction during DNA damage response and sensitizes cells to genotoxic stress, suggesting that SIRT7 catalytic desuccinylation of histone H3K122 is an important part of the DSB repair process. It promotes the stability of histone interactions with DNA in nucleosomes during the DDR and plays a key role in DDR and cell survival (Li et al., 2016b). SDH deficiency in tricarboxylic acid cycle (TCA) metabolism disrupts the distribution of succinyllysine in chromatin and the occurrence of hypersuccinylation modification, thereby hindering DNA repair activity, resulting in DNA repair damage and susceptibility to genotoxic drugs, consistent with the effects of chromatin hypersuccinylation observed in SIRT7 deficiency. Therefore, it is suggested that global succinylation of chromatin may be a mechanism by which metabolism regulates genome-wide transcription and DNA damage repair activity (Smestad et al., 2018). In addition, histone H4 at the nucleosomal DNA–histone interface also has a site-specific and evolutionally conserved succinylation modification, which also affects nucleosomal stability and nucleosome–chromatin dynamics. The succinylation modification of H4K77succ at the H4K77 site promotes the development rate of nucleosomes by reducing the interaction between DNA and histone octamers in the outer nucleosome region, and the DNA is unbuckled from the histone surface to increase the accessibility of DNA, allowing transcription factors and other factors to be quickly bound to nucleosome DNA. The same conclusion was obtained in simulated succinylated H4K77E mutants, which reduced nucleosome stability and

led to defects in DNA damage repair and telomere silencing in response to DDR in vivo (Jing et al., 2020).

Regulation of nonhistone succinylation in DDR

Recent studies have reported that succinylation of nonhistone proteins is also involved in DDR regulation. Based on TMT labeling and affinity enrichment combined with high-resolution LC–MS/MS analysis, we found significantly increased levels of abnormal acetylation or succinylation of proteins in breast cancer tissues compared to normal paracarcinoma tissues. Enrichment analysis showed that the modified proteins were mainly enriched in the three H2A.X complexes associated with DDR development, and nucleolus phosphoprotein NPM1 was a common protein of the three complexes. Its K27 site was highly conserved, and there was a significantly high succinylation modification, indicating that the NPM1 protein played a key role in the H2A.X complex. Abnormal modification of NPM1 protein in H2A.X complex can affect the abnormal DDR state of breast cancer, suggesting that this may provide potential new therapeutic targets for breast cancer research (Gao et al., 2020). Previous studies have reported that flap endonuclease-1 (FEN1), as a structure-specific multifunctional endonuclease, undergoes a variety of posttranslational modifications, including methylation, phosphorylation and SUMO-1, during DNA replication and damage repair (Guo et al., 2010). In addition, the FEN1 protein was found to induce S187 phosphorylation-dependent succinylation in response to DNA damage, including UV irradiation, hydroxyurea, CPT, and mitomycin C. Among them, CPT1A is a potential palmitoyl transferase that mediates succinylation at K200, a key site of the FEN1 protein. This modification enhanced the activity of interstitial endonuclease GEN and stimulated its interaction with the Rad9–Rad1–Hus1 complex, thereby rescuing stalled replication cleavage and initiating homologous directed repair. This indicates that the succinylation of the nonhistone FEN1 protein also plays an important role in regulating DNA damage repair and maintaining genome stability (Shi et al., 2020). In addition, the tumor suppressor p53, as a genomic guardian, responds to DNA damage by inducing cell cycle arrest, apoptosis, senescence, metabolism regulation, autophagy, iron death and other ways to maintain genomic stability (Kaiser & Attardi, 2018; Kastenhuber & Lowe, 2017; Wang et al., 2023a). Various PTMs of p53 are considered to be the most effective way to regulate p53 activation and affect its biological function, particularly in response to DNA damage (Abuetabth et al., 2022; Bode & Dong, 2004; Dai & Gu, 2010). In addition to classical PTMs, succinylation at K120 on p53 is also involved in DDR. Moreover, SIRT5 can interact with p53, mediating its succinylation without affecting the deacetylation of this site to inhibit the transcriptional activity of p53, thereby

affecting the activation of p53 and leading to the inactivation of p53 target gene expression and cell apoptosis in response to DNA damage. This indicates that succinylation of p53 is essential for its activation to complete DNA damage repair and further reveals the potential mechanism of SIRT5's carcinogenic function (Liu et al., 2022).

Lactylation

Mechanism of lactylation

Lactate, as the metabolic end product of the glycolytic pathway, was initially considered a metabolic waste, among which L-lactic acid is the main substance produced by glycolytic metabolism in humans and most mammals, and its mass production is associated with tumorigenesis, autoimmune diseases, sepsis, diabetes and other diseases (Brooks, 2018, 2020; Li et al., 2022). However, in recent years, lactate has been shown to have multiple biological functions, including being a carbon source for cell metabolism, signaling molecules and immunomodulatory molecules involved in life activities such as angiogenesis (Morland et al., 2017; Porporato et al., 2012), macrophage polarization (Certo et al., 2020; Zhang et al., 2020), hepatocyte differentiation (Lyll et al., 2018) and T-cell activation (Barbieri et al., 2023; Feng et al., 2022). In 2019, the occurrence of novel epigenetic ϵ -N-L-lactylation (K(L-la)) on histone lysine residues mediated by L-lactate, inducing a mass shift of 72.021 Da, was first identified based on HPLC–MS/MS analysis, which is believed to alter chromatin spatial structure, DNA accessibility, and is closely related to gene regulation and macrophage polarization-related gene expression (Zhang et al., 2019).

Lactyltransferase

Lactylation is highly sensitive to lactate produced by glycolysis, and L-lactic acid can be converted into L-lactyl-CoA. In the presence of acetyltransferase p300 as a potential lactylation “writer”, the lactyl group is transferred from lactyl-CoA to histone lysine to form a covalent coupling (Zhang et al., 2019). Therefore, the occurrence of K(L-la) lactylation mediated by L-lactic acid is considered to be catalyzed by enzymatic reactions. In addition to catalyzing histone lactylation, a large number of studies have found that p300/CBP can catalyze the lactylation of nonhistone proteins, including Snail (Fan et al., 2023), HMGB1 (Yang et al., 2022), YY1 (Wang et al., 2023b) and α -MHC (Zhang et al., 2023a). Furthermore, it has been found that the acyltransferase GCN5 can mediate the lactylation of histone H3K18 in monocyte-macrophages in the early postmyocardial infarction dependent on IL-1 β , thereby promoting

the transcription of downstream target genes Lrg1, Vegf-a and IL-10 and improving the dual repair activity of excessive inflammation and promoting angiogenesis after myocardial infarction (Wang et al., 2022). Tip60 catalyzes Vps34 lactylation at K356/K781 sites, thereby enhancing the binding of Vps34 with Beclin1, Atg14L and UVRAG and further improving Vps34 lipid kinase activity (Jia et al., 2023). Compared with normal serum lactate concentrations of 1–2 mmol/L and almost entirely in the form of L-lactic acid, D-lactic acid synthesized by the glycolytic byproduct methylglyoxal (MGO) via the glyoxalase pathway is only at the nanomolar level (11–70 nmol/L) (Ewaschuk et al., 2005). Methylglyoxal rapidly binds to glutathione via glyoxalase 1 (GLO1) to form lactoylglutathione (LGSH). LGSH is then hydrolyzed by glyoxalase 2 (GLO2), recycling glutathione and producing D-lactic acid. However, S-D-(R)-lactoylglutathione can mediate K(D-la) lactylation on lysine through a nonenzymatic reaction as a lactyl donor, but this type of modification has been found to occur only on cytoplasmic proteins that are in close contact with S-D-lactoylglutathione (Armeni et al., 2014; Gaffney et al., 2020).

Delactylases

To maintain the homeostasis of lactylation in vivo, delactylase enzymes are also needed. All 18 HDACs were screened as potential delactylases, and both class I HDACs (HDAC1-3) and class III HDACs (SIRT1-3) caused a decrease in histone lactylation levels in vitro. In addition, HDAC1-3 can also be used as “erasers” to regulate histone lysine ϵ -amino lactylation in vivo and are the most effective delactylases in cells. Moreover, HDAC1 and HDAC3 have site-specific delactylation activity, which can catalyze the delactylation of histone H4K5 but has no significant effect on the Lys18 lactylation of histone H3 (Moreno-Yruela et al., 2022). However, NAD⁺-dependent SIRT1 can also act as a nonhistone delactylase to mediate delactylation at the K1897 site of α -MHC, and the process occurs both in normal physiology and heart failure (Zhang et al., 2023a). Based on the quantitative proteomics of SILAC, researchers found that the deacetylase SIRT3 can mediate removal of the lactyl moiety at the K348 site on CCNE2 in hepatocellular carcinoma (HCC) cells, catalyze its delactylation, and thus induce HCC cell apoptosis, indicating that SIRT3 is considered to be a tumor suppressor in HCC (Jin et al., 2023) (Fig. 4).

Regulation of lactate-mediated lactylation in DDR

In recent years, lactate and its mediated lactylation have been shown to play a significant role in the regulation of cell metabolism and gene expression, providing a bridge between metabolic reprogramming and gene regulation and participating in the process of DNA damage repair

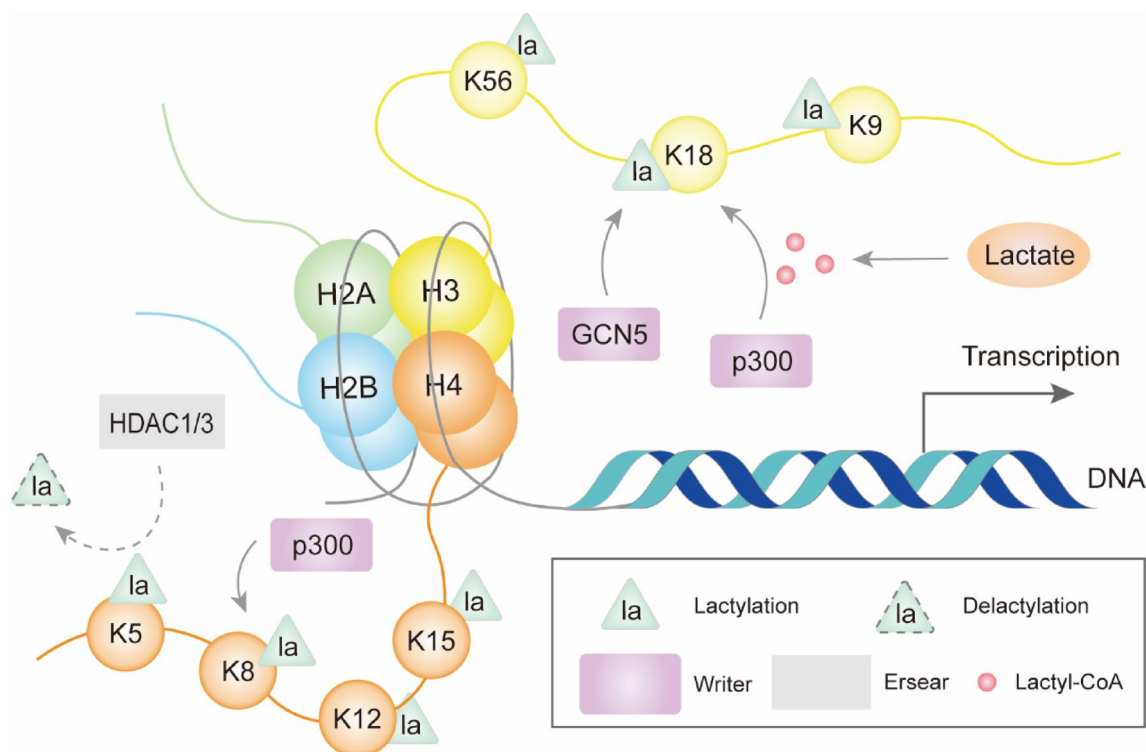


Fig. 4 Schematic of site-specific histone lactylation catalyzed by lactyltransferases (“writers”) and delactylases (“erasers”)

(Ciszewski et al., 2022; Wang et al., 2023c). Hydroxy-carboxylic acid receptor 1 (HCAR1), as a member of the G protein-coupled receptor family, mediates the improvement of DNA damage ability by stimulating the expression of DNA repair-related proteins, including BRCA1 and NBS1, in the lower reaches of the initial HeLa cells through lactate or DHBA ligands. This process indicates that the expression of the lactate receptor/HCAR1 can be involved in the regulation of the DNA repair pathway in cervical cancer cells and is an important mechanism of doxorubicin resistance (Wagner et al., 2017). In addition, the response of SW480 and HepG2 cells treated with lactate to cisplatin was also significantly inhibited, showing increased drug resistance, which was found to be related to the ability of tumor cells to cope with DNA damage and DNA recombination when exposed to lactate, as well as increased gene expression involved in DNA damage repair. In addition, these genes reverse cisplatin-induced DNA damage through mismatch and nucleotide excision repair pathways, thus affecting the antitumor effect of cisplatin, which is one of the vital reasons for tumor resistance (Govoni et al., 2021). Lactylation of the whole proteome was analyzed based on YnLac, a biological orthogonal chemical reporter of alkynyl functionalization, and a large number of novel modification sites on nonhistone proteins were found, among which polyribose polymerase 1 (PARP1) was a newly identified substrate of lactylation.

Previous studies have found that PARP1 can be activated by DNA damage and plays an important role in DDR by stimulating the ADP-ribosylation of itself and other proteins, thereby recruiting DNA damage repair factors and reshaping chromatin structure. However, the occurrence of lactate-mediated lactylation of PARP1 can also increase its ADP ribosylation activity, indicating that lactylation has a potential regulatory role in DNA damage repair (Sun et al., 2022). In addition, the involvement of histone lactylation in senescence is also thought to be related to the regulation of DNA repair, proteolysis and cell cycle-related genes (Zhang et al., 2023b).

Cross-talk involving novel PTMs in DDR

Cross-talk between crotonylation and ubiquitination

PTMs are considered to be the prominent regulators of DDR, and their coordination can jointly respond to DNA damage stress, participate in DDR and repair processes, and maintain genome integrity and stability. Ubiquitination at the histone H2AK119 site is a widely studied PTM related to DSB damage and is involved in the DNA damage repair process. However, it was found that lysine crotonylation at

this site and the transition between these two modifications are reversibly regulated by replication stress and involved in DDR. In response to DNA damage-induced replication pressure, the deacetylase SIRT1 mediates the deacetylation of H2AK119, leading to ubiquitination of this site mediated by BMI1. As a result, ubiquitination-modified histone H2A can weaken the DNA recombination, breakage, and mutation caused by replication transcription conflict with TRC by accumulating RNA Pol II in the reverse replication fork and inhibiting transcription near the replication fork, thereby maintaining genome stability (Hao et al., 2022).

Cross-talk among crotonylation, trimethylation and acetylation

Histone lysine crotonylation is recognized by the unique YEATS domain and is involved in regulating chromatin structure, gene transcription, and DDR processes. Studies have found that there are three types of acetylation, trimethylation, and crotonylation at the H3K27 site, which have different regulatory effects on the transcription status of genes in chromatin. H3K27 trimethylation mediates gene silencing of the chromatin closed state, acetylation mediates transcriptional activation of open chromatin, and H3K27 crotonylation can be selectively recognized by the GAS41 YEATS domain in the GAS41/SIN3A-HDAC1 coinhibitory factor complex, mediating gene inhibition of a unique open chromatin state, indicating an interactive relationship between H3K27 modifications. The degree of each modification type plays an important role in regulating gene transcription (Liu et al., 2023).

Cross-talk between succinylation and acetylation

Abnormal PTMs are closely related to genome instability and disease occurrence. Previous studies have found dynamic changes between acetylation and succinylation in organisms. However, researchers have quantitatively analyzed the overall changes in these two modifications in the UV-induced cell stress response using high-resolution nanoliquid chromatography–tandem mass spectrometry and affinity purification technology. A total of 3371 acetylation sites on 1446 proteins and 576 succinylation sites on 250 proteins were identified. Among them, 27 proteins contained both acetylation and succinylation sites, and a large number of modification sites were discovered for the first time. Unlike the decrease in most modification levels, the MDH2 acetylation level decreases in response to DNA damage induced by UV irradiation, but the succinylation level increases, indicating that MDH2 may participate in DNA damage repair as an important metabolic enzyme through its interaction or transformation between acetylation and succinylation (Xu et al., 2016). However, further research

is needed on the specific regulatory mechanism. H2A.X has been found to be activated during DNA damage and recruit numerous proteins to aggregate to form protein foci, mediating the DNA damage repair process. A large number of abnormal proteins detected in breast cancer tissues are highly acetylated and succinylated. The proteins involved in DNA damage repair were significantly upregulated, while the proteins sensitive to DNA damage were downregulated, indicating that abnormal DDR status in breast cancer tissues is closely related to the excessive modification of H2A.X complex and may be a crucial reason for breast cancer resistance to chemotherapy and radiotherapy. As a vital member of the H2A.X complex, nucleophosmin NPM1 is the only protein with acetylation and succinylation at the same lysine site (K27), whose modifications may regulate chromosome structure and affect the DDR process, but the interaction between modifications is still unclear (Gao et al., 2020).

Cross-talk between lactylation and acetylation

Lactylation, as a newly discovered type of PTM in recent years, has attracted increasing attention for its important functions in metabolic reprogramming and epigenetic regulation. However, its regulation in DDR has not yet been thoroughly studied, and it is currently reported that lactylation of the nonhistone protein PARP1 may be related to DNA damage repair. PARP1 is an important regulatory molecule in the process of DNA damage repair, and the PARylation activity of PARP1 activated by DNA damage mediates its own and other protein modifications. However, studies have found that seven lysine sites (K498, K505, K506, K508, K518, K521 and K524) on the self-modified domain of PARP1 can also undergo hyperacetylation and hyperlactylation, which affect its ADP-ribosylation activity but with different regulatory effects. The hyperacetylation state of PARP1 abolishes its autogenous ADP-ribosylation activity, while hyperlactylation of PARP1 can mediate an increase in its activity. Since both modifications occur at the same site, it is speculated that PARP1 lactylation may compete with its acetylation site to inhibit acetylation regulation and restore the ribosylation activity of PARP1, indicating that PARP1 lactylation may also be involved in the DNA damage repair process (Sun et al., 2022). However, the specific competitive mechanisms of its lactylation and acetylation, as well as the temporal regulatory relationship with ADP ribosylation, still need further clarification.

Conclusions and perspectives

To cope with various stress threats, cells have evolved a complex and elaborate system, known as DDR, to maintain the stability and integrity of the genome, in which DNA damage

repair and triggering programmed cell death or aging are the main forms of DDR (Huang & Zhou, 2021; Schumacher et al., 2021). PTMs of histones and nonhistones are crucial in DDR system. At present, classical PTMs, including phosphorylation, acetylation and ubiquitination, have been thoroughly studied for their regulatory mechanisms in DDR and play a significant role in cell fate determination and other life processes. With the continuous updating of enrichment

methods and mass spectrometry techniques, a growing number of novel modification types and modification sites have been identified, further enriching the diversity of proteomics and participating in regulating various biological processes. However, the regulatory mechanism of novel PTMs in the DDR has not been widely clarified. In this review, we summarize the latest progress on the mechanism of novel PTMs, including ufmylation, crotonylation, succinylation

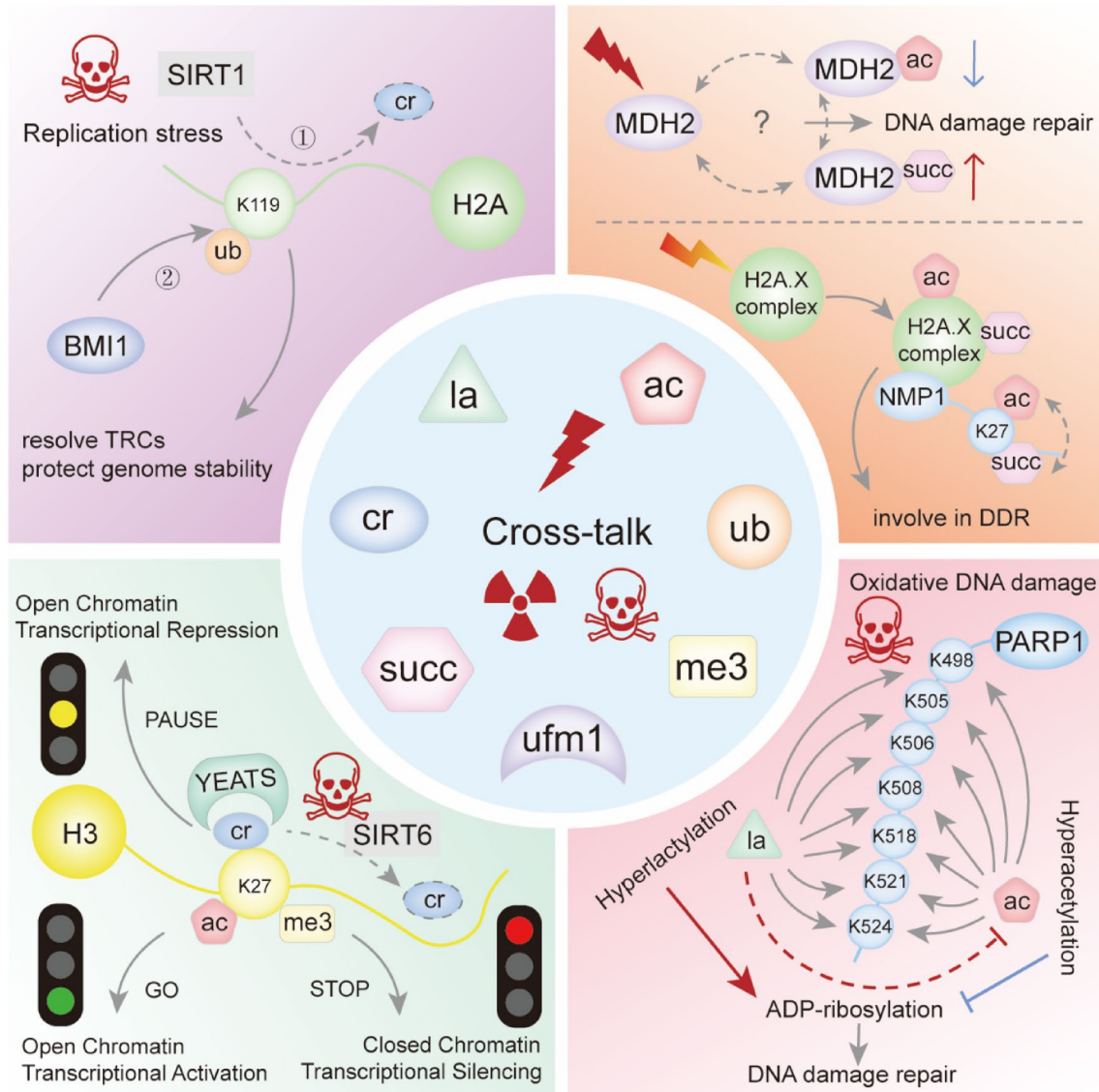


Fig. 5 Schematic of cross-talk involving novel PTMs in the DDR. The top left part depicts the cross-talk between crotonylation and ubiquitination in response to replication stress. SIRT1-catalyzed deacetylation of H2AK119 is a prerequisite for BMI1-mediated ubiquitination of the same site, thereby resolving TRCs and protecting genome stability. The top right part depicts the cross-talk between succinylation and acetylation in DNA damage. The MDH2 acetylation level is downregulated, and succinylation is upregulated. In addition, both acetylation and succinylation occur at the K27 site on NMP1 in the H2A.X complex, both of which are involved in DDR.

The bottom left depicts the cross-talk among crotonylation, trimethylation and acetylation. Each type of H3K27 modification represents distinct chromatin states for transcriptional repression, activation or silencing. SIRT6 catalyzes the deacetylation of H3K27 in response to DNA damage. The bottom right depicts the cross-talk between lactylation and acetylation in oxidative DNA damage. Seven lysine sites on PARP1 can be modified by hyperlactylation and hyperacetylation but have distinct effects on ADP-ribosylation, and PARP1 lactylation may compete for the inhibition of its acetylation, which may be involved in the DNA damage repair process

and lactylation, their regulatory roles and the cross-talk involving novel PTMs in DDR (Fig. 5).

Ufmylation, as a type of ubiquitin-like modification, has been found to regulate a variety of important biological functions (Gerakis et al., 2019; Millrine et al., 2023). Based on mass spectrometry, a large number of ufmylation-modified substrates have been discovered, and several proteins are related to DNA damage repair. However, current studies have reported that ufmylation of histone H4 and nonhistone MRE11 participates in the dynamic regulatory mechanism of DDR (Qin et al., 2019; Wang et al., 2019), and it is still necessary to further identify UFM1-mediated modification substrates related to DDR and clarify their participation in the regulatory process to enrich the functional network of histone and nonhistone ufmylation in DDR. Novel PTMs, including crotonylation, succinylation and lactylation, all belong to the acylation type and have similar mechanisms to classical acetylation. It has been found that these novel types of acylation may share identical modifying or demodifying enzymes as the widely studied lysine acetylation, and the processes of modifications all occur reversibly. For instance, acetyltransferase p300/CBP also exhibits crotonyltransferase activity (Sabari et al., 2015), succinyltransferase activity (Zorro Shahidian et al., 2021) and lactyltransferase activity (Zhang et al., 2019, 2023a), participating in various modification processes. Similarly, GCN5 also possesses catalytic activities toward multiple types of acylation (Kollenstart et al., 2019; Tong et al., 2020; Wang et al., 2017, 2022). In addition, deacetylase SIRT1-3 can also act as “erasers” for both crotonylation and lactylation on histones and nonhistone proteins (Bao et al., 2014; Feldman et al., 2013; Jin et al., 2023; Moreno-Yruela et al., 2022; Zhang et al., 2023a). However, due to the differences of the acyl-CoA (donor) forms, distinct structural or conformational alterations can be induced on the lysine residues by each PTM, which consequently determine the discrepancies of the modified proteins in their physiological functions. Notably, unlike other novel acylations, succinylation confers both negative charges and relatively large spatial changes on lysine residues, thereby resulting in stronger regulatory effects (Li et al., 2023). Thus, their roles in DDR could be more complex, including the response of cross-talk to DNA damage. Moreover, under different stress states, the selectivity of substrates and the catalytic activity exhibited by modifying enzymes may vary. Classic acetyltransferase p300 can also catalyze histone H3K18 crotonylation (H3K18cr), mediating stronger downstream gene transcription than p300, which is involved in histone acetylation (Sabari et al., 2015). However, the presence of lactylation catalyzed by GCN5 was likewise found to occur at the same modified site, mediating the transcription of downstream target genes (Wang et al., 2022). As a result, the specific conditions under which the dynamic changes in PTMs of different novel

histones at the same site occur have not been fully described and understood. This opens up more possibilities and challenges for us to understand the processes of life activities and disease occurrence. Based on the mechanisms of modifications, it is hypothesized that the following situations may exist. 1) Under different physiological or pathological conditions, the environment in which cells are located varies, such as differences in metabolic levels and stress states, which affect the occurrence of specific modification types. 2) Differences in donor levels of acyl-CoA involved in modification in cells result in different types of modifications. 3) Differential expression of modifying or demodifying enzymes affects the type and degree of modification. 4) Differences in activity between modifying and demodifying enzymes in a particular environment lead to differences in the strength of modifications. 5) Specific spatial structures in the vicinity of the modification site may also affect the affinity of different modifying or demodifying enzymes.

In addition, research on the regulation of DDR by various novel PTMs on similar modification sites and cross-talk between different modifications is still in the preliminary stage, and further exploration is needed. Currently, the novel PTMs involved in DDR regulation are still limited to hot spots on histones, and more functional expression of modification sites can be explored. Especially in recent years, the newly discovered small molecule metabolite lactate-mediated lactylation process has further opened up the “metabolic epigenetic” regulatory network in various disease studies. The involvement of lactylation in DDR system is also rewarding, but it is believed that further work will contribute to further expanding the study of novel modification regulation and expansion in DDR and provide promising treatment strategies for the diagnosis and treatment of various diseases.

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Declarations

Conflict of interest The authors declare no competing interests.

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