Review

Post-Translational Modifications of PCNA in Control of DNA Synthesis and DNA Damage Tolerance-the Implications in Carcinogenesis

Siyi Zhang1#, Tingting Zhou1#, Zhuo Wang1, Fei Yi1, Chunlu Li1, Wendong Guo1, Hongde Xu1, Hongyan Cui1, Xiang Dong1, Jingwei Liu1, Xiaoyu Song1# and Liu Cao2

1. Institute of Health Sciences, China Medical University, Shenyang, Liaoning Province, 110122, PR China.
2. College of Basic Medical Science, Key Laboratory of Cell Biology of Ministry of Public Health, Key Laboratory of Medical Cell Biology of Ministry of Education, Liaoning Province Collaborative Innovation Center of Aging Related Disease Diagnosis and Treatment and Prevention, China Medical University, Shenyang, Liaoning Province, 110122, PR China.

#These authors contributed equally to this work.

© The author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).
See http://ivyspring.com/terms for full terms and conditions.

Received: 2021.07.06; Accepted: 2021.09.19; Published: 2021.09.23

Abstract

The faithful DNA replication is a critical event for cell survival and inheritance. However, exogenous or endogenous sources of damage challenge the accurate synthesis of DNA, which causes DNA lesions. The DNA lesions are obstacles for replication fork progression. However, the prolonged replication fork stalling leads to replication fork collapse, which may cause DNA double-strand breaks (DSB). In order to maintain genomic stability, eukaryotic cells evolve translesion synthesis (TLS) and template switching (TS) to resolve the replication stalling. Proliferating cell nuclear antigen (PCNA) trimer acts as a slide clamp and encircles DNA to orchestrate DNA synthesis and DNA damage tolerance (DDT). The post-translational modifications (PTMs) of PCNA regulate these functions to ensure the appropriate initiation and termination of replication and DDT. The aberrant regulation of PCNA PTMs will result in DSB, which causes mutagenesis and poor response to chemotherapy. Here, we review the roles of the PCNA PTMs in DNA duplication and DDT. We propose that clarifying the regulation of PCNA PTMs may provide insights into understanding the development of cancers.

Key words: replication, DDT, ubiquitination, phosphorylation, acetylation, SUMOylation, NEDDylation, ISGylation

Introduction

Accurate DNA replication is essential for appropriate cell division. Human cells suffer from various “stress” at the replication fork, where the stress derives from replication obstacles and sources of damage. The replication obstacles contain re-replication, misregulated origin licensing, nucleotide depletion, and oncogene activation. The damage sources include reactive oxygen species (ROS), ultraviolet (UV), ionizing radiation (IR), and DNA damaging chemotherapy drugs. During replication, DNA synthesis stalls when replication machinery encounters lesions. Persistent replication stalling will cause DNA double-strand break (DSB). Because the failure of post-replication DNA damage repair leads to cell death or genomic instability, organisms evolve DNA damage tolerance (DDT) mechanisms to bypass DNA lesions. DDT pathways mainly consist of the error-prone translesion synthesis (TLS) and error-free template switching (TS) pathway.

Proliferating cell nuclear antigen (PCNA) plays a central role in DNA synthesis and DDT. PCNA serves as a moving platform recruiting various factors for replication or DDT. A conserved motif named PCNA-interacting protein box (PIP box) has been found in PCNA-binding partners. The core element is a consensus sequence Q1-x2-x2-h4-x5-x6-a7-a8, where “h” represents hydrophobic residues (L, M, I, or V) and “a” being aromatic amino acids (F, Y, or H) [1].
Figure 1. The Structure of PCNA Homo-trimer and the PTMs Sites. The crystal structure of Homo sapiens PCNA homo-trimer is shown as each monomer with different color (Protein Data Bank: 1VYM) [158]. The PTMs sites are highlighted in the amino acid sequence as colored characters (upper) and in the homo-trimer as colored balls (lower). Ubiquitination (K164), SUMOylation (K164), phosphorylation (Y211, Y60, Y133, Y250), acetylation (K13, K14, K77, K80, K20), methylation (K248, K110).

Figure 2. PCNA orchestrates DNA replication. PCNA binds to Polδ and Polε to orchestrate DNA lagging strand and leading strand synthesis, respectively.

During DNA replication, three monomers of PCNA form a ring-shaped homo-trimer (Figure 1), which encircles DNA and recruits B-family DNA polymerases (Polδ and Polε) to carry out the high fidelity DNA synthesis (Figure 2) [2]. In eukaryotes, Polδ is dominant over Polε in association with PCNA [3]. Polδ is responsible for the lagging strand synthesis, while Polε acts on the leading strand for replication [4, 5].

The replication fork stalls when the replication machinery confronts DNA lesions, and PCNA is mono-ubiquitinated at lysine residue (K) 164 by RAD6-RAD18 complex [6]. The mono-ubiquitinated PCNA switches its association from high fidelity polymerases to low fidelity ones, such as Polη, Polκ, REV1, and Polζ [7-9]. These polymerases help the replication fork bypass the damaged bases to continue DNA synthesis (Figure 3) [10-14]. However, every coin has two sides. The TLS pathway ensures uninterrupted replication and protects cells from DSB, while it also increases the frequency of mutations due to the low accuracy of TLS polymerases. In addition to the RAD6-RAD18 complex, the K63 polyubiquitination of PCNA at K164 requires the Mms2-Ubc13-Rad5 complex. The poly-ubiquitinated PCNA triggers the error-free TS (Figure 3) [15]. Thus, dysregulation of replication and the TLS/TS pathway will result in genomic instability and tumorigenesis.
Post-translational modifications (PTMs) possess various functions such as modulating protein activity, localization, stability, and interactions [16]. This review discusses the recent studies about the PTMs in regulating PCNA functions and the implications in tumor development.

Ubiquitination

Ubiquitin is an 8.5 kDa small protein with 76 amino acids. Protein ubiquitination, discovered in the early 1980s, is sequentially catalyzed by the ubiquitin system containing a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin ligase (E3). Initially, studies reported that ubiquitination mainly guides the tagged protein for 26S proteasome degradation. As the investigation develops in-depth, proteins are either modified with a single ubiquitin (mono-ubiquitination) or polymeric ubiquitin chains (poly-ubiquitination). Mono-ubiquitination serves as a signal for DNA damage response, such as mono-ubiquitinated H2AX/H2A/H2B and PCNA [17]. Additionally, it acts as a critical step for subsequent assembly of poly-ubiquitination [18]. Based on the different lysine (K) sites of the ubiquitin conjugation, poly-ubiquitination is diverse in various types, including K6, K11, K27, K29, K33, K48, and K63 poly-ubiquitination [19, 20]. Different ubiquitin linkages result in a diversity of cellular signals. The most well-studied types of poly-ubiquitination are the K48- and K63-ubiquitin chains. The K48 poly-ubiquitination labels substrates for proteasome degradation [21], while the K63 poly-ubiquitination participates in DNA damage response [22]. Protein ubiquitination is a reversible process that can be influenced by the ubiquitin-cleavage activity of deubiquitinating enzymes (DUBs).

In response to stalled replication forks, PCNA is mono-ubiquitinated by a sequential process mediated by an E1, RAD6 (E2), and RAD18 (E3) [6]. The human homolog of RAD6 is HHR6A and HHR6B [23]. The human RAD18, an approximately 54kDa protein consisting of 484 amino acids, is corresponded to yeast RAD18 [24]. PCNA is mono-ubiquitinated specifically at K164 during TLS [6, 12]. When PCNA is mono-ubiquitinated, it switches its interaction from high fidelity Polδ or Polε to low fidelity TLS polymerases (Polη, Polι, Polκ, Polλ, Polζ, or REV1). Then, these TLS polymerases proceed to bypass the lesions [10-14, 25].

High expressions of RAD6 and RAD18 are positively related to the development and therapy resistance of various tumors [26-36]. Sanders et al. identified the small molecular inhibitors of RAD6. Among them, SMI#9 shows a potent inhibition effect on the proliferation and migration of breast cancer cells [37]. It has been demonstrated that SMI#9 attenuates chemotherapy agent-induced PCNA mono-ubiquitination and enhances chemosensitivity of triple-negative breast cancer (TNBC) or ovarian cancer [35, 36, 38]. Inhibition of RAD18 has also been found to suppress gastric cancer progression and sensitize gastric cancer to chemotherapy via reduction of PCNA mono-ubiquitination [31]. However, the small molecular inhibitors of RAD18 have not been designed, which needs further studies to find effective ones for tumor treatment.

The K63-linked poly-ubiquitination of PCNA at its K164 site is involved in error-free lesion bypass via TS [15]. In addition to the RAD6-RAD18 complex,
PCNA poly-ubiquitination requires RING-finger E3 ligase Rad5, which recruits Ubc13 and Mms2 as an E2 complex [39]. The human homolog of Rad5 contains the helicase-like transcription factor (HLTF) and the SNF2 histone linker PHD RING helicase (SHPRH) to mediate PCNA poly-ubiquitination [40]. The mechanism of poly-ubiquitinated PCNA regulating lesion bypass has not been fully understood. One possibility is that poly-ubiquitinated PCNA may induce replication fork reversion to promote a recombination-like process using the undamaged DNA duplex as a template.

DNA binding is critical for PCNA mono-ubiquitination, as mono-ubiquitination of free PCNA is inhibited by the overabundance of free replication protein A (RPA) through its interaction with RAD18 under native conditions [41]. RPA, the main ssDNA-binding protein, is required for DNA replication, recombination, DNA damage repair, cell cycle checkpoints, and DNA damage checkpoints [42]. RPA undergoes a conformational change during replication stress when it binds to ssDNA and forms filaments at TLS sites. RAD6-RAD18 complex is recruited to the RPA filaments and then promotes the mono-ubiquitination of PCNA on DNA [41, 43].

The chromatin microenvironment modulated by various factors may be crucial for the regulation of PCNA ubiquitination. The chromatin structure modulated by histone modifications and nucleosome remodeling is essential for the control of replication, transcription, and DNA damage repair [44-46]. The cells with the mutations of histone H3/H4, which participated in packaging DNA into chromatin, showed a reduced level of PCNA mono- and di-ubiquitination after treating with methyl methanesulfonate (MMS) or UV. Accordingly, the H3/H4 mutants exhibited decreased MMS-induced mutagenesis compared to wild-type ones [47].

WRN protein is one member of the RecQ helicase family. The mutation of the WRN gene causes Werner Syndrome (WS) with premature aging and predisposition to cancer [48, 49]. In response to DNA damage agents, WRN forms discrete nuclear foci only during the S phase [50, 51], implying the essential roles of WRN in replication-post DNA damage repair. WRN interacts with PCNA in the absence of DNA damage. ATM-mediated phosphorylation of WRN causes its degradation in response to DNA damage. This degradation results in the release of PCNA from WRN that probably promotes PCNA ubiquitination. The WS and WRN knockdown cells show high-level ubiquitination of PCNA even without DNA damage [51]. These results indicate that WRN may inhibit aging and carcinogenesis via PCNA ubiquitination regulation. A small molecular inhibitor NSC19630 against WRN effectively inhibits tumor cell growth and induces apoptosis via impairing the response to DNA damage and replication stress [52]. Novel small molecular inhibitors targeting WRN have also been screened out, prohibiting the proliferation of cancer cells [53]. However, the underlying mechanisms require further investigation.

The human PTIP protein, the homolog of the Xenopus Swift with six BRCT domains participating in DNA damage repair, is an adaptor for ATM/ATR kinase [54-56]. The investigation from PTIP/Swift deleted mouse and yeast models reveals that PTIP/Swift plays a significant role in DNA damage repair during replication [57, 58]. Depleting PTIP/Swift in Xenopus and human cells compromised the mono- and di-ubiquitination of PCNA in response to UV [59], raising the potential role of PTIP/Swift in DDT.

RNF8 is an E3 ligase and a key DNA damage response factor [60]. It was identified as a novel ligase for mono- and poly-ubiquitination of PCNA using UbcH5c and Ubc13/Uev1a as an E2 ubiquitin-conjugating enzyme, respectively [60]. Similar to the RAD6-RAD18 complex, RNF8-UbcH5c-mediated mono-ubiquitination of PCNA serves as a substrate for poly-ubiquitination [60]. In medulloblastoma cells, knockdown of RNF8 inhibited IR-induced PCNA ubiquitination while enhanced cell cycle arrest and apoptosis [61]. However, the deletion of RNF8 barely affected PCNA ubiquitination in DT40 chicken cells after exposure to UV [62]. The effects of RNF8 on PCNA ubiquitination call for further research in different species of cells.

The insulin-like growth factor (IGF) signal pathway primarily functions in cell proliferation, survival, apoptosis, differentiation, metabolism, and migration [63]. It also participates in PCNA ubiquitination. After exposure to UVB, the skin of individuals over the age of 65 shows an increased level of PCNA mono-ubiquitination [64]. The geriatric skin contains a decreased expression of IGF-1 and reduced activation of IGF-1 receptor (IGF-1R) [65]. Both deprivation of IGF-1 and inhibition of IGF-1R result in elevated UVB-induced PCNA mono-ubiquitination in human skin and keratinocytes [64]. Exposure to UV is the primary cause of non-melanoma skin cancer, which possesses mutagenic photoproducts in DNA. These findings suggest that the IGF signaling pathway may prevent UV-induced carcinogenesis by regulating PCNA ubiquitination.

Phosphorylation kinases are emerging as targets attracting the most attention for cancer therapy development [66, 67]. By screening the library of kinase inhibitors, the inhibitor targeting pro-survival kinase Akt has been shown to impair the
monoo-ubiquitination of PCNA in cells after UV exposure. Persistent inhibition of Akt resulted in the blockage of TLS Pol\(\eta\) recruitment to DNA damage sites and further perturbation of DNA replication processivity, which led to synthetic lethality in homologous recombination (HR)-deficient cells [68]. However, this study did not unravel whether Akt could phosphorylate PCNA to regulate its ubiquitination. Nevertheless, it points to the field of modification crosstalk targeting PCNA for TLS regulation.

The deubiquitination enzyme USP1 reversely regulates PCNA ubiquitination in the S phase, which is critical to prevent unwanted mutagenesis. After the cells are exposed to UV, USP1 is downregulated by autocleavage and accompanied by upregulation of PCNA ubiquitination [69]. USP1-induced deubiquitination of PCNA is positively regulated by ELG1, the component of the RFC complex. ELG1 directs USP1-UAF1 complex for PCNA [70]. USP1 is upregulated in BRCA1 deficient tumors. The USP1-mediated restriction of PCNA ubiquitination protects replication fork in BRCA1-deficient cells because persistent ubiquitination of PCNA recruits Pol\(\kappa\) and REV1, which contributes to replication fork instability. Thus, the USP1 inhibitor may be beneficial for a subset of PARP inhibitor-resistant BRCA1-deficient tumors [71].

Kashiwaba et al. have identified another DUB USP7, which is responsible for the reduction of H\(_2\)O\(_2\)-induced PCNA mono-ubiquitination. Suppression of USP7 increases H\(_2\)O\(_2\)-induced mutagenesis in cells [72]. It has also been found that USP7 removes K63 poly-ubiquitination of PCNA [73]. Surprisingly, USP7 can also facilitate UV-induced mono-ubiquitination of PCNA via deubiquitinating and stabilizing Pol\(\kappa\) and REV1, which contributes to replication fork instability. Thus, the USP1 inhibitor may be beneficial for a subset of PARP inhibitor-resistant BRCA1-deficient tumors [71].

PCNA SUMOylation occurs during the S phase or in the presence of DNA damage. In yeast, the SUMOylated PCNA cooperates with the helicase Srs2 to inhibit HR repair during the S phase [86]. The recruitment of Srs2 by SUMOylated PCNA at replication sites disrupts Rad51 nucleoprotein filaments and dissociates Pol\(\kappa\)/Pol\(\eta\) from the repair synthesis machinery [87]. This mechanism controls the spontaneous HR [88], which contributes to maintaining genomic stability.

The replication factor C (RFC) is a conserved chaperone-like complex, which loads PCNA onto chromatin. PCNA SUMOylation by SUMO1 is facilitated by RFC. The association of PCNA to RFC is the prerequisite for SUMOylation, while DNA binding is not necessary [84]. The human SUMO1-PCNA has also been found to suppress inappropriate HR by recruiting the helicase PARI (Figure 4A) [83]. Furthermore, it has been demonstrated that the expression of SUMO1-PCNA fusion protein in human cells inhibits HR in response to DSB and reduces MMS-induced \(\gamma\)H2A foci and fragmented double-stranded DNA. The mutation at K164 for PCNA SUMOylation in Rad18/ cells eliminates the ubiquitination at this site and increases the MMS-induced \(\gamma\)H2A foci [84]. However, rather than generating DSB directly, MMS-induced DSB derives from replication fork stalling [89]. Thus, these observations indicate that the SUMOylation of PCNA by SUMO1 may be critical in preventing the replication fork collapse to DSB to maintain genome stability.

**SUMOylation**

The SUMOylation is a reversible and dynamic PTM that conjugates small ubiquitin-related modifier (SUMO) to the lysine of target proteins. The nascent SUMO is proteolytically cleaved by SUMO-specific proteases (SUPs) to expose the C-terminal glycine-glycine (GG) motif. The SUMO E1 enzyme activates the mature SUMO. Then, it is transferred by the SUMO E2 and E3 enzyme to the substrate. SUMOylation is involved in several biological processes, including protein-protein interactions, protein-DNA binding, subcellular localization, transcriptional regulation, and DNA repair [79]. The aberrant regulation of SUMOylation is frequently related to cancer, cardiac disease, and neurodegenerative disease [80-82].

The human SUMO molecules contain four paralogs (SUMO1-4). PCNA can be conjugated with both SUMO1 and SUMO2 [83-85]. PCNA is SUMOylated at K164 and K127. Distinctively from K164 being both ubiquitinated and SUMOylated, K127 is only SUMOylated but cannot be ubiquitinated [6]. By co-expressing ectopic PCNA with SUMO1, SUMO2, and SUMO3 in human cells, it was found that SUMO1 is predominantly conjugated to PCNA, while SUMO2 or SUMO3 is weakly attached to PCNA even with UV treatment. Furthermore, the in vitro PCNA SUMOylation assay revealed that the E1 SUMO-activating enzyme (SAE1/SAE2), the E2 SUMO-conjugating enzyme (Ubc9), and the E3 SUMO-ligases (Pias1, Pias2, Pias3, and Pias4) participate in SUMO1 conjugating to PCNA [84].

The replication factor C (RFC) is a conserved chaperone-like complex, which loads PCNA onto chromatin. PCNA SUMOylation by SUMO1 is facilitated by RFC. The association of PCNA to RFC is the prerequisite for SUMOylation, while DNA binding is not necessary [84]. The human SUMO1-PCNA has also been found to suppress inappropriate HR by recruiting the helicase PARI (Figure 4A) [83]. Furthermore, it has been demonstrated that the expression of SUMO1-PCNA fusion protein in human cells inhibits HR in response to DSB and reduces MMS-induced \(\gamma\)H2A foci and fragmented double-stranded DNA. The mutation at K164 for PCNA SUMOylation in Rad18/ cells eliminates the ubiquitination at this site and increases the MMS-induced \(\gamma\)H2A foci [84]. However, rather than generating DSB directly, MMS-induced DSB derives from replication fork stalling [89]. Thus, these observations indicate that the SUMOylation of PCNA by SUMO1 may be critical in preventing the replication fork collapse to DSB to maintain genome stability.
DSB can also occur in unperturbed growing cells induced by transcription-replication conflicts (TRCs). The TRCs cause replication fork stalling leading to replication fork collapse, which results in DSB formation and genomic instability [90, 91]. The E3 ligase TRIM28 catalyzes the SUMO2 conjugation to PCNA, which is triggered by transcription to resolve the TRCs [92]. The SUMOylation of PCNA by SUMO2 is induced by the RNA polymerase II (RNAPII)-bound helicase RECQ5, which suppresses transcription-related DSB and functions as a tumor suppressor [93-97]. SUMO2-PCNA temporarily dissociates RNAPII in the collision path by enriching the histone chaperone FACT to remove parental histones ahead of the replication fork and CAF1 to deposit repressive histone marks at the replication site, that ensures the progression of the replication fork avoiding DSB risks (Figure 4B) [97].

The SUMOylation can also serve as a signal for ubiquitination. The SUMO-targeting ubiquitin ligases (STUbLs) harboring SUMO-interaction motifs (SIMs) mediate the ubiquitination of SUMO moieties or SUMOylated proteins [98, 99]. The mutant of the K164/K127 of PCNA or SIM of RAD18 reduces PCNA ubiquitination, suggesting that the SUMOylation of PCNA can direct RAD18 to target PCNA [100]. This observation implies the crosstalk between SUMOylation and ubiquitination of PCNA.

**Phosphorylation**

Phosphorylation, discovered in the mid-1950s, is a principal way to regulate most cellular functions, including protein stability, subcellular localization, and protein-protein interaction. It reversibly confers phosphate group to substrate proteins at a serine, threonine, or tyrosine residue [101].

The chromatin binding is essential for PCNA to exert DNA replication and DDT functions. PCNA is phosphorylated when it binds to DNA synthesis sites [102, 103]. In 1995, Zophonias O. Jonsson et al. found that the Y211 site of PCNA is critical for chromatin loading, as the Y211A mutation of PCNA completely abolishes its trimer formation, which then cannot be loaded onto DNA by RFC [104]. Subsequently, it was found that the Y211 site of PCNA can be phosphorylated by the epidermal growth factor (EGF) receptor (EGFR) to stabilize the chromatin-bound PCNA. The increased PCNA Y211 phosphorylation is frequently correlated to prostate cancer and breast cancer [105, 106]. Blocking PCNA Y211 phosphorylation by the Y211F PCNA peptide, the proliferation of prostate cancer and TKI-resistant triple-negative breast cancer (TNBC) cells is significantly restrained [106, 107]. The treatment of cells with EGFR inhibitors such as AG1478 or lapatinib suppresses Y211 phosphorylation of PCNA and downregulates chromatin-bound PCNA [108].

In the absence of EGFR, the phosphorylation of PCNA at the Y211 site is also catalyzed by the non-receptor tyrosine kinase c-Abl when the upstream Ron receptor is activated by its ligand hepatocyte growth factor-like protein (HGFL). Imatinib, a pharmacological inhibitor of c-Abl, suppresses the HGFL-induced proliferation of breast cancer cells [88]. Thus, the Y211 site of PCNA may be an effective target to develop cancer therapy.

PCNA can also be phosphorylated at Y60, Y133, and Y250 sites by the nuclear IGF-1R (nIGF-1R). The phosphorylation of PCNA leads to the recruitment of E2 conjugating enzyme (UBC13) and E3 ligases...
(RAD18, SHPRH, and HLTF) for PCNA mono- and poly-ubiquitination. In IGF-1R negative cells, DNA damage induces G1 arrest and S phase replication fork stalling, while the nIGF-1R activation can rescue the stalled replication fork [109]. IGF-1R and PCNA are colocalized in many cancer types. The colocalization increases in the tumor tissues compared to the adjacent normal tissues. This colocalization is frequently lost in carcinomas with poor chemotherapy response and a majority of metastatic lesions. However, the stronger colocalization of IGF-1R and PCNA is correlated to the higher overall survival in cancer patients [110].

These findings suggest that the phosphorylation of PCNA is crucial for its loading onto chromatin, which serves as a prerequisite for the succeeding functions of PCNA. Moreover, the phosphorylation may also have crosstalk with the ubiquitination of PCNA.

Acetylation

The acetylation was first identified on histones in 1964, followed by the discovery of acetylation of non-histone proteins such as high-mobility group (HMG) proteins, tubulin, and p53 [111-115]. Lysine acetylation is dynamically controlled by two groups of enzymes. The enzymes are lysine acetyltransferases (HATs) and lysine deacetylases (HDACs) [116]. The non-histone protein acetylation is implicated in diverse functions, including gene transcription, cell cycle regulation, DNA damage repair, protein stability, protein folding, protein aggregation, cytoskeleton organization, RNA processing, RNA stability, and autophagy [117].

Hoyun Lee and colleagues found that mammalian PCNA is not regulated by phosphorylation but by acetylation. They found that PCNA may be acetylated and deacetylated by p300 and HDAC1, respectively [118]. The acetylated PCNA shows a high affinity to Polβ and Polδ, which is important for DNA replication. The acetylation of PCNA at K14 is induced after UV damage and promotes PCNA for degradation to inhibit DNA replication [119]. Ennio Prosperi and colleagues further clarified that CBP and p300 are required for the acetylation of PCNA. CBP- and p300-mediated acetylation of PCNA at K13, K14, K77, and K80 promotes the removal of chromatin-bound PCNA and its degradation after DNA synthesis [120]. Thus, the acetylation of PCNA avoids the excessive retention of PCNA on chromatin, preventing genomic instability.

In response to MMS-induced DNA damage, the sliding surface of PCNA is acetylated at K20 by Eco1, which stimulates HR and suppresses the DNA damage sensitivity of the DDT mutant yeast cells [121]. Depleting HAT3 in Leishmania donovani caused the loss of PCNA ubiquitination in response to UV [122]. This observation indicates that the HAT3-mediated PCNA acetylation may be a prerequisite for PCNA ubiquitination. This study links the acetylation to the ubiquitination of PCNA in eukaryotes.

Other PTMs

Methylation

The understanding of protein methylation is expanding these years since the first report of flagellar protein methylation in 1959. The methyl group is transferred by methyltransferase from S-adenosylmethionine (SAM) to lysine, arginine, aspartic acid, or glutamic acid. The methylation is reversible as the methyl group can be removed from substrates by demethylases.

PCNA was found to be methylated at K248 by histone H4K20 methyltransferase SETD8, and this methylation allows the stabilization of PCNA expression [123, 124]. SETD8-mediated PCNA methylation increased the interaction between PCNA and flap endonuclease FEN1. This effect is essential for DNA replication and repair. The methylation-inactive mutation of PCNA causes impaired DNA replication and induced DNA damage [123]. Depleting SETD8 results in decreased cell proliferation and increased DNA damage during the S phase [125]. SETD8 is overexpressed in various types of cancers. The expression of SETD8 and PCNA are correlated in cancer samples, implying the crucial role of SETD8-mediated methylation of PCNA in tumor development [123]. Various SETD8 inhibitors have been discovered, such as Nahuoic acid A, UNC0379, SPS811–3, MS453 [126-129]. Among these inhibitors, UNC0379 shows an anti-growth effect on neuroblastoma and ovarian cancer [130, 131]. The functional effect of other inhibitors on tumor development and whether they target the regulatory sites of PCNA by SETD8 requires further identification.

The methyltransferase enhancer of zeste homolog 2 (EZH2) is responsible for the trimethylation of histone H3 at K27 [132-134]. EZH2 is positively related to cancer cell proliferation and localizes at the replication fork in response to DNA damage [135-138]. EZH2 directly binds to PCNA via PIP-box and methylates PCNA at K110. The EZH2-mediated methylation of PCNA stabilizes the PCNA trimer to enhance its interaction with Polδ and DNA replication [139]. Inhibitors targeting EZH2 such as 3-Deazaaneplanocin A (DZNep), GSK126, El1, and UNC1999 exhibit a proliferation suppression effect on various tumor cells or cancer models, including Acute
Myeloblastic Leukemia (AML), lymphoma, gastric cancer, breast cancer, and MLL-rearranged leukemia [140-145]. Additionally, the inhibitor CPI-1205 being in clinical trials has shown a potent anti-tumor effect [146].

**NEDDylation**

The neuronal precursor cell-expressed developmentally down-regulated protein 8 (NEDD8) is a small ubiquitin-like molecule. NEDD8 is reversibly conjugated to a lysine residue of target proteins [147]. Similar to the ubiquitin system, NEDDylation is also a process triggered by the NEDD8-activating enzyme (E1), NEDD8-conjugating enzyme (E2), and NEDD8 ligase (E3). NEDDylation participates in DNA damage response, such as the decision between non-homologous end joining (NHEJ) and HR [148-150]. PCNA can be NEDDylated by RAD18. The deNEDDylation of PCNA is catalyzed by the deNEDDylase NEDP1. In response to H2O2-induced oxidative stress, NEDP1 dissociates with PCNA causing elevated RAD18-dependent NEDDylation of PCNA. The NEDDylation antagonizes the ubiquitination of PCNA. In addition, the NEDDylation of PCNA disrupts the interaction between PCNA and Polŋ. The impairment of PCNA NEDDylation reduces the cell sensitivity to oxidative stress and vice versa [151]. This crosstalk between NEDDylation and ubiquitination of PCNA may be a mechanism to confine the error-prone TLS to maintain genomic stability.

**ISGylation**

ISGylation resembles the ubiquitination process. The interferon-stimulated gene 15 (ISG15) ubiquitin-like modifier is conjugated sequentially by an E1 (ubiquitin-activating enzyme E1-like protein, Ube1L), an E2 (ubiquitin-carrier protein H8, UbcH8), and an E3 (HECT domain and RCC1-like domain-containing protein 5, HERC5, or estrogen-responsive finger protein, EFP) [152-156]. The primary function of ISGylation is to impede the infection of intracellular pathogens. ISGylated proteins are also involved in physiological processes such as TLS, autophagy regulation, protein synthesis, cytoskeletal dynamics, and secretion. Moreover, the dysregulation of ISGylation is closely related to cancer proliferation, migration, and sensitivity to chemotherapy [157]. Upon UV irradiation, the ISGylation of PCNA is induced by EFP, which binds to mono-ubiquitinated PCNA. The ISGylated PCNA recruits USP10 to deubiquitinate PCNA, which results in Polŋ disassociation from PCNA. This dissociation leads to the termination of TLS. Ultimately, PCNA is deISGylated by UBP43 for reloading of replicative DNA polymerases to resume normal replication. By ISGylation-defective mutation in PCNA or silencing ISG15/EFP, the compromise of PCNA ISGylation causes persistent retention of PCNA and Polŋ to nuclear foci. The prolonged recruitment of PCNA and Polŋ on chromatin increases mutation frequency [77]. This investigation indicates that ISGylation of PCNA is essential for controlling the appropriate TLS and preventing mutagenesis.

**Conclusions and Perspectives**

PCNA is the master of DNA replication and DDT during the S phase. The faithful replication and timely lesion bypass if the replication fork encounters lesions are indispensable events for cell survival and proliferation. PCNA trimer serves as a coordinator recruiting DNA polymerases and slides on the DNA to facilitate DNA synthesis. When replication fork stalls induced by endogenous or exogenous sources of damage, PCNA is ubiquitinated and transfers to bind DDT DNA polymerases or mediate template switching. This process ensures the replication processivity. The ubiquitination of PCNA is removed by DUBs to restrict unwanted mutagenesis [69, 71, 75]. Thus, the appropriate regulation of PCNA in the control of DNA replication and DDT is critical for normal cellular function and preventing mutagenesis.

PTMs dynamically regulate the functions of target proteins to fine-tune biological processes in response to environmental changes. In this review, we discuss the regulation and functions of PCNA PTMs. PCNA can be modified by ubiquitination, SUMOylation, phosphorylation, acetylation, methylation, NEDDylation, and ISGylation (Table 1), and the modification sites are presented in Figure 1. Chromatin loading is a requisite for PCNA to exert replication and repair functions. PCNA phosphorylation catalyzed by EGFR or c-Abl is critical for PCNA loading onto chromatin [88, 105]. The affinity to Polŋ, responsible for DNA replication, is regulated by PCNA acetylation [118]. The acetylation of PCNA also promotes its degradation to suppress DNA synthesis in response to DNA damage [119], which may provide time for DNA damage response and repair.

When the replication fork stalls, the ubiquitination allows PCNA to recruit TLS polymerases or promote TS to tolerate the DNA lesions. The DNA binding of PCNA and the histones are essential for the ubiquitination of PCNA [41, 47]. Several DNA damage response factors (WRN, PTIP, and RNF8) and kinases (IGF-1R and Akt) related to cell growth signal function in regulating PCNA ubiquitination [51, 59-61, 64, 68].
The SUMOylation of PCNA with SUMO1 is regulated by RFC [84]. The SUMO1-PCNA negatively regulates HR to suppress genome rearrangements and DSB [83, 86]. The SUMO2 is conjugated to PCNA by TRIM28 E3 ligase and induced by RECQ5 [92, 97]. The SUMO2-PCNA ensures replication progression by resolving the collision between transcription and replication machinery [97].

SETD8 and EZH2, which are positively correlated to tumor development, can catalyze PCNA methylation. The methylation causes PCNA stabilization and increases its interaction with FEN1 and Polδ for DNA replication and DDT [123, 139]. The NEDDylation of PCNA is dynamically regulated by the E3 ligase RAD18 and the deNEDDylase NEDP1. The NEDDylation of PCNA impairs the association between PCNA and Polη [151], which may be essential to control TLS.

The modification crosstalk also takes place between the SUMOylation and the ubiquitination of PCNA. The SUMOylation serves as a signal for PCNA ubiquitination, as it activates RAD18 to target SUMO-PCNA [100]. The nuclear IGF-1R-mediated phosphorylation of PCNA promotes its ubiquitination by recruiting UBC13, RAD18, SHPRH, and HLTF. The colocalization of PCNA and IGF-1R is deficient in cancer tissues from patients with poor chemotherapy response, suggesting that the crosstalk between phosphorylation and ubiquitination of PCNA may predict the high overall survival of cancer patients [110]. PCNA ubiquitination is deficient in acetyltransferase HAT3-null Leishmania donovani [122]. This result indicates that acetylation may promote the ubiquitination of PCNA. In contrast, NEDDylation suppresses the ubiquitination of PCNA in response to oxidative stress [151], which suggests a negative regulation of the NEDDylation for ubiquitination of PCNA. In addition, ISGylation antagonizes the ubiquitination of PCNA directly. The ISGylated PCNA recruits USP10 to reduce the ubiquitination of PCNA in response to oxidative stress [151], which suggests a negative regulation of the NEDDylation for ubiquitination of PCNA. In addition, ISGylation antagonizes the ubiquitination of PCNA directly. This result indicates that acetylation may promote the ubiquitination of PCNA. In contrast, NEDDylation suppresses the ubiquitination of PCNA in response to oxidative stress [151], which suggests a negative regulation of the NEDDylation for ubiquitination of PCNA. In addition, ISGylation antagonizes the ubiquitination of PCNA directly. The ISGylated PCNA recruits USP10 to reduce the ubiquitination of PCNA and leads to the termination of TLS subsequently [77].

The PTMs of PCNA and the related regulators described above have several implications in carcinogenesis. These modifications can be aberrantly utilized by cancer cells to exert unlimited growth and resistance to chemotherapy (Table 2). Consequently, elucidating the regulation and mechanism of PCNA PTMs will provide insights to develop cancer therapies. As summarized in Table 3, the Y211F PCNA peptide targeting the phosphorylation site Y211 of PCNA inhibits the proliferation of prostate cancer and TNBC cells effectively [106, 107]. A variety of small molecular inhibitors against the enzymes or regulators of PCNA PTMs, including SMI#9 (RAD6, ubiquitination), AG41478 (phosphorylation, EGFR), and Lapatinib (phosphorylation, EGFR), have been identified that they suppress cell proliferation or
enhance chemosensitivity via inhibition of these PCNA PTMs [35-38]. Although WRN, c-Abl, SETD8, and EZH2 show regulation of PCNA PTMs (Table 1) and potent anti-growth effects on various tumors (Table 2), the anti-tumor mechanisms directly correlated to the regulation of PCNA PTMs need further investigation. Moreover, novel inhibitors or specific blocking peptides suppressing tumor development via inhibition of PCNA PTMs require identification.

### Table 2, The implications of PCNA PTMs in carcinogenesis

| PTM            | Site in PCNA | Implication in Carcinogenesis                                                                 | Reference |
|----------------|--------------|-----------------------------------------------------------------------------------------------|-----------|
| Ubiquitination | RAD6, RAD18 | Development and therapy resistance of tumors.                                                  | [26-36]   |
|                | H454/455     | MMS-induced mutagenesis.                                                                      | [47]      |
|                | WRN          | Modification predisposition to cancer                                                          | [48]      |
|                | RNF8         | Cell cycle arrest and apoptosis of medulloblastoma cells                                      | [61]      |
|                | IGF1         | Cell proliferation, survival, apoptosis, differentiation, metabolism, migration                | [63]      |
| SUMOylation     | K164, K127   | Prevention of the replication fork collapse to DSB.                                            | [83, 84, 89] |
|                |              | Prevention of the replication fork collapse to DSB.                                            | [90-92, 97] |
| Phosphorylation | Y211         | Increased phosphorylation-prostate and breast cancer                                           | [105, 106] |
|                | Y60, Y133,   | IGF-1R and PCNA                                                                               | [109, 110] |
|                | Y250         | Colocalization to many cancer types, types of IGF-1R receptor response, high overall survival |           |
| Acetylation     | K13, K14, K77, K80 | Removal of chromatin-bound PCNA-savodiance of excessive retention of PCNA and genomic instability | [120]     |
| Methylation     | K248         | SETD8-proliferation, high expression in many types of cancers                                 | [125, 123] |
|                | K110         | EZH2-cansee cell proliferation                                                                  | [135-138] |
| NEDDylation     |              | Cell sensitivity to oxidative stress, maintain genomic stability                               | [151]     |
| ESgylation      |              | Control of appropriate TLS and prevention of mutagenesis                                       | [77, 157] |

### Table 3, Inhibitors or blocking peptides relating to PTMs and regulators for cancer therapy

| Inhibitor | Affected PTM | Target | Cancer Type/Cancer Cell Type | Reference |
|-----------|--------------|--------|-----------------------------|-----------|
| SM1@9     | Ubiquitination | RAD6   | breast cancer, ovarian cancer | [35-38]   |
| N3C19630  | Ubiquitination | WRN    | HeLa cells                  | [52]      |
| Y211PCNA  | Phosphorylation | Y211 site in PCNA | prostate cancer, breast cancer | [106, 107] |
| AG1478    | Phosphorylation | EGFR   | MDA-MB-468 cells            | [108]     |
| Lapatinib | Phosphorylation | c-Abl  | breast cancer                | [88]      |
| Imatinib  | Methylation | SETD8  | neuroblastoma, ovarian cancer | [130, 131] |
| UNC0379   | Methylation | EZH2   | AML, lymphoma, gastric cancer, breast cancer, MLL-rearranged leukemia | [140-146] |

According to these investigations, the regulation outline of PCNA PTMs gradually becomes clear. The modulations and the crosstalk in these PTMs of PCNA orchestrate its functions in DNA replication and DDT. However, the dysregulation of PCNA PTMs will lead to mutagenesis and ultimately result in carcinogenesis. Thus, clarifying the mechanisms of PCNA PTMs is critical for understanding tumor development, which may provide effective targets for cancer therapy.

### Abbreviations

PTMs: post-translational modifications; PCNA: proliferating cell nuclear antigen; TLS: translesion synthesis; TS: template switching; ROS: reactive oxygen species; UV: ultraviolet; IR: ionizing radiation; DSB: DNA double-strand break; DDT: DNA damage tolerance; PI: PCNA-interacting protein box; K: lysine; DUBs: deubiquitination enzymes; HLF: helicase like transcription factor; SHPRH: SNF2 histone linker PHD RING helicase; RPA: replication protein A; MMS: methyl methanesulfonate; WS: Werner Syndrome; IGF: insulin-like growth factor; IGF-1 receptor: IGF-1R; HR: homologous recombination; SUMO: small ubiquitin-related modifier; SUPs: SUMO-specific proteases; RFC: replication factor C; STUbLs: SUMO-targeting ubiquitin ligases; SIMs: SUMO-interaction motifs; TRCs: transcription-replication conflicts; RNAPII: RNA polymerase II; EGF: epidermal growth factor; EGFR: EGF receptor; TNBC: triple-negative breast cancer; HGFL: hepatocyte growth factor-like protein; nIGF1R: nuclear IGF-1R; HMG: high-mobility group; HATs: lysine acetyltransferases; HDACs: lysine deacetylases; H2A.Z: heterochromatin protein 1; H2A.Z: heterochromatin protein 2; EZH2: enhancer of zeste homolog 2; DZNept: 3-Deazaneplanocin A; AML: Myeloblastic Leukemia; NEDD8: neuronal precursor cell-expressed developmentally down-regulated protein 8; NHEJ: non-homologous end joining; ISG15: interferon stimulated gene 15.

### Acknowledgements

We receive fundings from National Key R&D Program of China (2016YFC1302400), National Natural Science Foundation of China (82030091, 81770001, and Z18-4-021), and the Ministry of Education Innovation Team Development Plan (IRT_17R107&IRT13101) for L.C.; National Natural Science Foundation of China (81502438) for T.Z., (81502414) for F.Y.; Foundation of Liaoning Province of China (LFWK201725) for X.S., (QN2019039) for T.Z., (QN2019038) for F.Y.; Foundation of Liaoning Province of China (LFWK201725) for X.S., (QN2019039) for T.Z., (QN2019038) for F.Y., (LQNK201747) for Z.W.

### Competing Interests

The authors have declared that no competing interest exists.
1. Warbrick E. PCNA binding through a conserved motif. Bioessays. 1998; 20: 11-18.
2. Moldovan GL, Pfander B, Jentsch S. PCNA, the maestro of the replication fork. Cell. 2007; 129: 665-79.
3. Georgescu RE, Langston L, Yao NY, Yuervia O, Zhang D, Finkelshtein J, et al. Mechanism of asymmetric polymerase assembly at the eukaryotic replication fork. Nat Struct Mol Biol. 2014; 21: 664-70.
4. Garg P, Stith CM, Sabouri N, Johansson E, Burgess PM. Idling by DNA polymerase delta maintains a ligatable nick during lagging-strand DNA replication. Genes Dev. 2004; 18: 2764-73.
5. Purnell ZF, Ioa L, Lundstrom EB, Johansson E, Kunkel TA. Yeast DNA polymerase epsilon particles in leading-strand DNA replication. Science. 2007; 317: 127-30.
6. Hoege C, Pfander B, Moldovan GL, Pyrovakalis G, Jentsch S. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature. 2002; 419: 135-41.
7. Kannouche PL, Wing J, Lehmann AR. Interaction of human DNA polymerase eta with mono-ubiquitinated PCNA: a possible mechanism for the polymerase assembly on DNA damage. Mol Cell. 2004; 14: 491-500.
8. Watanabe K, Tateishi S, Kawasuji M, Tsurimoto T, Inoue H, Yamaizumi M. Rad18 gene product interacts with HHR6A and HHR6B. Nucleic Acids Res. 2000; 28: 536-42.
9. Bienko M, Green CM, Crosetto N, Rudolf F, Zapart G, Coull B, et al. Ubiquitin-binding domains in Y-family polymerases regulate translesion synthesis. Science. 2005; 310: 1821-4.
10. Zhang Y, Yuan F, Wu X, Taylor JS, Wang Z. Response of human DNA polymerase alpha to DNA damage. Proc Natl Acad Sci USA. 2001; 98: 2938-43.
11. Maq G, Villani G, Ramadán K, Shevelev L, Tanguy Le Gac N, Blanco L, et al. Human DNA polymerase lambda functionally and physically interacts with proliferating cell nuclear antigen in normal and translesion DNA synthesis. J Biol Chem. 2017; 292: 489-493.
12. Garg P, Burgers PM. Ubiquitinated proliferating cell nuclear antigen activates target DNA polymerases and eta. Proc Natl Acad Sci U S A. 2005; 102: 18361-6.
13. Pires MC, Blans P, Zink CN, Hollis J, Fishekind JC, Perrino FW. Lesion bypass polymerase eta with mono-ubiquitinated PCNA and DNA damage-induced response. Oncogene. 2004; 23: 3986-96.
14. Sigismund S, Polo S, Di Fiore PP. Signaling through monoubiquitination. Curr Opin Cell Biol. 2002; 14: 195-9.
15. Giannattasio M, Zwicky K, Follonier C, Foiani M, Lopes M, Branzei D. Monoubiquitination of proliferating cell nuclear antigen in normal and translesion DNA synthesis. Sci Signal. 2014; 7: ra51.
16. Zhang Y, Yu an F, Wu X, Taylor JS, Wang Z. Response of human DNA polymerase alpha to DNA damage. Proc Natl Acad Sci USA. 2001; 98: 2938-43.
57. Cho EA, Prindle MJ, Dressler GR. BRCT domain-containing protein PTIP is essential for transcription through mitosis. Mol Cell Biol. 2003; 23: 1666-73.
58. Hanway D, Chun JK, Xia G, Oshiro G, Winzeler EA, Romesberg FE. Previously uncharacterized genes in the UV- and H2O2-induced DNA damage response in yeast. Proc Natl Acad Sci U S A. 2002; 99: 10605-10.
59. Gali H, Juhasz S, Murocz M, Hajdu I, Fatyol K, Szukacsov V, et al. Role of HER2 and RNFLP ubiquitin E3 ligases in the promotion of translation of DNA synthesis in the chicken DT40 cell line. DNA Repair (Amst). 2011; 10: 75-77.
60. Zhang S, Chea J, Meng X, Zhou Y, Lee EY, Lee MY. PCNA is ubiquitinated by RNR. Cell Cycle. 2008; 7: 3999-404.
61. Li F, Liu B, Zhou X, Yu Q. Silencing of ESC Ubiquitin Ligase RNFL8 Enhances Immortalizing, Radiation-Sensitivity of Medulloblastoma Cells by Promoting the Deubiquitination of PCNA. Onco Res. 2018; 2: 1365-73.
62. Mohiuddin, Tumoz IM, Rouse J, Blow J. PTIP/Swift is required for efficient PCNA ubiquitination in response to DNA damage. DNA Repair (Amst). 2008; 7: 751-4.
63. Dutta D, Shatalin K, Epshtein V, Gottesman ME, Nu dler E. Linking RNA polymerase backtracking to genome instability in e. coli. Cell. 2011; 146: 533-43.
64. Li M, Xu X, Chang CW, Liu Y. TRIM28 functions as the SUMO E3 ligase for PCNA in prevention of transcription induced DNA breaks. Proc Natl Acad Sci U S A. 2011; 21: 23588-96.
65. Hickey CM, Wilson NR, Hochstrasser M. Function and regulation of SUMO proteases. Nat Rev Mol Cell Biol. 2012; 13: 755-66.
66. Alvarez V, Vinas L, Gallego-Sanchez A, Andres S, Sacristan MP, Bueno A. Preferential digestion of PCNA-ubiquitin and p53-ubiquitin links by USP7 to remove polyubiquitin chains from substrates. J Biol Chem. 2011; 294: 4177-87.
67. Durando M, Tateishi S, Vaziri C. A non-catalytic role of DNA polymerase eta in replication forks. Nucleic Acids Res. 2013; 41: 10360-9.
68. Durando M, Tateishi S, Vaziri C. A non-catalytic role of DNA polymerase eta in replication forks. Nucleic Acids Res. 2013; 41: 10360-9.
69. Huang TT, Nijman SM, Mirchandani KD, Galardy PJ, Cohn MA, Haas W, et al. USP7 modulates PCNA ubiquitination in response to DNA damage. Mol Cell. 2014; 54: 626-38.
70. Kashiwaba S, Kanao R, Masuda Y, Kusumoto-Matsuo R, Hanaoka F, Masutani C. Preferential digestion of PCNA-ubiquitin and p53-ubiquitin links by USP7 to remove polyubiquitin chains from substrates. J Biol Chem. 2011; 294: 4177-87.
71. Lim KS, Li H, Roberts EA, Gaudiano EF, Clairmont C, Sambel LA, et al. USP1 is a Suppressor of PCNA Ubiquitination and Oxidative-Stress-Induced Mutagenesis in Human Cells. Cell Rep. 2015; 13: 2072-80.
72. Park JM, Yang SW, Yu KR, Ka SH, Lee SW, Seol JH, et al. Modification of PCNA by BSG15 plays a crucial role in termination of error-prone transcription DNA syntheses. Mol Cell. 2014; 54: 626-38.
73. Masuda Y, Kanao R, Masuda Y, Kusumoto-Matsuo R, Hanaoka F, Masutani C. USP7 Is a Suppressor of PCNA Ubiquitination and Oxidative-Stress-Induced Mutagenesis in Human Cells. Cell Rep. 2015; 13: 2072-80.
74. Sugie N, Kimura T, Kimura Y, Nemoto S, Honda H, Seiki M. The presence of acetyl groups of histones. Biochem J. 1963; 87: 263-71.
75. Cho EA, Prindle MJ, Dressler GR. BRCT domain-containing protein PTIP is essential for transcription through mitosis. Mol Cell Biol. 2003; 23: 1666-73.
76. Hanway D, Chun JK, Xia G, Oshiro G, Winzeler EA, Romesberg FE. Previously uncharacterized genes in the UV- and H2O2-induced DNA damage response in yeast. Proc Natl Acad Sci U S A. 2002; 99: 10605-10.
77. Gali H, Juhasz S, Murocz M, Hajdu I, Fatyol K, Szukacsov V, et al. Role of HER2 and RNFLP ubiquitin E3 ligases in the promotion of translation of DNA synthesis in the chicken DT40 cell line. DNA Repair (Amst). 2011; 10: 75-77.
78. Dutta D, Shatalin K, Epshtein V, Gottesman ME, Nu dler E. Linking RNA polymerase backtracking to genome instability in e. coli. Cell. 2011; 146: 533-43.
79. Li M, Xu X, Chang CW, Liu Y. TRIM28 functions as the SUMO E3 ligase for PCNA in prevention of transcription induced DNA breaks. Proc Natl Acad Sci U S A. 2011; 21: 23588-96.
80. Hickey CM, Wilson NR, Hochstrasser M. Function and regulation of SUMO proteases. Nat Rev Mol Cell Biol. 2012; 13: 755-66.
81. Vinas L, Gallego-Sanchez A, Andres S, Sacristan MP, Bueno A. Preferential digestion of PCNA-ubiquitin and p53-ubiquitin links by USP7 to remove polyubiquitin chains from substrates. J Biol Chem. 2011; 294: 4177-87.
82. Durando M, Tateishi S, Vaziri C. A non-catalytic role of DNA polymerase eta in replication forks. Nucleic Acids Res. 2013; 41: 10360-9.
83. Lim KS, Li H, Roberts EA, Gaudiano EF, Clairmont C, Sambel LA, et al. USP1 Is Required for Replication Fork Protection in BRCA1-Deficient Tumors. Mol Cell. 2016; 62: 2529-41.
84. Kashivsaka S, Kanao R, Masuda Y, Kusumoto-Matsuo R, Hanaoka F, Masutani C. USP7 Is a Suppressor of PCNA Ubiquitination and Oxidative-Stress-Induced Mutagenesis in Human Cells. Cell Rep. 2015; 13: 2072-80.
85. Durando M, Tateishi S, Vaziri C. A non-catalytic role of DNA polymerase eta in replication forks. Nucleic Acids Res. 2013; 41: 10360-9.
86. Harmeyer J, Ballest K, Hignite E, Hignite J. The presence of acetyl groups of histones. Biochem J. 1963; 87: 263-71.
87. Sekler JS, Dejan A. SUMO and the robustness of cancer. Nat Rev Cancer. 2017; 17: 184-97.
88. Da Silva-Ferrada E, Ribeiro-Rodrigues TM, Rodriguez MS, Girao H. SUMO and the robustness of cancer. Nat Rev Cancer. 2017; 17: 184-97.
89. Hendriks IA, D’Souza RC, Chang JG, Mann M, Vertegaal AC. System-wide identification of wild-type SUMO-2 conjugation sites. Nat Commun. 2015; 6: 7289.
90. Pander B, Molsdov GL, Sacher M, Hoegg C, Tjentch S. SUMO-modified PCNA recruits Snr2 to prevent recombination during S phase. Nature. 2005; 436: 428-33.
91. Burkovics P, Sebesta M, Sisakova A, Plautl N, Szukacs V, Robert T, et al. Snr2 mediates PCNA-SUMO-dependent inhibition of DNA repair synthesis. EMBO J. 2013; 32: 742-55.
92. Zhou H, Chen MS, Lo YH, Waltz SE, Wang J, Ho PC, et al. The Ron receptor tyrosine kinase activates c-Abl to promote cell proliferation through tyrosine phosphorylation of PCNA in breast cancer. Oncogene. 2014; 33: 1429-37.
93. Nikolaov T, Ensminger M, Lobrich M, Kaina B. Homologous recombination protects mammalian cells from replication-associated DNA double-strand breaks arising in response to methyl methanesulfonate. DNA Repair (Amst). 2010; 9: 1050-63.
94. French S. Consequences of replication fork movement through transcription initiation units. Biochimie. 1992; 74: 258-68.
115. Gu W, Roeder RG. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell. 1997; 90: 595-606.

116. Gil J, Ramirez-Torres A, Encarnacion-Guevara S. Lysine acetylation and cancer: a proteomic perspective. J Proteomics. 2017; 150: 267-309.

117. Narita T, Weintert BT, Choudhary C. Functions and mechanisms of non-histone protein acetylation. Nat Rev Mol Cell Biol. 2019; 20: 156-74.

118. Naryzhny SN, Lee H. The post-translational modifications of proliferating cell nucleotide antigen expression, not phosphorylation, plays an important role in the regulation of its function. J Biol Chem. 2004; 279: 20194-9.

119. Yu Y, Cai JP, Tu B, Wu L, Zhao Y, Liu X, et al. Proliferating cell nuclear antigen is protected from degradation by forming a complex with Murf Homolog2. J Biol Chem. 2009; 284: 1910-20.

120. Cazzalini O, Sommati S, Tillmon D, Muto I, Bachi A, Rapp A, et al. CBP and p300 acetylate PCNA to link its degradation with nucleotide excision repair synthesis. Nucleic Acids Res. 2014; 42: 8433-48.

121. Bilton P, Li J, Lambert JP, Chen Y, Trenblay VL, Brunzelle JS, et al. Acetylation of PCNA Sliding Surface by Ecol Promotes Genome Stability through Homologous Recombination. Mol Cell. 2017; 65: 78-90.

122. Kumar D, Saha S. HAT3-mediated acetylation of PCNA precedes PCNA monoubiquitination following exposure to UV radiation in Leishmania donovani. Nucleic Acids Res. 2015; 43: 5423-45.

123. Takawa M, Cho HS, Hayami S, Toyokawa G, Kogure M, Yamane Y, et al. Histone lysine methyltransferase SETDB2 promotes carcinogenesis by deregulating PCNA expression. Carcinogenesis. 2012; 32: 2317-27.

124. Couture JF, Colla E, Brunzelle JS, Patel RC. Structural and functional analysis of SET8, a histone H4 Lys-20 methyltransferase. Genes Dev. 2005; 19: 1455-65.

125. Jorgensen S, Elvers I, Trelle MB, Menzel T, Eskildsen M, Jensen ON, et al. The epigenetic modifier SETD8 regulates Pol (ADP ribose)-regulated recruitment and regulates DNA double strand break repair pathway choice. Nucleic Acids Res. 2015; 43: 967-89.

126. Guan J, Yu S, Zhang X. NEDDylation antagonizes ubiquitination of proliferating cell nuclear antigen and regulates the recruitment of polymerase eta in response to oxidative DNA damage. Protein Cell. 2018; 9: 365-79.

127. Yuan W, Krijn RM. Influenza B virus NS1 protein inhibits conjugation of the interferon (IFN)-induced ubiquitin-like R51 protein. EMBO J. 2001; 20: 362-71.

128. Kim KS, Giannakopoulos NV, Virgin HW, Zhang DE. Interferon-inducible ubiquitin E3 ligase (E6-AP) is a selective SAM-dependent inhibitor of the histone methyltransferase EZH2. Mol Cell Biol. 2004; 57: 6922-33.

129. Zhao C, Beaudenon SL, Kelley ML, Waddell MB, Yuan W, Schulman BA, et al. The UbcH8 ubiquitin E2 enzyme is also the E2 enzyme for R51, an IFN-alpha/beta-induced ubiquitin-like protein. Proc Natl Acad Sci U S A. 2004; 101: 17529-34.

130. Guan J, Yu S, Zhang X. NEDDylation antagonizes ubiquitination of proliferating cell nuclear antigen and regulates the recruitment of polymerase eta in response to oxidative DNA damage. Protein Cell. 2018; 9: 365-79.

131. Yuan W, Krijn RM. Influenza B virus NS1 protein inhibits conjugation of the interferon (IFN)-induced ubiquitin-like R51 protein. EMBO J. 2001; 20: 362-71.

132. Kim KS, Giannakopoulos NV, Virgin HW, Zhang DE. Interferon-inducible ubiquitin E3 ligase (E6-AP) is a selective SAM-dependent inhibitor of the histone methyltransferase EZH2. Mol Cell Biol. 2004; 57: 6922-33.

133. Zhao C, Beaudenon SL, Kelley ML, Waddell MB, Yuan W, Schulman BA, et al. The UbcH8 ubiquitin E2 enzyme is also the E2 enzyme for R51, an IFN-alpha/beta-induced ubiquitin-like protein. Proc Natl Acad Sci U S A. 2004; 101: 17529-34.

134. Wu J, Pung YF, Sze NS, Chin KC. HECT oncogene (E3) ligase that mediates type I IFN-induced ISGylation of protein targets. Proc Natl Acad Sci U S A. 2006; 103: 10735-40.

135. Zou W, Zhang DE. The interferon-inducible ubiquitin-protein isopeptide ligase (E3) EFP also functions as an ISG15 E3 ligase. J Biol Chem. 2006; 281: 3899-94.

136. Villarroya-Beltri C, Guerra S, Sanchez-Madrid F. ISGylation - a key to lock the cell gate for preventing the spread of threats. J Cell Sci. 2017; 130: 2961-9.

137. Kontopidis G, Wu SY, Zheleva DI, Taylor P, McInnes C, Lane DP, et al. Structural and biochemical studies of human proliferating cell nuclear antigen complexes provide a rationale for cyclin association and inhibitor design. Proc Natl Acad Sci U S A. 2003; 105: 18717-22.

138. Kirk KM, Verma A, Mundi PS, et al. Precision Targeting with EZH2 and HDAC Inhibitors in Epigenetically Dysregulated Lymphomas. Clin Cancer Res. 2019; 25: 5271-83.

139. Yang Y, Zhu F, Wang Q, Ding Y, Ying R, Zeng L. Inhibition of EZH2 and EZFGR produces a synergistic effect on cell apoptosis by increasing autophagy in gastric cancer cells. Onco Targets Ther. 2018; 11: 8455-63.