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Review

SUMOylation and de-SUMOylation in response to DNA damage

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ABSTRACT

To maintain genomic integrity, a cell must utilize multiple mechanisms to protect its DNA from the damage generated by environmental agents or DNA metabolism. SUMO (small ubiquitin-like modifier) can regulate protein stability, protein cellular location, and protein–protein interactions. In this review, we summarize the current understanding of the roles of SUMOylation and de-SUMOylation in DNA damage response (DDR) and DNA repair with a specific focus on the role of RPA SUMOylation in homologous recombination (HR).

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1. Introduction

DNA damage response (DDR) and DNA repair are important mechanisms that protect genomic integrity from DNA damage generated by environmental agents or DNA metabolism [1,2]. They are precisely regulated by the post-translational modifications of target proteins, including phosphorylation, ubiquitination, methylation, acetylation and protein-based modification. The first protein-based modification to be described was ubiquitin [3–6]. Over past several years modification of SUMO (small ubiquitin-like modifier), one member of UBLs (ubiquitin-like proteins), has emerged as a critical factor in multiple biological processes such as DNA transcription, DNA replication, and the events following DNA damage [7–10]. In this review, we will discuss the current understanding of the roles of several critical components in the cycle of SUMOylation and de-SUMOylation and recent developments of SUMOylation and de-SUMOylation in DDR or DNA repair. More specifically, we will focus on the role of RPA SUMOylation in homologous recombination (HR) and roles of SENPs (SUMO-specific proteases) in response to DNA damage stress.

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2. SUMOylation and de-SUMOylation

There are more than a dozen UBLs in mammals [11,12]. The conjugation of ubiquitin or UBLs to lysine side chains in target proteins is a protein-based posttranslational modification, which greatly confers the functional and dynamic diversities of modified proteins. Since the discovery of SUMO 14 years ago, its roles in different biological processes are extensively investigated [7–10]. Budding yeast expresses one SUMO protein (Smt3p). Mammalian cells have four SUMO paralogs: SUMO-1 (also called Sentrin 1); SUMO-2 (also known as Sentrin 3) and SUMO-3 (also known as Sentrin 2) [7]. The matured SUMO-2 and SUMO-3 are ~95% identical to each other and are ~45% identical in sequence with SUMO-1. SUMO-4 is probably not conjugated under physiological conditions, so its biological role is unclear [13].

SUMO conjugation is regulated through an enzyme-controlled cycle. Like most other UBLs, SUMO paralogs are synthesized as precursors that must be processed to reveal the C-terminal di-glycine motif to become the matured forms. Next, the matured SUMOs are activated by a SUMO-activating enzyme (E1), a heterodimer containing SAE1 (SUMO-activating enzyme subunit 1) and SAE2, transferred by a SUMO-conjugating enzyme (E2), UBC9, to substrate to form an isopeptide bond between the C-terminal carboxy group of SUMO and the ε-amino group of lysine in the substrate protein that is usually with the help of a SUMO protein ligase (E3) [7,14]. Typically, lysine residues modified by SUMO are found within a SUMO modification consensus motif, ψKXE (where ψ is a

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large hydrophobic residue and X is any residue), although modification at non-consensus sites has been reported [15,16]. SUMO-2 and SUMO-3, like Smt3p, can form chains in vitro and in vivo, primarily through a conserved lysine (Lys15 in Smt3p, Lys11 in SUMO-2 or SUMO-3) [17–19]. SUMO conjugation is usually short-lived and SUMOylated proteins will be rapidly deconjugated by the SUMO/Sentrin-specific proteases (SENPs) [9,20].

Six SENPs have been identified. SENP1, SENP2, SENP3, and SENP5 are more closely related to the yeast ULP1, whereas SENP6 and SENP7 are related to ULP2 [9]. SENP1 and SENP2 process both C-terminal hydrolysis and isopeptidase activity in mammalian cells [9,20]. SENP1 is more efficient at processing both SUMO-1 and SUMO-2 than SUMO-3 [21]. In contrast, SENP2 preferentially processes SUMO-2 among SUMO paralogs. Structure analysis indicates that two residues at the C-terminal side of the cleavage site make a significant contribution to SUMO process by SENP1 [21]. These sequences are His-Ser in SUMO-1, Val-Tyr in SUMO-2, and Val-Pro in SUMO-3. They also likely determine the efficiency of SENP2 activity [22]. SENP1-(415–643) deconjugates SUMO-1-conjugated and SUMO-2-conjugated RanGAP1 indistinguishably in vitro [21], but it can discriminate between SUMO-1-conjugated RanGAP1 and Sp100 [23], suggesting that the structure of its target proteins also impacts the isopeptidase activity of SENP1. SENP2 deconjugates SUMO-1-conjugated RanGAP1 more efficiently than it does SUMO-2-conjugated in vitro, in accordance with its higher hydrolytic activity in processing SUMO-2 [22]. The isopeptidase activity of SENP1 and SENP2 seems to be related to their interacting interface with SUMOs. During deconjugation of RanGAP1–SUMO-2/3 and RanGAP1–SUMO-1, there is an increased interface between SUMO-2/3 and SENP2 corresponding to the preferential deconjugation of RanGAP1–SUMO-2/3 by SENP2 compared with that of RanGAP1–SUMO-1 [22]. However, the interfaces between SUMOs and SENP1 are essentially same, corresponding to the indistinguishable capability of SENP1 to deconjugate both RanGAP1–SUMO-1 and RanGAP1–SUMO-2 [21]. In addition, the catalytic interface of SENP1 and SENP2 appears to be favorable for the lysine side-chain, with higher degrees of freedom than the more-rigid peptide extension of unprocessed SUMOs. This explains why the isopeptidase activities of both SENP1 and SENP2 are much stronger than their C-terminal hydrolase activity [21,22]. Compared with SENP1 and SENP2, SENP5 efficiently processes the precursor of SUMO-3 only [24,25]. SENP6 and SENP7 show very low processing activity [26,27]. SENP3 and SENP5 are more active in deconjugating SUMO-2/3-conjugated targets than SUMO-1-containing species [24,25]. In yeast, ULP2 deletion causes an accumulation of high-molecular-weight conjugated species of Smt3p. In mammalian cells, SENP6 and SENP7, the members of ULP2 family, show stronger activity on poly-SUMO-2/3 chain than on single SUMO moieties and SUMO-1 chains [26,27]. The structure of SENP7 is unique compared to other well characterized family members such as SENP1, SENP2, and ULP1. The deletion of a distinct region in SENP7, which appears to be in a suitable position to explain its specificity via interactions with an extended SUMO chain, had no effect on the activities of SENP7, indicating either that the link between the SENP7's structure and its specific activity on poly-SUMO-2/3 chain is not clear, or that high poly-SUMO2/3 chain deconjugation activities observed for SENP6 and SENP7 may simply reflect a preference for flexible isopeptide linked substrates [27]. It is also unclear whether or not that SENP6 and SENP7 functions redundantly in vivo.

3. SUMOylation in DDR

The cascade of DDR signaling is reflected by that DDR proteins are orderly recruited and function at DNA damage sites that are modulated by the post-translation modifications, including phosphorylation, acetylation, methylation, ubiquitination and poly(ADP-ribosylation) [2,28–31]. Roles of ATM and ATR kinases have been extensively studied compared to DNA-PK that primarily regulates a smaller group of proteins involved in DSB end joining. In response to DSBs, PARP1 initiates the recruitment of the MRN/ATM complex at DSBs [32,33]. Next, activation of the ATM by MRN and TIP60 triggers a signaling orchestra involving in the phosphorylation of CHK2, p53 and H2AX [34–36]. The presence of γ-H2AX is essential for accumulation and retention of signaling and repair factors at the break, which promotes an ordered recruitment of MDC1 [37–39], RNF8 [40–42], RNF168 [43], BRCA1, and 53BP1 [44].

In response to replication stress, the accumulation of RPA-coated ssDNA regions at stalled replication fork, recruits the ATR/ATRIP and the RAD17/RFC2–5 complexes [45]. Next, loading of the 9-1-1 complex by RAD17/RFC2–5 together with the 9-1-1-associated proteins [46,47] results in the activation of the ATR signaling cascade and CHK1 phosphorylation. Through these signal transduction pathways, DDR channels physiological processes to different directions, such undergo apoptosis, enter senescence, activate heightened immune surveillance, as well as initiate DNA repair [48–50].

Recently, Morris et al. and Galanty et al. showed that SUMO, UBC9, the SUMO-ligase enzymes, PIAS1 and PIAS4, and two of their substrates, BRCA1 and 53BP1, relocated to γ-H2AX foci after DNA damage. Additionally, they found that PIAS4 regulated events correlated with SUMO-1 and 53BP1 modification, and PIAS1 with SUMO-2/3 and BRCA1 modification. Moreover, Morris et al. identified two consensus SUMO-conjugation sites in BRCA1 and documented their important roles for regulating BRCA1's ubiquitin ligase activity. Notably, they observed that depletion of PIAS1 and PIAS4 caused the defect of RNF8/HERC2/RNF168-dependent ubiquitination and accumulation of subsequent proteins at DNA damage sites. Consistent with this, PIAS depleted cells impaired kinetics of DNA repair and enhanced sensitivity to genotoxic insults [51,52].

Given the effects of PIAS1/4 depletion on the activities of RNF8/RNA168, RNF8/RNF168 complex could also be regulated by SUMOylation. Could more SUMO substrates be involved in the pathway of DDR signaling? In fact, many proteins in DDR signaling cascade were reported to be SUMOylated. In response to heat shock, PIASy-promoted poly-SUMOylation of PARP1 induces RNF4-mediated ubiquitination of PARP1, which decreases PARP1 stability and regulates its transcription function [53]. In addition, SUMOylation of PARP1 does not affect its ADP-ribosylation activity in vitro, but restrains its transcriptional coactivator function by abrogating its p300-mediated acetylation [54] and alters the capacity of PARP1 to modify other chromatin-associated proteins [55]. Therefore, SUMOylation can modulate PARP1 via several different mechanisms involving in altering protein degradation and affecting other modifications. Apart from PARP1, in response to UV, the site-specific SUMOylation of TIP60 at lysines 430 and 451 initiates its relocation from nucleoplasm to the promyelocytic leukemia (PML) body and the overexpression of non-SUMOylatable mutant abrogates the p53-dependent DNA damage response, revealing that the SUMOylation of TIP60 augments its acetyltransferase activity in UV-irradiated DNA damage response [56]. In addition, both RNF168 and RAP80 have been shown to be SUMOylated [57,58]. It will be interesting to test if these SUMOylations function in DDR signaling. Roles of SUMOylated p53, the well known effector in DDR signaling, have been extensively studied and excellently reviewed in several publications [59,60].

4. SUMOylation in DNA repair

SUMOylation has been involved in many DNA repaired pathways. For example, SUMOylation affects the cellular localization

and the affinity for the AP site of thymidine glycosylase (TDG), one of DNA glycosylases in base excision repair (BER) [61–63]; XPC SUMOylation increases its stability and decreases its affinity for the formed complex of nucleotide excision repair (NER) for reutilization [64] ; the non-SUMOylable mutant of XRCC4, a non-homologous end joining (NHEJ)-related factor, cannot be translocated from cytoplasm to nucleus, leading to radiation sensitivity and failure to complete V(D)J recombination [65]. In this review, we will focus on role of SUMOylation in HR.

HR is initiated by the MRE11–RAD50–NBS1 (MRN) complex that stabilizes DNA ends, initiates the DNA resection, and promotes the recruitment of ATM [66]. DNA end resection is carried out by CtIP [67]. In S and G_2, CtIP associates with BRCA1, which facilitates DNA end processing [67]. In addition, more other protein factors are also involved in the entire process of DSB resection [68–70]. Next, the single strand DNA-binding protein replication protein A (RPA) binds to the ssDNA overhangs, and RAD51/RAD52 are then recruited to DSBs. Both RPA and RAD52 help load RAD51 onto ssDNA to form nucleoprotein filaments which search for the homologous duplex DNA in the undamaged sister chromatid to facilitate strand invasion that forms D loop [71]. In yeast, RAD52 is an essential recombination-mediator, but *MmRAD52* null mice are viable and display no abnormalities in fertility and in development of the immune system [72]. *MmRAD52*$^{-/-}$ ES cells are also not hypersensitive to agents that induce DSBs. Therefore, the presence of genes functionally related to MmRAD52 can partially compensate for the absence of MmRAD52 protein [73]. The potential candidates are human RAD51 paralogs or BRCA2 [73]. D loop could be dissembled by RTEL to generate non-crossover [74]. The newly synthesized fragments ends joined by DNA ligases could form double Holliday Junctions (dHJs) [71]. HJ intermediates could be dissolved by the BLM/TOPOIII complex or cleaved by the endonucleases GEN1, MUS81/EME1, or SLX1/SLX4 to generate either crossover or non-crossover products [75–77].

PIAS1 or PIAS4 depletion impairs RPA accumulation in laser-lines. Consistent with this finding and the involvement of RPA-coated single strand DNA in homologous recombination, PIAS1 or PIAS4 depletion reduced homologous recombination [51,52]. Both RAD51 and RAD52 have been shown to interact with UBC9 and SUMO directly [78–82]. Overexpression of both SUMO and non-conjugated SUMO decrease radioresistance by down-regulating DSB-induced HR [83]. While we and other group found that SUMOylation mutant proteins inhibit HR [81,82]. This controversy may be related to artificially overexpressed SUMO that can enhance numerous proteins' SUMOylation and affect HR indirectly. In line with our findings, defect of UBC9 or MMS21 causes the significant disruption of RAD51 intracellular trafficking, resulting in markedly inhibition of DNA damage-induced RAD51 nuclear foci [84] or the hyperactivation of ATM/ATR signaling pathways, resulting in a consequent DNA damage-induced apoptosis [85].

Recently, role of SUMO in proteins involving in resolution of dHJs has also been shown. As we described before, the BLM/TOPO-III complex can dissolve dHJs at the late stage of in HR. BLM interacts directly with the RAD51 and in vitro it can displace RAD51 from ssDNA and unwind the invading DNA strand of a D-loop formed by RAD51 [86]. Interestingly, BLM also collaborates with exonucleases that process DSBs to generate ssDNA for a formation of RAD51 filament [87]. Thus, BLM has both anti- and pro-recombinogenic functions in HR, raising an interesting question how these different functions of BLM in HR are controlled. SUMOylation of BLM had been shown to regulate BLM's cellular distribution [88]. Further studies by Ouyang et al. showed that HU treatment failed to induce SCEs (sister chromatid exchanges) in non-SUMOylable BLM cells compared to normal BLM cells due to impaired RAD51 localization to HU-induced repair foci. They also found that in vitro RAD51 directly interacts with SUMO and that SUMOylation enhances the binding of RAD51 to BLM. The data demonstrates that SUMOylation switches BLM's pro- and anti-recombinogenic functions in HR; alternatively, BLM SUMOylation relieves its inhibitory effects on HR, and promotes RAD51 function. Additionally, SUMOylation also regulates the transport of Werner's helicase (WRN) [89,90], another member of the RecQ family of DNA helicases that partially co-localizes with DNA repair proteins, such as RAD51 and RAD52, and may be involved in several nuclear functions, such as HR, restoration of stalled replication forks, repair of DNA strand breaks, and telomere maintenance.

HR mechanism also involves in maintaining telomere length in ALT (alternative lengthening of telomeres) cells [91]. Telomeres are repetitive DNA elements, involving the repeat sequence TTAGGG in humans, which comprise 5–15 kilobases at the ends of chromosomes [92]. Telomeres are shortened due to the end-replication problem of the lagging strand and critically short telomere induces cellular senescence in mammalian cells [93,94]. The transcriptional upregulation of telomerase is the major mechanism to overcome this replication-mediated defect. Some cancer cells rely on an ALT mechanism to elongate telomeres due to the defect of up-regulating telomerase, which facilitates telomere elongation by recruiting telomerase into nuclear PML bodies and then initiating HR by the participation of HR proteins [95]. The Shelterin/Telosome complex, consisting of six core proteins, TRF1, TRF2, TIN2, TPP1, RAP1, and POT1, binds to telomeric DNA and prevents it from being recognized as DSBs [96]. SUMOylation of TRF1, TRF2, TIN2, and RAP1 is enhanced by MMS21 [97]. Inhibition of MMS21 blocks recruitment of telomeres to APBs (ALT-associated promyelocytic leukemia nuclear bodies). It is therefore proposed that SUMOylation of the Shelterin/Telosome complex could promote the relocalization of telomerase to PML bodies due to the high affinity of proteins within PML bodies for SUMO [97].

Roles of SUMO in HR have been extensively studied in yeast. The *ubc9* cells exhibit a RAD51-dependent accumulation of cruciform structures in response to replication stress, indicating an important role of SUMOylation in resolution of recombination intermediates during DNA replication [98]. Consistently, UBC9 and SUMOylated PCNA are essential for RAD18-MMS2-mediated damage-bypass through SCJs (sister chromatid junctions), a RAD51-dependent recombination event [99]. SUMO has also been demonstrated to impact yeast RAD52 from different aspects, such as enhances its stability, decreases its binding affinity to DNA, and promotes repair within regions of repetitive DNA [100–102]. In addition, Ohuchi et al. reported that SUMOylation of RAD52 occurs only in the S phase by induction of MMS and is important for MMS-induced interchromosomal homologous recombination, which may be led by the RAD52 SUMOylation-mediated an efficient recruitment of RAD51 [103]. However, no evidence shows that mammalian RAD52 could be SUMOylated in response to DNA damage, which is consistent with the case of PNCA that we discuss elsewhere and that a set of important proteins involved in HR events, lack clear homologs in yeast, such as the tumor-suppressor and cell cycle proteins p53, BRCA1 and BRCA2 [104]. Thus, the roles of SUMO in HR might be variable in different species from an evolutionary point.

5. RPA-SUMOylation in HR

RPA, the main eukaryotic ssDNA binding protein complex, consists of three subunits, RPA1 (RPA70), RPA2 (RPA32), and RPA3 (RPA14). RPA70 is the major ssDNA binding subunit and is involved primarily in interactions with other DNA metabolism proteins [105,106]. The hyper-phosphorylation of RPA32 may redirect RPA from DNA replication to DNA repair [107]. RPA14 is required for stable heterotrimer formation [105,108]. We first noted the

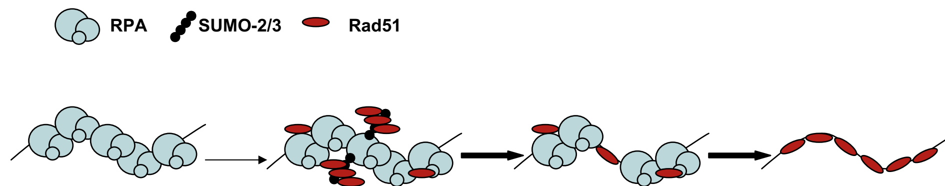

Fig. 1. Role of RPA SUMOylation in DNA repair. Single strand DNA is coated by RPA that is SUMOylated by an unknown E3. RPA70 SUMOylation serves as a HR mediator to facilitate recruitment of RAD51 to initiate strand invasion. This model does not show the roles of other HR mediators.

RPA70 SUMOylation in SENP6-knockdown cells [81]. SENP6-knockdown cells reveal the significant phenotype of DDR, including the appearance of γ-H2AX foci, enhanced phosphorylation of CHK1 Ser-345 and CHK2 Thr-68 and defective cell cycle progression in both S and G_2/M phases. In addition, SENP6-knockdown induces the co-localization of RPA70 and SUMO-2/3 at many punctate foci. These observations therefore led us to examine the biological relevance between RPA70 SUMOylation and DNA damage. Indeed, RPA70 can be modified in vivo on its lysine residues 449 and K577 with K449 being the major site of SUMOylation and RPA70 SUMOylation is augmented by replication-mediated DSB. Endogenous RPA70 is modified by SUMO-2/3. The major form of SUMOylated RPA70 is di-SUMOylated RPA70 that is also induced by heat shock [58]. Consistent with the observation from Ellis' group [82], we also found that RAD51 directly interacts with SUMOs in vitro. Importantly, SUMOylation enhances the binding of RAD51 to RPA70 and facilitates the formation of RAD51 filament in vitro; RPA70(ΔSUMO) delays formation of RAD51 foci and inhibits its HR events in vivo. We therefore propose that RPA70 SUMOylation may serve as HR mediator to facilitate recruitment of RAD51 to the DNA damage foci to initiate DNA repair through HR (Fig. 1). It will be interesting to study if RPA SUMOylation also functions in other DNA repair pathways, what the E3 for RPA70 is, and what the interplay is between SUMOylation and phosphorylation in RPA complex.

The functional connection between SUMOylated PCNA and HR is well established in budding yeast. PCNA SUMOylation recruits Srs2 through a conserved SUMO-interaction motif in its carboxy-terminus to prevent unscheduled recombination events at replication fork [10]. We again didn't observe SUMOylation of PCNA in our experiment. Although the failure to detect PCNA SUMOylation in mammalian cells or even in fission yeast is no proof for its absence, the action of SUMO on PCNA is very likely limited to the budding yeast system, because no sequence or function related helicase is found to exert as Srs2 on the RAD6 pathway [10]. Two subunits of yeast RPA, RFA1 and RFA2, are also sumoylated in a SLX5–SLX8-dependent manner after MMS treatment [109]. Since SLX5–SLX8-dependent sumoylation disfavors SSA (single-strand annealing) and RAD51-independent BIR (break-induced replication) [109]. The studies that address the functions of RFA1, RFA2 in the RAD51-dependent repair processes by mapping and mutating the SUMOylation sites will be interesting.

6. SENPs in the response to DNA damage stress

The *ulp2Δ* cells are permanently arrested as large-budded cells regardless of whether the DNA DSB was repairable or not, and exhibit wildtype RAD53 phosphorylation and dephosphorylation kinetics. The induction of a non-repairable DNA DSB causes approximate 50% ulp2Δ cells arrested in metaphase and in post-metaphase, respectively, which depends on checkpoint proteins, such as MEC1, RAD53, DUN1, or PDS1. These evidences suggest that ULP2 functions after the termination of DNA damage checkpoint and lead to propose that ULP2 restarts the cell cycle and may also be required during a later stage of mitosis to promote successful spindle development though its unknown substrates [110,111].

Knocking down SENP6, a member of ULP2 family in mammals, actives both CHK1 and CHK2 suggesting that SENP6 does not affect upstream of DDR, which is consistent with the observations that formations of γ-H2AX and RPA foci are still available after deletion of SENP6 [81]. In terms of DNA repair, SENP6-knockdown increases SCE and does not inhibit formation of RAD51 foci, indicating that SENP6 has minor effect at the early stage of HR [81]. In actuality, SENP6 is an editor of SUMO polychain induced by different stresses. Thus, SENP6 most likely functions at terminal stage of DNA damage singling pathway for cell to restart its normal processes.

Both SENP1 and SENP2 regulate p53 in response to DNA damage. Knocking down SENP1 by siRNA reduces p53-dependent apoptosis through SIRT1 SUMOylation [112]. An isoform of SENP2 not only associates with MDM2 but also modulates its SUMO conjugation of MDM2 that prevents its self-ubiquitination and increases its ability to ubiquitinate p53 [113]. DNA damage stresses also regulate activities of SENPs. UV and H_2O_2 promote the association of SENP1 with its substrate, SIRT1 to reduce p53-dependent apoptosis [112]. H_2O_2 inhibits the activity of SENP1 by inducing the formation of an intermolecular disulfide linkage between Cys-603 and Cys-613 [114]. This reversible modification has also been observed in the SUMO E1 subunit UBA2, E2-conjugating enzyme UBC9, and ULP1 [114]. Additionally, in presence of exogenous SUMO-1, doxorubicin stimulates ATM-dependent Ser-824 phosphorylation of KAP1 and represses its SUMOylation through SENP1 [115]. It will be interesting to define if this phosphorylation plays a role in recruitment of SENP1. Moreover, oxidative stress also induces the interaction between SENP3 and HSP90 that blocks ubiquitin-mediated proteasomal degradation through impairing the E3 ligase function of CHIP [116].

7. Conclusion and perspectives

The findings cited in this review suggest that SUMOylation and de-SUMOylation play important roles in regulating of DNA damage-induced events, including DDR and DNA repair. It is obvious that much is not known about how DNA damage signaling regulates several critical components in SUMO pathway. For instance, is SUMO-E1 also involved the events in response to DNA damage? How does DDR signaling direct UBC9 and SUMO E3 ligases to function in damage sites? How do different SENPs play their roles in response to different DNA damage stresses? Clinically, there have been two physical abnormalities related to the translocation of the SENP gene. Translocation of SENP1 generates SENP1–MESDC2 [117], causing a form of sacrococcygeal teratoma; SENP6 is fused to TBCA1 during T-cell lymphoma, thus linking to developmental delay and recurrent infections [118]. In addition, overexpressed SENP1 has been found in prostate cancer [119]. Moreover, increased expression of UBC9 and PIASs has also noted in cancers [120,121]. Thus, further exploration in this field will identify potential new approaches to both prevention and treatment of human diseases.

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