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Review

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Role of deubiquitinating enzymes in DNA double-strand break repair

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Abstract: DNA is the hereditary material in humans and almost all other organisms. It is essential for maintaining accurate transmission of genetic information. In the life cycle, DNA replication, cell division, or genome damage, including that caused by endogenous and exogenous agents, may cause DNA aberrations. Of all forms of DNA damage, DNA double-strand breaks (DSBs) are the most serious. If the repair function is defective, DNA damage may cause gene mutation, genome instability, and cell chromosome loss, which in turn can even lead to tumorigenesis. DNA damage can be repaired through multiple mechanisms. Homologous recombination (HR) and non-homologous end joining (NHEJ) are the two main repair mechanisms for DNA DSBs. Increasing amounts of evidence reveal that protein modifications play an essential role in DNA damage repair. Protein deubiquitination is a vital post-translational modification which removes ubiquitin molecules or polyubiquitinated chains from substrates in order to reverse the ubiquitination reaction. This review discusses the role of deubiquitinating enzymes (DUBs) in repairing DNA DSBs. Exploring the molecular mechanisms of DUB regulation in DSB repair will provide new insights to combat human diseases and develop novel therapeutic approaches.

Key words: Deubiquitinating enzymes (DUBs); DNA double-strand breaks (DSBs); DNA repair; Non-homologous end joining (NHEJ); Homologous recombination (HR)

1 Introduction

Human body suffers hundreds and thousands of occurrences of DNA damage every day, which will eventually cause DNA aberrations (Lindah and Barnes, 2000). Some DNA lesions arise via natural metabolic processes, such as reactive oxygen species (ROS), alkylation agents, and lipid peroxidation productions (Cadet et al., 1997). DNA also can be damaged by environmental agents. This exogenous damage is caused by ultraviolet (UV) radiation and ionizing radiation (IR), viruses, and hydrolysis. DNA double-strand break (DSB) induced by IR is one of the most serious kinds of damage. IR generates many DNA lesions, including base changes, deoxyribose changes, DNA strand cross-links, DNA protein cross-links, and DNA strand breakage, due to the deoxyribose destruction of deoxyribose or the breaking of phosphodiester bonds (Khanna and Jackson, 2001). Living organisms have developed DNA repair mechanisms in order to maintain DNA integrity. If the repair function is not effective to fix DNA damage, accumulation of DNA lesions evidently causes gene mutation, genome instability, cell chromosome loss, and cell apoptosis; these lead to aging and age-related diseases such as tumorigenesis (Schmitt et al., 2007).

There are five types of DNA repair in eukaryotes: nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), single-strand break repair (SSBR), and DSB repair (DSBR). NER can remove large fragments of DNA damage, BER can repair individual base damage, and MMR is used to repair base mismatches. SSBR can repair DNA single-strand break. DSBR includes three mechanisms: non-homologous end joining (NHEJ), homologous recombination (HR), and microhomology-mediated end joining (MMEJ) (Harrison and Haber, 2006; Kawanishi et al., 2006; Harper and Elledge,

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2007; Gupta and Heinen, 2019; Olivieri et al., 2020). Posttranslational modification (PTM) of proteins, especially ubiquitination, is essential for DNA repair, protein stability, and cell communication in the cellular DNA damage response (DDR). The process of deubiquitination, the removal of ubiquitin chains from proteins, is important to prevent the degradation of targeted proteins. In this review, we will discuss the role of deubiquitination modification in DNA DSBR in regulating DNA damage response and maintaining genomic stability.

2 Repair of DNA DSBs

NHEJ and HR are two major mechanisms in DNA DSBR (Lieber, 2008; san Filippo et al., 2008; Scully et al., 2019). NHEJ occurs throughout the cell cycle. It is a rapid and highly efficient pathway to DNA sequences of minor reference alleles. NHEJ is often considered to be error-prone (Karanam et al., 2012; Chiruvella et al., 2013). By contrast, HR is restricted to the S and G2 phases due to the requirement for a sister chromatid. It repairs precisely by using the sequence template from sister chromatids. As a result, HR is a high-fidelity repair pathway compared to NHEJ (Kakarougkas and Jeggo, 2014).

NHEJ mainly requires a DNA-dependent protein kinase catalytic subunit (DNA-PKcs), Ku70, Ku80, DNA ligase IV (LIG4), Artemis X-ray repair cross-complementing protein 4 (XRCC4), and XRCC4-like factor (XLF). The DSB is repaired by blunt end ligation independent of sequence homology (Ceccaldi et al., 2016). Ku70 and Ku80 make up the Ku70/80 heterodimer, which is the core NHEJ protein. The Ku70/80 complex initiates NHEJ by binding to DNA DSB ends. The Ku70/80 heterodimer is a sensor which activates and recruits the DNA-PKcs to the DNA ends (Gottlieb and Jackson, 1993). The DNA-PKcs autophosphorylates and activates itself, which in turn regulates NHEJ end processing. It also facilitates recruitment of LIG4, XRCC4, and XLF for a ligation complex. There are some additional proteins, such as polynucleotide kinase 3' phosphatase and Artemis, that also contribute to end processing (Cottarel et al., 2013). Artemis is a nuclease, which is essential for DNA end joining (Riballo et al., 2004). In conclusion, the mechanism of NHEJ is simple and does not rely on a template. The NHEJ process is very active in organisms with more complex genomes, compared with HR. However, in organisms with simpler genomes, especially in single-cell form, NHEJ is likely to destroy the integrity of original sequences, so it is not favored (Britton et al., 2013).

HR restores the missing sequence information at the DSB site using a non-damaged sister chromatid as a template. The following factors or complexes play a crucial role in HR repair: meiotic recombination 11 (MRE11)/DNA repair protein RAD50 homolog (RAD50)/Nijmegen breakage syndrome 1 (NBS1) (MRN), CtBP-interacting protein (CtIP), replication protein A (RPA), RAD51, RAD52, breast cancer susceptibility 1 (BRCA1)/partner and localizer of BRCA2 (PALB2)/BRCA2, and XRCC2/3 (Symington and Gautier, 2011). MRN complex and CtIP bind the single-stranded DNA (ssDNA) on the DNA breaks, and initiate HR (Takeda et al., 2007). MRN-mediated resection generates 3' overhangs by MRE11 exonuclease activity on the DNA breaks. Resected DNA is further processed and extended the length of ssDNA by DNA2 and exonuclease 1 (EXO1) (Symington and Gautier, 2011). Next, the 3' overhangs of ssDNA are coated by RPA, which prevents the ssDNA from forming secondary structures. RAD51 displaces RPA in dependent of BRCA1/PALB2/BRCA2 complex, XRCC2/3 complex, RAD52, and other proteins (Pellegrini et al., 2002). RAD51 loading mediates strand invasion and strand displacement on the undamaged homologous template. Subsequently, extension of the D-loop helps to generate a Holliday junction and capture the second end lead for repair (Kennedy and Andrea, 2005). In summary, as mentioned previously, HR repair reaches a high degree of accuracy by using homologous sequences, such as DNA replication from sister chromatids, as templates for synthesizing DNA. Therefore, HR is considered the safest and most reliable DSB repair pathway.

3 Deubiquitinating enzymes

Protein ubiquitin modification is involved in almost all cellular processing, including cell cycle regulation, cell proliferation, cell death, differentiation, and metastasis; it acts by regulating protein stability, protein localization, and signal transduction (Welchman

et al., 2005). Similar to phosphorylation, methylation, and acetylation, protein ubiquitination is also a reversible process. To reverse the ubiquitination reaction, the deubiquitinating enzymes (DUBs) remove ubiquitin molecules or polyubiquitin chains from substrates (Nishi et al., 2014; Hanpude et al., 2015). In total, there are around 100 DUBs. These DUBs are grouped into six sub-families, which include the ubiquitin-specific proteases (USPs), the Machado-Joseph Disease protein domain proteases (MJDs), the Jun activation domain-binding protein 1 (Jab1)/myeloproliferative neoplasm (MPN)/Mov34 family (JAMM/MPN$^+$), the ubiquitin C-terminal hydrolases (UCHs), the ovarian tumor proteases (OTUs) (Nijman et al., 2005b; Mevissen et al., 2013), and the motif interacting with Ub-containing novel DUB family (MINDY) family (Rehman et al., 2016). Deubiquitinases interact with substrates by recognizing specific domains and motifs, including the ubiquitin interacting motif (UIM) domain, ubiquitin-E2-like variant (UEV) domain, and ubiquitin-associated (UBA) domain (Hurley et al., 2006; Komander et al., 2009). DUBs also play a crucial role in the DNA repair process and DNA damage response pathway (Table 1) (Nowsheen et al., 2020). Here, we will discuss the role of DUBs in DNA DSBR.

Table 1 DUBs involved in DNA damage

DUB	Substrate	Reference
USP1	CHK1	Nijman et al., 2005a; Guervilly et al., 2011
USP3	CHK1; RNF168	Doil et al., 2009; Cheng and Shieh, 2018
USP4	CtIP and MRN complex	Liu et al., 2015; Wijnhoven et al., 2015
USP7	TIP60; MRN-MDC1 complex	Sun et al., 2005
USP11	BRCA1-PALB2-BRCA2 complex	Schoenfeld et al., 2004; Orthwein et al., 2015
USP13	RAP80-BRCA1 complex	Li et al., 2017
USP14	Ku70	Sharma et al., 2020
USP16	RNF8/RNF168	Shanbhag et al., 2010
USP20	Claspin	Yuan et al., 2014
USP28	Claspin; PIRH2 and CHK2	Zhang et al., 2006; Wang et al., 2018
USP38	HDAC1	Yang et al., 2020
USP39	CHK2	Wu JH et al., 2019
USP47	IK	Ka et al., 2020
USP50	Ku70	Cai et al., 2018
USP51	H2AK13,15ub	Wang et al., 2016
BRCC36	BRCA1-RAP80 complex	Dong et al., 2003; Sobhian et al., 2007; Wang and Elledge, 2007; Cooper et al., 2009; Shao et al., 2009; Feng et al., 2010; Hu X et al., 2011
OTUB1	E2s	Juang et al., 2012; Sato et al., 2012; Wiener et al., 2012
OTUB2	RNF168, BRCA1/RAP80 complex	Altun et al., 2015
OTUD4	Unclear	Wu ZQ et al., 2019
OTUD5	Ku80	Li et al., 2019
UCHL3	Rad51/Ku80	Luo et al., 2016
POH1	RNF8 and Rad51	Butler et al., 2012
BAP1	BRCA1, RAD51 and RPA	Ismail et al., 2014

DUB: deubiquitinating enzyme; USP: ubiquitin-specific protease; BRCA: breast cancer susceptibility; BRCC36: BRCA1/2-containing complex 36; OTUB: otubain; OTUD: ovarian tumor domain-containing protein; UCHL3: ubiquitin C-terminal hydrolase L3; POH1: Pad1 homologue; BAP1: BRCA1-associated protein 1; CHK: checkpoint kinase; RNF: RING finger protein; MRN: meiotic recombination 11 (MRE11)/DNA repair protein RAD50 homolog (RAD50)/Nijmegen breakage syndrome 1 (NBS1); TIP60: Tat-interactive protein-60 kDa; MDC1: mediator of DNA damage checkpoint protein 1; PALB2: partner and localizer of BRCA 2; RAP80: receptor-associated protein 80; PIRH2: p53 induced RING-H2 protein; HDAC1: histone deacetylase 1; RPA: replication protein A; CtIP: CtBP-interacting protein; H2AK13,15ub: ubiquitylation on histone H2A at K13 and K15; E2s: E2 ubiquitin enzymes.

4 Deubiquitination modification of the core proteins in DNA repair pathways

4.1 Regulation of ATM and ATR kinases

As the key DDR kinases, ataxia-telangiectasia mutated (ATM) and ATM and Rad3-related (ATR) are important in initiating the DNA DSBR mechanism (Durocher and Jackson, 2001; Kerzendorfer and O'Driscoll, 2009). By screening most of the DUBs, Yuan et al. (2014) confirmed that USP20 participates in the ATR-related DDR pathway as a major regulator. ATR phosphorylates USP20, and E3 ubiquitin ligase homologous to E6-AP carboxyl terminus (HECT) and RLD domain-containing E3 ubiquitin protein ligase 2 (HERC2) disassociates from USP20 in order to stabilize USP20. In turn, USP20 activates ATR-checkpoint kinase 1 (CHK1) signaling through deubiquitinating and stabilizing Claspin. In addition, another study showed that USP28 can also stabilize Claspin by promoting the ATR-mediated activation of CHK1 and maintaining the G2 arrests (Wang et al., 2018).

Multiple DUBs are reported to regulate ATM signaling. USP7 is shown to stabilize ATM to promote DNA damage signaling (Sun et al., 2005). IK is a spliceosomal component which is important for maintaining the proper splicing of the ATM pre-messanger RNA (pre-mRNA) in proteins related to DDR. Depleting IK can induce ATM protein deficiency. USP47 is reported to interact directly with IK and stabilize it through deubiquitination (Ka et al., 2020). Moreover, a recent study revealed that ovarian tumor domain-containing protein 4 (OTUD4) regulates DNA repair and radio-sensitivity in non-small cell lung cancer (NSCLC) cells via ATM/CHK2/p53 signaling (Wu ZQ et al., 2019).

4.2 Regulation of CHK1 and CHK2

CHK1 is a serine (Ser)/threonine (Thr) protein kinase which regulates the cell cycle checkpoint response as well as DDR. CHK1 is phosphorylated and activated by the upstream kinase ATR, leading to the initiation of DNA repair and cell cycle checkpoints. CHK1 is reported to be modified by the K63-linked Ub chain following cellular stress treatment (Latif et al., 2004; Rodriguez and Meuth, 2006; Meuth, 2010). USP3, the DUB, reduces K63 poly-ubiquitination of CHK1. In addition, loss of USP3 elevates CHK1 ubiquitination, which in turn leads to prolonged CHK1 chromatin association and activation (Cheng and Shieh, 2018). On the other hand, USP1 is reported to regulate DNA repair through deubiquitinating fanconi anemia complementation group D2 (FANCD2), which in turn stabilizes CHK1 (Nijman et al., 2005a; Guervilly et al., 2011).

CHK2 is also a Ser/Thr kinase, and functions as a key mediator in DDR. Compared to CHK1, CHK2 is phosphorylated and activated by ATM. A recent study has reported that deubiquitination plays a critical role in CHK2 regulation. USP28 stabilizes CHK2 and tumor protein p53-binding protein 1 (53BP1) to induce apoptosis following DNA damage (Zhang et al., 2006). Moreover, USP28 interacts with p53-induced RING-H2 protein (PIRH2) and CHK2, which in turn stabilizes CHK2 through blocking PIRH2-induced ubiquitination and degradation of CHK2 (Wang et al., 2018). Recently, USP39 has been reported as a new deubiquitinase of CHK2. USP39 deubiquitinates CHK2 and enhances its stability. In addition, after ablation of USP39, CHK2 is degraded, which leads to decreased apoptosis and makes cancer cells resistant to ionizing radiation and chemotherapy drugs (Wu JH et al., 2019).

4.3 Regulation of RING finger protein 8/168

The RING finger protein 8 (RNF8)/RNF168 members of E3 ligase cascade promote H2A ubiquitination and recruit BRCA1 complex and 53BP1, which are critical for DNA damage signaling transduction and activation of HR and NHEJ (Mattiroli et al., 2012). Mounting evidence reveals that DUBs (such as Pad1 homologue (POH1), USP3, BRCA1/2-containing complex 36 (BRCC36), USP16, otubain 1/2 (OTUB 1/2), and USP51) function as the negative regulators of the RNF8 pathway. USP3 and USP16 oppose H2A ubiquitylation induced by the RNF8/RNF168 pathway (Weake and Workman, 2008). The RNF168 accumulation at DSB sites is blocked when USP3 is overexpressed (Doil et al., 2009). In addition, RNF8/RNF168-mediated transcriptional silencing is reversed by USP16 (Shanbhag et al., 2010). Another two DUBs, BRCC36 and POH1, also display strong deubiquitination activity for RNF8/RNF168-mediated K63 ubiquitination at DSB sites (Dong et al., 2003; Sobhian et al., 2007; Wang and Elledge, 2007; Cooper et al., 2009; Shao et al., 2009; Feng et al., 2010; Hu X

et al., 2011; Butler et al., 2012). Another study found that USP51 directly binds H2A-H2B and deubiquitinates RNF168-mediated $H2AK^{15ub}$ at DSB sites. Following DNA damage, USP51 is recruited to DSB sites and deubiquitinates $H2AK^{15ub}$, which in turn regulates the irradiation-induced foci (IRIFs) of BRCA1 and 53BP1 (Wang et al., 2016).

OTUB1, an ovarian tumor (OTU)-family DUB, negatively regulates RNF168-dependent ubiquitination. Interestingly, OTUB1 interacts with E2 ubiquitin enzymes (UBCH5 and UBC13) and blocks ubiquitin transfer for RNF168. In addition, OTUB1 also inhibits the UBE2D and UBE2E, and another E2 families (Nakada et al., 2010; Juang et al., 2012; Wiener et al., 2012). OTUB2 is also an OTU-family DUB, which specifically recognizes K63-ubiquitin chains at DSB sites (Kato et al., 2014). OTUB2 depletion is important for the IRIFs of 53BP1, BRCA1, and RNF168, but not the upstream factors, mediator of DNA damage checkpoint protein 1 (MDC1) or RNF8 (Altun et al., 2015).

4.4 Regulation of the core factors in HR pathways

CtIP endonuclease activity is essential for DNA end processing and HR repair (Sartori et al., 2007). USP4 binds to CtIP and regulates DNA end resection following DNA damage. Knocking down USP4 impairs the recruitment of CtIP to DSB sites. Auto-deubiquitination of USP4 can stimulate interaction with CtIP/MRN, but USP4 CA mutant (a catalytically inactive mutant) cannot mediate its auto-deubiquitination and has lost the ability to interact with CtIP/MRN complex (Liu et al., 2015; Wijnhoven et al., 2015). In addition, USP7 interacts with the MRN/MDC1 complex and USP7 deubiquitinates and stabilizes MDC1. USP7 depletion leads to MDC1 degradation, which in turn impairs the foci formation of 53BP1 and BRCA1 (Su et al., 2018). DUB Ataxin-3 is recruited to DNA damage sites that depend on SUMOylation by small ubiquitin-like modifier 1 (SUMO1). Ataxin-3 antagonises RNF4 activity and negatively regulates ubiquitylation of MDC1, which is important to increase staining intensity of MDC1 foci and MDC1-dependent repair of DSBs (Pfeiffer et al., 2017).

BRCA1 is an important regulator in DNA damage repair, DNA replication, cell growth and apoptosis (Hu YD et al., 2011). BRCA1 interacts with DDR proteins such as receptor-associated protein 80 (RAP80), CtIP, Abraxas (CCDC98), and Broad complex, Tramtrack, Bric-a-brac (BTB) domain and cap'n'collar (CNC) homolog 1 (BACH1). BRCC36 is a K63-specific DUB and its localization relies on RAP80. BRCC36 initially forms a "BRCA1-A complex," including the HR-promoting factors BARD1, mediator of RAP80 interactions and targeting 40 kDa (MERIT40), BRCC45, BRCA1, and CCDC98 (Chen et al., 2006; Kim et al., 2007b; Liu et al., 2007). BRCA1 colocalizes with CCDC98 (Abraxas), which is essential for the formation of BRCA1 foci in DSB sites. RAP80 and CCDC98 form complexes with BRCA1, which in turn are recruited to DSBs through RAP80's UIM domain binding to H2A ubiquitination chains (Kim et al., 2007a; Wang et al., 2007; Coleman and Greenberg, 2011). USP13 is an important DUB which is phosphorylated by ATM to facilitate USP13 recruitment to DSBs. In turn, USP13 deubiquitinates RAP80 and promotes RAP80 recruitment to DSBs following DNA damage. Taken together, USP13 regulates RAP80-BRCA1 foci formation by a phosphorylation-deubiquitination axis (Li et al., 2017). USP48 deubiquitinates $H2AK^{125/127/129Ub}$ and inhibits the function of the BRCA1 E3 ligase. Loss of USP48 and 53BP1 increases retention at the break site and DNA resection lengths are extended (Uckelmann et al., 2018). USP15 deubiquitinates BRCA1-associated RING domain protein 1 (BARD1) and promotes the retention of BRCA1/BARD1 at DSBs. In the USP15 mutation of cancer cells, the interaction between USP15 and BARD1 is decreased, and the sensitivity of poly(ADP-ribose) polymerase (PARP) inhibitors is increased (Peng et al., 2019). In cancer cells, BRCA1 interacts with USP9X, a DUB that regulates HR repair, and is involved in the sensitivity of cancer cells to PARP inhibitors (Lu et al., 2019). USP26 and USP37 are critical to DSB repair in the BRCA1-Abraxas-RAP80-MERIT40 complex. As the core DUBs, USP26 and USP37 promote interaction of BRCA1 with the PALB2 complex and ultimately, HR repair at DSBs (Typas et al., 2015). In addition, BRCA1 interacts with the BRCA2/PALB2 complex and facilitates RAD51 loading. Kelch-like ECH-associated protein 1 (KEAP1), an E3 ubiquitin ligase, ubiquitinates PALB2, which limits binding between PALB2 and BRCA1. However, USP11 deubiquitinates PALB2 and counteracts the process. Furthermore, the ubiquitination-mediated blocking interaction of BRCA1 and PALB2 limits DNA end

resection in G1 (Schoenfeld et al., 2004; Orthwein et al., 2015).

Ubiquitin C-terminal hydrolase L3 (UCHL3) is a novel deubiquitinase that regulates the BRCA2-RAD51 pathway following DNA damage. ATM phosphorylates and activates UCHL3, and subsequently UCHL3 deubiquitinates RAD51, promoting interaction between BRCA2 and RAD51 (Luo et al., 2016). In addition, POH1 (PSMD14) also enhances the loading of RAD51 to DNA damage sites and facilitates the HR repair pathway, which is a 53BP1-independent mechanism (Butler et al., 2012). Loss of POH1 reduces the foci formation of RAD51 to DSBs, compromises HR repair ability, and enhances the sensitivity of cells to DNA damage. POH1 is reported to remove ubiquitin chains and RAP80 foci, which in turn switches NHEJ to HR (Kakarougkas et al., 2013). Another study shows that ATM phosphorylates the deubiquitinase BRCA1-associated protein 1 (BAP1), which is rapidly recruited to DSB sites and promotes DNA repair (Yu et al., 2014). Depletion of BAP1 leads to impairment of the foci formation of BRCA1, RPA, and RAD51, which in turn reduces HR repair and sensitizes cells to IR (Ismail et al., 2014).

4.5 Regulation of the core factors in the NHEJ pathway

NHEJ is initiated by the Ku heterodimer. Ku80 ubiquitination causes removal of Ku70/80 from DNA damage sites, which contributes to selection of the DNA repair pathway. The deubiquitinases UCHL3 and OTUD5 deubiquitinate and stabilize Ku80 directly, and thus serve as important regulators of NHEJ repair. Depletion of UCHL3 reduces the foci formation of Ku80, decreases NHEJ efficiency, and makes cells sensitive to IR (Nishi et al., 2018). OTUD5 depletion impairs NHEJ repair during the S/G2 phase, but not HR repair (Li et al., 2019). Another Ku heterodimer subunit, Ku70, is also reported to be modified by deubiquitination. USP14 deubiquitinates and stabilizes Ku70. AKT mediates USP14 Ser432 phosphorylation, which is required for the foci formation of USP14 (Sharma et al., 2020). Overexpression of USP50 is reported to downregulate Ku70 protein levels by promoting Ku70 degradation, but apparently has no effect on mRNA levels, which suggests that USP50 may indirectly regulate Ku70 protein stability (Cai et al., 2018). In addition, following DNA damage, USP38 interacts with and deubiquitinates HDAC1 directly, which in turn increases the deacetylase activity of HDAC1 and promotes NHEJ (Yang et al., 2020).

5 Role of DUBs in therapeutics

Ubiquitin-proteasome system is an essential regulator system for protein degradation in cells. The dynamic balance of protein ubiquitination and deubiquitination regulates most cellular processes, such as cell growth, signal transduction, and cell development. If DUB functions become abnormal, a series of human diseases may be caused. Recently, a series of DUBs have been reported as potential therapeutic targets, and validation of the biological substrate of these DUBs is solidifying (Cohen and Tcherpakov, 2010; Farshi et al., 2015; Huang and Dixit, 2016). A number of putative small-molecular inhibitor DUBs have been identified, including USP1, USP5, USP9X, USP10, USP13, USP25/28, USP26, USP47, UCHL1, and UCHL5. In recent years, DUBs have been regarded as potential targets of tumor therapy. A variety of DUB small molecular inhibitors may have potential anti-tumor activity. For example, WP1130 can inhibit the activity of deubiquitinasing enzymes USP9X, USP14, USP5, and UCH37, which decrease the level of myeloid cell leukemia sequence 1 (Mcl-1, an anti-apoptotic protein) and increase the level of tumor suppressor p53, in turn killing cancer cells (Nicholson et al., 2008; Kapuria et al., 2010; Lee et al., 2010; Liu et al., 2011; Chauhan et al., 2012; Wrigley et al., 2017; Wang et al., 2018).

In conclusion, mounting evidence shows that DUBs are important signal molecules, especially in DNA repair and human disease therapy. Further exploration of the molecular mechanisms of DUB regulation will provide new insights into human diseases. However, at the moment it is difficult to select DUB inhibitors for clinical development since the crystal structure of the DUB substrate complex is not known (Harrigan et al., 2018). Therefore, future studies and efficient technologies should be developed to clarify the molecular structure of DUBs.

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Author contributions

Yunhui LI participated in searching and summarizing the relevant literature as well as designing and writing the manuscript. Jian YUAN provided the theme and design, and edited the manuscript. Both authors have read and approved the final manuscript.

Compliance with ethics guidelines

Yun-hui LI and Jian YUAN declare that they have no conflicts of interest.

This article does not contain any studies with human or animal subjects performed by either of the authors.

References

Altun M, Walter TS, Kramer HB, et al., 2015. The human otubain2-ubiquitin structure provides insights into the cleavage specificity of poly-ubiquitin-linkages. *PLoS ONE*, 10:e0115344. https://doi.org/10.1371/journal.pone.0115344

Britton S, Coates J, Jackson SP, 2013. A new method for high-resolution imaging of Ku foci to decipher mechanisms of DNA double-strand break repair. *J Cell Biol*, 202(3): 579-595. https://doi.org/10.1083/jcb.201303073

Butler LR, Densham RM, Jia JY, et al., 2012. The proteasomal de-ubiquitinating enzyme POH1 promotes the double-strand DNA break response. *EMBO J*, 31(19):3918-3934. https://doi.org/10.1038/emboj.2012.232

Cadet J, Berger M, Douki T, et al., 1997. Oxidative damage to DNA: formation, measurement, and biological significance. *Rev Physiol Biochem Pharmacol*, 131:1-87. https://doi.org/10.1007/3-540-61992-5

Cai JT, Wei JX, Schrott V, et al., 2018. Induction of deubiquitinating enzyme USP50 during erythropoiesis and its potential role in the regulation of Ku70 stability. *J Investig Med*, 66(1):1-6. https://doi.org/10.1136/jim-2017-000622

Ceccaldi R, Rondinelli B, D'Andrea AD, 2016. Repair pathway choices and consequences at the double-strand break. *Trends Cell Biol*, 26(1):52-64. https://doi.org/10.1016/j.tcb.2015.07.009

Chauhan D, Tian Z, Nicholson B, et al., 2012. A small molecule inhibitor of ubiquitin-specific protease-7 induces apoptosis in multiple myeloma cells and overcomes bortezomib resistance. *Cancer Cell*, 22(3):345-358. https://doi.org/10.1016/j.ccr.2012.08.007

Chen XW, Arciero CA, Wang CR, et al., 2006. BRCC36 is essential for ionizing radiation-induced BRCA1 phosphorylation and nuclear foci formation. *Cancer Res*, 66(10): 5039-5046. https://doi.org/10.1158/0008-5472.CAN-05-4194

Cheng YC, Shieh SY, 2018. Deubiquitinating enzyme USP3 controls CHK1 chromatin association and activation. *Proc Natl Acad Sci USA*, 115(21):5546-5551. https://doi.org/10.1073/pnas.1719856115

Chiruvella KK, Liang ZB, Wilson TE, 2013. Repair of double-strand breaks by end joining. *Cold Spring Harbor Perspect Biol*, 5(5):a012757. https://doi.org/10.1101/cshperspect.a012757

Cohen P, Tcherpakov M, 2010. Will the ubiquitin system furnish as many drug targets as protein kinases? *Cell*, 143(5):686-693. https://doi.org/10.1016/j.cell.2010.11.016

Coleman KA, Greenberg RA, 2011. The BRCA1-RAP80 complex regulates DNA repair mechanism utilization by restricting end resection. *J Biol Chem*, 286(15): 13669-13680. https://doi.org/10.1074/jbc.M110.213728

Cooper EM, Cutcliffe C, Kristiansen TZ, et al., 2009. K63-specific deubiquitination by two JAMM/MPN+ complexes: BRISC-associated Brcc36 and proteasomal Poh1. *EMBO J*, 28(6):621-631. https://doi.org/10.1038/emboj.2009.27

Cottarel J, Frit P, Bombarde O, et al., 2013. A noncatalytic function of the ligation complex during nonhomologous end joining. *J Cell Biol*, 200(2):173-186. https://doi.org/10.1083/jcb.201203128

Doil C, Mailand N, Bekker-Jensen S, et al., 2009. RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. *Cell*, 136(3):435-446. https://doi.org/10.1016/j.cell.2008.12.041

Dong YS, Hakimi MA, Chen XW, et al., 2003. Regulation of BRCC, a holoenzyme complex containing BRCA1 and BRCA2, by a signalosome-like subunit and its role in DNA repair. *Mol Cell*, 12(5):1087-1099. https://doi.org/10.1016/S1097-2765(03)00424-6

Durocher D, Jackson SP, 2001. DNA-PK, ATM and ATR as sensors of DNA damage: variations on a theme? *Curr Opin Cell Biol*, 13(2):225-231. https://doi.org/10.1016/S0955-0674(00)00201-5

Farshi P, Deshmukh RR, Nwankwo JO, et al., 2015. Deubiquitinases (DUBs) and DUB inhibitors: a patent review. *Expert Opin Ther Pat*, 25(10):1191-1208. https://doi.org/10.1517/13543776.2015.1056737

Feng L, Wang JD, Chen JJ, 2010. The Lys^{63}-specific deubiquitinating enzyme BRCC36 is regulated by two scaffold proteins localizing in different subcellular compartments. *J Biol Chem*, 285(40):30982-30988. https://doi.org/10.1074/jbc.M110.135392

Gottlieb TM, Jackson SP, 1993. The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. *Cell*, 72(1):131-142. https://doi.org/10.1016/0092-8674(93)90057-W

Guervilly JH, Renaud E, Takata M, et al., 2011. USP1 deubiquitinase maintains phosphorylated CHK1 by limiting its DDB1-dependent degradation. *Hum Mol Genet*, 20(11): 2171-2181. https://doi.org/10.1093/hmg/ddr103

Gupta C, Heinen CD, 2019. The mismatch repair-dependent DNA damage response: mechanisms and implications.

DNA Repair, 78:60-69. https://doi.org/10.1016/j.dnarep.2019.03.009

Hanpude P, Bhattacharya S, Dey AK, et al., 2015. Deubiquitinating enzymes in cellular signaling and disease regulation. *IUBMB Life*, 67(7):544-555. https://doi.org/10.1002/iub.1402

Harper JW, Elledge SJ, 2007. The DNA damage response: ten years after. *Mol Cell*, 28(5):739-745. https://doi.org/10.1016/j.molcel.2007.11.015

Harrigan JA, Jacq X, Martin NM, et al., 2018. Deubiquitylating enzymes and drug discovery: emerging opportunities. *Nat Rev Drug Discov*, 17(1):57-78. https://doi.org/10.1038/nrd.2017.152

Harrison JC, Haber JE, 2006. Surviving the breakup: the DNA damage checkpoint. *Annu Rev Genet*, 40:209-235. https://doi.org/10.1146/annurev.genet.40.051206.105231

Hu X, Kim JA, Castillo A, et al., 2011. NBA1/MERIT40 and BRE interaction is required for the integrity of two distinct deubiquitinating enzyme BRCC36-containing complexes. *J Biol Chem*, 286(13):11734-11745. https://doi.org/10.1074/jbc.M110.200857

Hu YD, Scully R, Sobhian B, et al., 2011. RAP80-directed tuning of BRCA1 homologous recombination function at ionizing radiation-induced nuclear foci. *Genes Dev*, 25(7):685-700. https://doi.org/10.1101/gad.2011011

Huang XD, Dixit VM, 2016. Drugging the undruggables: exploring the ubiquitin system for drug development. *Cell Res*, 26(4):484-498. https://doi.org/10.1038/cr.2016.31

Hurley JH, Lee S, Prag G, 2006. Ubiquitin-binding domains. *Biochem J*, 399(Pt 3):361-372. https://doi.org/10.1042/BJ20061138

Ismail IH, Davidson R, Gagné JP, et al., 2014. Germline mutations in BAP1 impair its function in DNA double-strand break repair. *Cancer Res*, 74(16):4282-4294. https://doi.org/10.1158/0008-5472.CAN-13-3109

Juang YC, Landry MC, Sanches M, et al., 2012. OTUB1 co-opts Lys48-linked ubiquitin recognition to suppress E2 enzyme function. *Mol Cell*, 45(3):384-397. https://doi.org/10.1016/j.molcel.2012.01.011

Ka HI, Lee S, Han S, et al., 2020. Deubiquitinase USP47-stabilized splicing factor IK regulates the splicing of *ATM* pre-mRNA. *Cell Death Discov*, 6:34. https://doi.org/10.1038/s41420-020-0268-1

Kakarougkas A, Jeggo PA, 2014. DNA DSB repair pathway choice: an orchestrated handover mechanism. *Br J Radiol*, 87(1035):20130685. https://doi.org/10.1259/bjr.20130685

Kakarougkas A, Ismail A, Katsuki Y, et al., 2013. Co-operation of BRCA1 and POH1 relieves the barriers posed by 53BP1 and RAP80 to resection. *Nucleic Acids Res*, 41(22):10298-10311. https://doi.org/10.1093/nar/gkt802

Kapuria V, Peterson LF, Fang DX, et al., 2010. Deubiquitinase inhibition by small-molecule WP1130 triggers aggresome formation and tumor cell apoptosis. *Cancer Res*, 70(22):9265-9276. https://doi.org/10.1158/0008-5472.CAN-10-1530

Karanam K, Kafri R, Loewer A, et al., 2012. Quantitative live cell imaging reveals a gradual shift between DNA repair mechanisms and a maximal use of HR in mid S phase. *Mol Cell*, 47(2):320-329. https://doi.org/10.1016/j.molcel.2012.05.052

Kato K, Nakajima K, Ui A, et al., 2014. Fine-tuning of DNA damage-dependent ubiquitination by OTUB2 supports the DNA repair pathway choice. *Mol Cell*, 53(4):617-630. https://doi.org/10.1016/j.molcel.2014.01.030

Kawanishi S, Hiraku Y, Pinlaor S, et al., 2006. Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. *Biol Chem*, 387(4):365-372. https://doi.org/10.1515/BC.2006.049

Kennedy RD, D'Andrea AD, 2005. The Fanconi Anemia/BRCA pathway: new faces in the crowd. *Genes Dev*, 19(24):2925-2940. https://doi.org/10.1101/gad.1370505

Kerzendorfer C, O'Driscoll M, 2009. Human DNA damage response and repair deficiency syndromes: linking genomic instability and cell cycle checkpoint proficiency. *DNA Repair (Amst)*, 8(9):1139-1152. https://doi.org/10.1016/j.dnarep.2009.04.018

Khanna KK, Jackson SP, 2001. DNA double-strand breaks: signaling, repair and the cancer connection. *Nature Genet*, 27(3):247-254. https://doi.org/10.1038/85798

Kim H, Huang J, Chen JJ, 2007a. CCDC98 is a BRCA1-BRCT domain-binding protein involved in the DNA damage response. *Nat Struct Mol Biol*, 14(8):710-715. https://doi.org/10.1038/nsmb1277

Kim H, Chen JJ, Yu XC, 2007b. Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. *Science*, 316(5828):1202-1205. https://doi.org/10.1126/science.1139621

Komander D, Clague MJ, Urbé S, 2009. Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol*, 10(8):550-563. https://doi.org/10.1038/nrm2731

Latif C, den Elzen NR, O'Connell MJ, 2004. DNA damage checkpoint maintenance through sustained Chk1 activity. *J Cell Sci*, 117(Pt 16):3489-3498. https://doi.org/10.1242/jcs.01204

Lee BH, Lee MJ, Park S, et al., 2010. Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature*, 467(7312):179-184. https://doi.org/10.1038/nature09299

Li FZ, Sun QQ, Liu K, et al., 2019. The deubiquitinase OTUD5 regulates Ku80 stability and non-homologous end joining. *Cell Mol Life Sci*, 76(19):3861-3873. https://doi.org/10.1007/s00018-019-03094-5

Li YH, Luo KT, Yin YJ, et al., 2017. USP13 regulates the RAP80-BRCA1 complex dependent DNA damage response. *Nat Commun*, 8:15752. https://doi.org/10.1038/ncomms15752

Lieber MR, 2008. The mechanism of human nonhomologous DNA end joining. *J Biol Chem*, 283(1):1-5. https://doi.org/10.1074/jbc.R700039200

Lindah T, Barnes DE, 2000. Repair of endogenous DNA damage. *Cold Spring Harb Symp Quant Biol*, 65:127-133. https://doi.org/10.1101/sqb.2000.65.127

Liu HL, Zhang HX, Wang XH, et al., 2015. The deubiquitylating enzyme USP4 cooperates with CtIP in DNA double-strand break end resection. *Cell Rep*, 13(1):93-107. https://doi.org/10.1016/j.celrep.2015.08.056

Liu JL, Xia HG, Kim M, et al., 2011. Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. *Cell*, 147(1):223-234. https://doi.org/10.1016/j.cell.2011.08.037

Liu ZX, Wu JX, Yu XC, 2007. CCDC98 targets BRCA1 to DNA damage sites. *Nat Struct Mol Biol*, 14(8):716-720. https://doi.org/10.1038/nsmb1279

Lu Q, Zhang FL, Lu DY, et al., 2019. USP9X stabilizes BRCA1 and confers resistance to DNA-damaging agents in human cancer cells. *Cancer Med*, 8(15):6730-6740. https://doi.org/10.1002/cam4.2528

Luo KT, Li L, Li YH, et al., 2016. A phosphorylation-deubiquitination cascade regulates the BRCA2-RAD51 axis in homologous recombination. *Genes Dev*, 30(23): 2581-2595. https://doi.org/10.1101/gad.289439.116

Mattiroli F, Vissers JHA, van Dijk WJ, et al., 2012. RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. *Cell*, 150(6):1182-1195. https://doi.org/10.1016/j.cell.2012.08.005

Meuth M, 2010. Chk1 suppressed cell death. *Cell Div*, 5:21. https://doi.org/10.1186/1747-1028-5-21

Mevissen TET, Hospenthal MK, Geurink PP, et al., 2013. OTU deubiquitinases reveal mechanisms of linkage specificity and enable ubiquitin chain restriction analysis. *Cell*, 154(1):169-184. https://doi.org/10.1016/j.cell.2013.05.046

Nakada S, Tai I, Panier S, et al., 2010. Non-canonical inhibition of DNA damage-dependent ubiquitination by OTUB1. *Nature*, 466(7309):941-946. https://doi.org/10.1038/nature09297

Nicholson B, Leach CA, Goldenberg SJ, et al., 2008. Characterization of ubiquitin and ubiquitin-like-protein isopeptidase activities. *Protein Sci*, 17(6):1035-1043. https://doi.org/10.1110/ps.083450408

Nijman SMB, Huang TT, Dirac AMG, et al., 2005a. The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. *Mol Cell*, 17(3):331-339. https://doi.org/10.1016/j.molcel.2005.01.008

Nijman SMB, Luna-Vargas MPA, Velds A, et al., 2005b. A genomic and functional inventory of deubiquitinating enzymes. *Cell*, 123(5):773-786. https://doi.org/10.1016/j.cell.2005.11.007

Nishi R, Wijnhoven P, le Sage C, et al., 2014. Systematic characterization of deubiquitylating enzymes for roles in maintaining genome integrity. *Nat Cell Biol*, 16(10): 1016-1026. https://doi.org/10.1038/ncb3028

Nishi R, Wijnhoven PWG, Kimura Y, et al., 2018. The deubiquitylating enzyme UCHL3 regulates Ku80 retention at sites of DNA damage. *Sci Rep*, 8:17891. https://doi.org/10.1038/s41598-018-36235-0

Nowsheen S, Deng M, Lou ZK, 2020. Ubiquitin and the DNA double-strand break repair pathway. *Genome Instab Dis*, 1(2):69-80. https://doi.org/10.1007/s42764-019-00007-5

Olivieri M, Cho T, Álvarez-Quilón A, et al., 2020. A genetic map of the response to DNA damage in human cells. *Cell*, 182(2):481-496.e21. https://doi.org/10.1016/j.cell.2020.05.040

Orthwein A, Noordermeer SM, Wilson MD, et al., 2015. A mechanism for the suppression of homologous recombination in G1 cells. *Nature*, 528(7582):422-426. https://doi.org/10.1038/nature16142

Pellegrini L, Yu DS, Lo T, et al., 2002. Insights into DNA recombination from the structure of a RAD51-BRCA2 complex. *Nature*, 420(6913):287-293. https://doi.org/10.1038/nature01230

Peng YH, Liao QC, Tan W, et al., 2019. The deubiquitylating enzyme USP15 regulates homologous recombination repair and cancer cell response to PARP inhibitors. *Nat Commun*, 10:1224. https://doi.org/10.1038/s41467-019-09232-8

Pfeiffer A, Luijsterburg MS, Acs K, et al., 2017. Ataxin-3 consolidates the MDC1-dependent DNA double-strand break response by counteracting the SUMO-targeted ubiquitin ligase RNF4. *EMBO J*, 36(8):1066-1083. https://doi.org/10.15252/embj.201695151

Rehman SAA, Kristariyanto YA, Choi SY, et al., 2016. MINDY-1 is a member of an evolutionarily conserved and structurally distinct new family of deubiquitinating enzymes. *Mol Cell*, 63(1):146-155. https://doi.org/10.1016/j.molcel.2016.05.009

Riballo E, Kühne M, Rief N, et al., 2004. A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to γ-H2AX foci. *Mol Cell*, 16(5):715-724. https://doi.org/10.1016/j.molcel.2004.10.029

Rodriguez R, Meuth M, 2006. Chk1 and p21 cooperate to prevent apoptosis during DNA replication fork stress. *Mol Biol Cell*, 17(1):402-412. https://doi.org/10.1091/mbc.e05-07-0594

san Filippo J, Sung P, Klein H, 2008. Mechanism of eukaryotic homologous recombination. *Annu Rev Biochem*, 77: 229-257. https://doi.org/10.1146/annurev.biochem.77. 061306.125255

Sartori AA, Lukas C, Coates J, et al., 2007. Human CtIP promotes DNA end resection. *Nature*, 450(7169):509-514. https://doi.org/10.1038/nature06337

Schmitt E, Paquet C, Beauchemin, M, et al., 2007. DNA-damage response network at the crossroads of cell-cycle checkpoints, cellular senescence and apoptosis. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 8(6):377-397. https://doi.org/10.1631/jzus.2007.B0377

Schoenfeld AR, Apgar S, Dolios G, et al., 2004. BRCA2 is ubiquitinated in vivo and interacts with USP11, a deubiquitinating enzyme that exhibits prosurvival function in the cellular response to DNA damage. *Mol Cell Biol*, 24(17):7444-7455. https://doi.org/10.1128/MCB.24.17.7444-7455.2004

Scully R, Panday A, Elango R, et al., 2019. DNA double-strand break repair-pathway choice in somatic mammalian cells. *Nat Rev Mol Cell Biol*, 20(11):698-714. https://doi.org/10.1038/s41580-019-0152-0

Shanbhag NM, Rafalska-Metcalf IU, Balane-Bolivar C, et al., 2010. ATM-dependent chromatin changes silence transcription in *cis* to DNA double-strand breaks. *Cell*, 141(6):970-981. https://doi.org/10.1016/j.cell.2010.04.038

Shao G, Lilli DR, Patterson-Fortin J, et al., 2009. The Rap80-BRCC36 de-ubiquitinating enzyme complex antagonizes RNF8-Ubc13-dependent ubiquitination events at DNA double strand breaks. *Proc Natl Acad Sci USA*, 106(9): 3166-3171. https://doi.org/10.1073/pnas.0807485106

Sharma A, Alswillah T, Kapoor I, et al., 2020. USP14 is a deubiquitinase for Ku70 and critical determinant of non-homologous end joining repair in autophagy and PTEN-deficient cells. *Nucleic Acids Res*, 48(2):736-747. https://doi.org/10.1093/nar/gkz1103

Sobhian B, Shao G, Lilli DR, et al., 2007. RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. *Science*, 316(5828):1198-1202. https://doi.org/10.1126/science.1139516

Su DX, Ma S, Shan L, et al., 2018. Ubiquitin-specific protease 7 sustains DNA damage response and promotes cervical carcinogenesis. *J Clin Invest*, 128(10):4280-4296. https://doi.org/10.1172/JCI120518

Sun YL, Jiang XF, Chen SJ, et al., 2005. A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. *Proc Natl Acad Sci USA*, 102(37):13182-13187. https://doi.org/10.1073/pnas.0504211102

Symington LS, Gautier J, 2011. Double-strand break end resection and repair pathway choice. *Annu Rev Genet*, 45:247-271. https://doi.org/10.1146/annurev-genet-110410-132435

Takeda S, Nakamura K, Taniguchi Y, et al., 2007. Ctp1/CtIP and the MRN complex collaborate in the initial steps of homologous recombination. *Mol Cell*, 28(3):351-352. https://doi.org/10.1016/j.molcel.2007.10.016

Typas D, Luijsterburg MS, Wiegant WW, et al., 2015. The de-ubiquitylating enzymes USP26 and USP37 regulate homologous recombination by counteracting RAP80. *Nucleic Acids Res*, 43(14):6919-6933. https://doi.org/10.1093/nar/gkv613

Uckelmann M, Densham RM, Baas R, et al., 2018. USP48 restrains resection by site-specific cleavage of the BRCA1 ubiquitin mark from H2A. *Nat Commun*, 9:229. https://doi.org/10.1038/s41467-017-02653-3

Wang B, Elledge SJ, 2007. Ubc13/Rnf8 ubiquitin ligases control foci formation of the Rap80/Abraxas/Brca1/Brcc36 complex in response to DNA damage. *Proc Natl Acad Sci USA*, 104(52):20759-20763. https://doi.org/10.1073/pnas.0710061104

Wang B, Matsuoka S, Ballif BA, et al., 2007. Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. *Science*, 316(5828):1194-1198. https://doi.org/10.1126/science.1139476

Wang XF, Liu ZY, Zhang L, et al., 2018. Targeting deubiquitinase USP28 for cancer therapy. *Cell Death Dis*, 9:186. https://doi.org/10.1038/s41419-017-0208-z

Wang ZQ, Zhang HL, Liu J, et al., 2016. USP51 deubiquitylates H2AK13, 15ub and regulates DNA damage response. *Genes Dev*, 30(8):946-959. https://doi.org/10.1101/gad.271841.115

Weake VM, Workman JL, 2008. Histone ubiquitination: triggering gene activity. *Mol Cell*, 29(6):653-663. https://doi.org/10.1016/j.molcel.2008.02.014

Welchman RL, Gordon C, Mayer RJ, 2005. Ubiquitin and ubiquitin-like proteins as multifunctional signals. *Nat Rev Mol Cell Biol*, 6(8):599-609. https://doi.org/10.1038/nrm1700

Wiener R, Zhang XB, Wang T, et al., 2012. The mechanism of OTUB1-mediated inhibition of ubiquitination. *Nature*, 483(7391):618-622. https://doi.org/10.1038/nature10911

Wijnhoven P, Konietzny R, Blackford AN, et al., 2015. USP4 auto-deubiquitylation promotes homologous recombination. *Mol Cell*, 60(3):362-373. https://doi.org/10.1016/j.molcel.2015.09.019

Wrigley JD, Gavory G, Simpson I, et al., 2017. Identification and characterization of dual inhibitors of the USP25/28 deubiquitinating enzyme subfamily. *ACS Chem Biol*, 12(12):3113-3125. https://doi.org/10.1021/acschembio.7b00334

Wu JH, Chen YP, Geng GH, et al., 2019. USP39 regulates DNA damage response and chemo-radiation resistance by deubiquitinating and stabilizing CHK2. *Cancer Lett*, 449:114-124. https://doi.org/10.1016/j.canlet.2019.02.015

Wu ZQ, Qiu MH, Guo Y, et al., 2019. OTU deubiquitinase 4 is silenced and radiosensitizes non-small cell lung cancer cells via inhibiting DNA repair. *Cancer Cell Int*, 19:99. https://doi.org/10.1186/s12935-019-0816-z

Yang YF, Yang CZ, Li TT, et al., 2020. The deubiquitinase USP38 promotes NHEJ repair through regulation of HDAC1 activity and regulates cancer cell response to genotoxic insults. *Cancer Res*, 80(4):719-731. https://doi.org/10.1158/0008-5472.CAN-19-2149

Yu H, Pak H, Hammond-Martel I, et al., 2014. Tumor suppressor and deubiquitinase BAP1 promotes DNA double-strand break repair. *Proc Natl Acad Sci U S A*, 111(1):285-290. https://doi.org/10.1073/pnas.1309085110

Yuan J, Luo KT, Deng M, et al., 2014. HERC2-USP20 axis regulates DNA damage checkpoint through Claspin. *Nucleic Acids Res*, 42(21):13110-13121. https://doi.org/10.1093/nar/gku1034

Zhang D, Zaugg K, Mak TW, et al., 2006. A role for the deubiquitinating enzyme USP28 in control of the DNA-damage response. *Cell*, 126(3):529-542. https://doi.org/10.1016/j.cell.2006.06.039