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Review

DNA damage response: The emerging role of c-Abl as a regulatory switch?

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ABSTRACT

A complex regulatory network of signaling pathways safeguards genome integrity following DNA damage. When double strand breaks occur several enzymes and mediators are recruited to the sites of lesion to release a network of DNA repair processes referred to as DNA damage response (DDR). c-Abl interacts in the nucleus with several proteins implicated in distinct aspects of DNA repair. This suggests that c-Abl may be involved in the regulation of double strand break repair. The involvement of c-Abl in DNA repair mechanisms came into the spotlight in female germ cells under genotoxic stress. Recent findings have implicated c-Abl in a cisplatin-induced signaling pathway eliciting death of immature oocytes. Pharmacological inhibition of c-Abl by Imatinib (STI571) protects the ovarian reserve from the toxic effect of cisplatin. This implies that the extent of c-Abl catalytic outcomes may tip the balance between survival (likely through DNA repair) and activation of a death response. Many observations indicate that timely ubiquitin-modifications and signal decoding are implicated in regulating DNA repair. Here, we discuss some connections between phosphorylation- and ubiquitin-mediated signaling at the damaged sites. We speculate about multiple interactions that may occur between c-Abl (and 'sensor' kinases) with ubiquitin-related proteins involved in DDR. Additional work is required to understand the complexity of the physiological outcomes of c-Abl in DDR. However, a fine-tuning of nuclear outcomes, through pharmacological inhibition of c-Abl, may provide novel paradigms for DDR and, potentially, therapeutic strategies for cancer treatment.

Contents

Abbreviations: 53BP1, Tumor suppressor p53-binding protein 1; ATM, Ataxia telangiectasia mutated; ATR, Ataxia telangiectasia and Rad3-related protein; BARD, BRCA1-associated RING domain protein 1; BER, Base excision repair; BRCA1, Breast cancer type 1 susceptibility protein; BRCC36, BRCA1–BRCA2 containing complex subunit 36; CtIP, CtBP-interacting protein; DDB1, DNA damage-binding protein 1; DDB2, DNA damage-binding protein 2; DNAPK, DNA-dependent protein kinase; DSBR, Double-strand break repair; ERCC6, Excision repair cross-complementing 6; HERC2, HECT domain and RCC1-like domain-containing protein 2; MDM2, Double minute 2 protein; MMR, Mismatch repair; MRN, Mre11/Nbs1/Rad50 complex; MSH5, DNA Mismatch repair protein 5; NER, Nucleotide excision repair; PIAS, Protein inhibitor of activated STAT; RAP 80, Receptor-associated protein 80; RNF8, RING finger protein 8; RNF20, RING finger protein 20; RNF40, RING finger protein 40; RNF168, RING finger protein 168; TopBP1, DNA topoisomerase 2-binding protein 1; UBC13, Ubiquitin-conjugating enzyme E2 13; USP1, Ubiquitin-specific-processing protease 1; WRN, Werner syndrome ATP-dependent helicase; YAP1, Yes-associated protein.

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1. The emerging central role of c-Abl in modulating the cell response to DNA damage

The cellular response to DNA damage (DDR) relies on a network of multiple interconnected signaling pathways acting in concert to minimize the dangerous effects of DNA double strands breaks (DSBs). The phosphatidylinositol 3-kinases (PI3K)-related kinases ataxia-telangiectasia mutated (ATM), ATM and Rad3-related (ATR) and DNA-activated protein kinase (DNAPK) are activated early by distinct DNA lesions and start a cascade of events signaled by the rapid phosphorylation of several proteins implicated in processes such as DNA repair, cell cycle arrest and apoptosis [1–3]. Although the PI3K related enzymes are considered major players in the DNA damage cell response, a fourth unrelated kinase, c-Abl, has more recently been associated to various aspects of the DDR [4]. c-Abl is a non-receptor tyrosine kinase that has the potential to bind to several proteins [5]. It has been implicated in several cellular pathways, including those originating from growth factor stimulation, cell adhesion, oxidative stress and DNA damage [6–9]; its activity is tightly regulated and it can be promptly activated following ionizing radiation and other types of genotoxic insults [10,11]. c-Abl accumulation leads to cell cycle arrest and to programmed cell death in cultured cells. Several c-Abl targets (YAP1, TP73, TP63, MDM2) are indeed important modulators of DNA damage-induced apoptosis. At the same time, many partners and substrates of c-Abl are known mediators of DNA repair [5] (among them, DDB1, DDB2, ERCC6, RAD9A, RAD51, RAD52 and WRN, ATM, ATR, DNAPK, BRCA1, TopBP1, and MSH5, see Fig. 1), suggesting that c-Abl may be implicated in the regulation and/or assembly of DNA repair complexes. In spite of its emerging central role in DNA repair, the mechanistic details are still poorly understood and the physiological functions, if any, of many of the interactions that have been reported remains elusive [12,13]. Wang et al. have recently reported that c-Abl is involved in the activation of ATM and ATR kinases following doxorubicin treatment. c-Abl deficient primary MEFs, following genotoxic stress, failed to activate both ATM and ATR and their downstream effectors [14]. These observations suggest that c-Abl may have a significant role in the activation of the key upstream molecular events governing the initiation and propagation of DDR [12]. Additional insights on the central role played by c-Abl in modulating the interplay between DNA repair and induction of apoptosis came from the study of female germ cells under genotoxic stress [15]. Intraperitoneal injection of cisplatin in newborn female mice leads to depletion of the follicle reserve and to long-term infertility. Recent findings have implicated c-Abl in a cisplatin-induced signaling pathway eliciting death of immature oocytes [16]. A p53-related protein, TAp63, is a critical downstream effector of this pathway. Inhibition of c-Abl by Imatinib (STI571) protects the ovarian reserve from the toxic effect of cisplatin. This implies that the extent of c-Abl catalytic outcomes may tip the balance between survival (likely through DNA repair) and activation of a death response. Our current model suggests that c-Abl may function as a hub assisting the progression of repair but eventually promoting cell death when DNA breaks prove irreparable [13]. Although we have shown that co-treatment with Imatinib has a protective effect on the ovarian reserve [17], we need to clarify the mechanisms underlying such an effect. The kinetics of c-Abl activation following DNA damage represents an important immediate issue to be addressed. Additional work is required to understand the complexity of the physiological role of c-Abl in DDR, and its involvement in the modulation of the many posttranslational mechanisms, including ubiquitination, underlying the DDR.

1.1. Surfing at the break point

Chromatin is a complex scaffold formed by chromosomal DNA wrapped around the histone core. This scaffold is not static. Chromatin modifications are essential for modulation of many cellular processes including transcription, replication and DNA repair. Two classes of enzymes can modify chromatin structure. One class consists of large multi-protein complexes that use ATP hydrolysis to alter the nucleosome position or composition within chromatin [18]. The second class mediates covalent modifications of histone tails. Posttranslational modifications of histones are implicated in the DNA damage response [19,20]. In particular, histone modification induced by members of the ubiquitin enzyme family is one of the main defensive strategies adopted by DNA-injured cells [21]. Ubiquitin-conjugation seems to modulate the

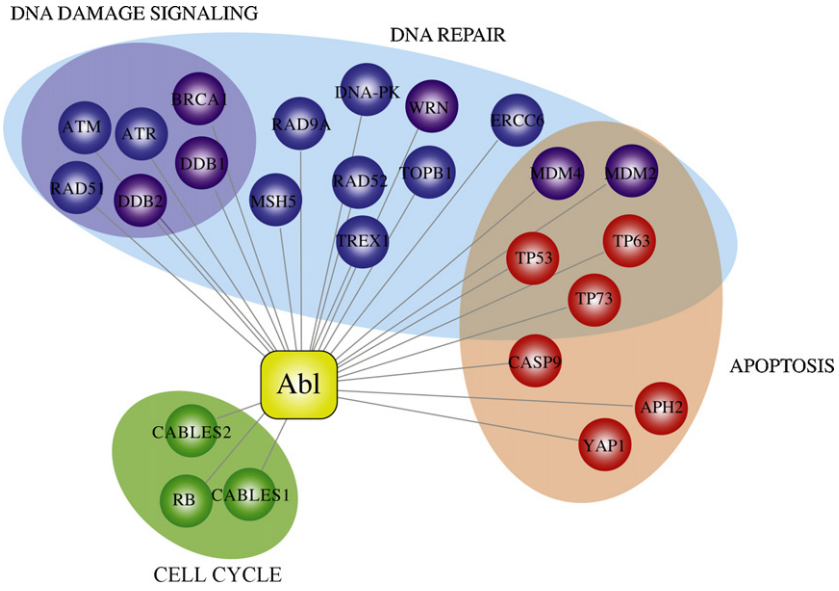

Fig. 1. Abl-interacting proteins in the DNA damage response. In red ellipse proteins involved in apoptosis, in blue those involved in DNA damage signaling and DNA repair. Green ellipse contains proteins involved in cell cycle arrest. Proteins directly involved in ubiquitin-signaling of DDR are in violet. All these proteins have also been reported as Abl substrates with the exception of TREX1, CABLE2, BRCA1, and DDB2.

assembly of the many components of the genome surveillance system. Several ubiquitin-signaling paths influence various aspects of genome-integrity maintenance and both monoubiquitylation and polyubiquitylation are emerging as versatile strategies to modulate protein–protein interaction networks [22–28]. A model of a complex 'ubiquitin landscape' at the damaged sites is emerging, albeit incomplete and poorly understood [29,30]. Particularly noteworthy is the extensive crosstalk between ubiquitin-modifications and phosphorylation-mediated pathways in DDR. A complex web of molecular interactions determines whether and how to repair the damage or rather let the injured cell die [31–34]. Here, we discuss some connections between phosphorylation- and ubiquitin-dependent signaling at the damage sites. We speculate about multiple interactions that may occur between c-Abl (and 'sensor' kinases) with ubiquitin-related proteins involved in DDR.

2. DNA damage response: sensing, repairing or signaling to death

Intricate mechanisms are set in motion for counteracting the potentially dangerous effects of DNA lesions. These mechanisms are challenged in chemotherapy regimens for cancer treatment. Crosslinking agents are among the most widely used and most effective anticancer drugs. They form covalent adducts on cellular DNA either on the same strand (intrastrand) or between the two complementary strands (interstrand). How are they repaired? The main players are nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR) and double strand break (DSB) repair. Interstrand crosslinks may induce double strand breaks as an intermediate step during repair. So, cells may use several DNA repair pathways in a concerted way. It is beyond the scope of this review to discuss these repair mechanisms in detail. Interested readers are directed to several reviews on this subject [35–40]. Here, we will focus on DSBs since very recent studies have indicated that transient abrogation of c-Abl activity modulates DSB repair pathway mediated by either homologous recombination repair (HRR) or nonhomologous end-joining (NHEJ) mechanisms [41,42]. In addition, in germ cells, DSBs occur normally during meiosis to promote homologous recombination and by doing so genetic diversity [43]. Mice deficient in c-Abl exhibit defects in spermatogenesis [44]. This suggests that c-Abl has a role in the maintenance of genome integrity by dealing with DSBs in meiotic cells.

Three distinct protein complexes act as *sensors*, *transducers* and *effectors* of DDR induced by DSBs. Many components of these three layers interact with each other and converge toward different outcomes depending on the severity of the damage and on the cell type. The activation of checkpoints slows down cell cycle progression until lesions are resolved. If unrepaired DSBs persist, cells undergo either apoptosis or senescence to prevent the accumulation of potentially tumorigenic mutations [45–47]. Female germ cells are extremely sensitive to DNA insults compared with somatic cells [48]. In line with this, ovarian failure and infertility are often off-target consequences of chemotherapeutic treatment. Oocytes from follicle reserve are arrested in meiosis I; DNA damage is either quickly repaired or triggers a robust cell degeneration. Intriguingly, abrogation of c-Abl activity has a protective effect on the ovarian reserve under genotoxic stress. Despite the diversity regarding the cell type, the efficiency of repair and signaling of the breaks is enhanced by the concentration of factors in the proximity of the lesion. At the damaged site, the DDR can be presented as a sequential assembly of protein complexes (Fig. 2).

DNA repair initiated by sensors of breaks, – including MRN complex, ATM – relies on the activity of different E3-ligases namely

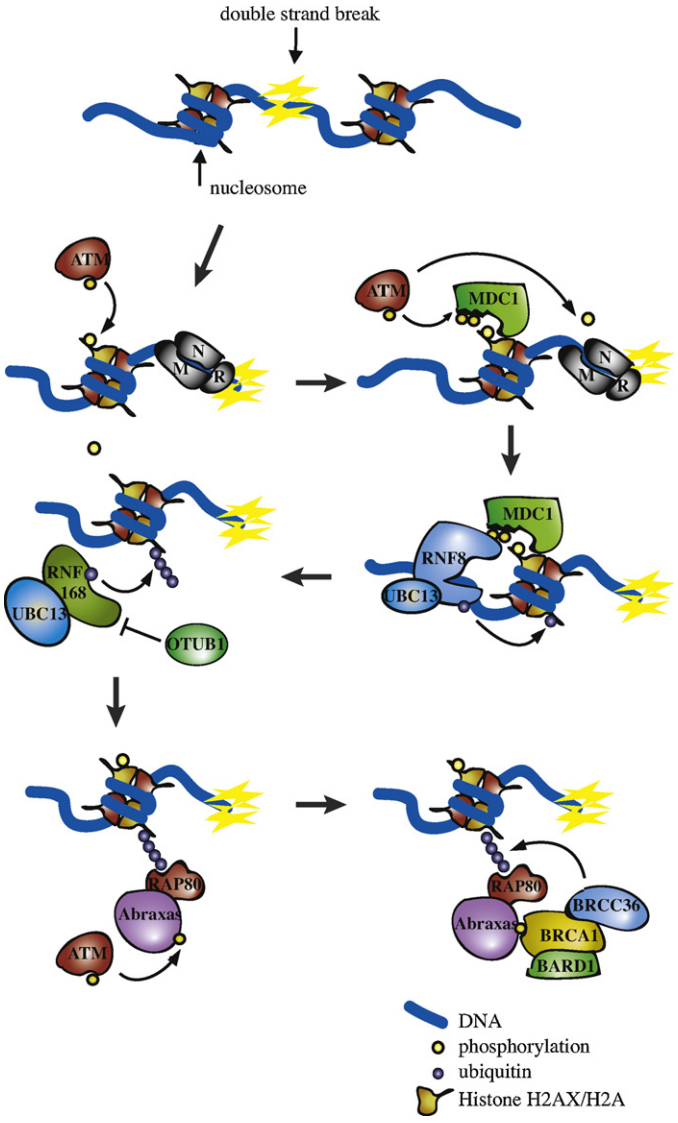

Fig. 2. DSBs are recognized by Mre11–Rad50–Nbs1 (MRN) complex, which promotes the activation of ATM. H2AX phosphorylation by ATM provides a docking site for MDC1. The ubiquitin ligase RNF8 (recruited through its FHA domain) in tandem with UBC13 ubiquitylated H2A and γH2AX. Signaling of the breaks is then enhanced by the recruitment of E3 ligase enzyme RNF168 (through its MIU domain) that acts by extending K-63 ubiquitin chains. The deubiquitinating enzyme OTUB1 suppresses RNF168-dependent ubiquitination by direct inhibition of the E2 ligase UBC13. RAP80 associates to ubiquitin by its UIM domain and recruits the BRCA1-A complex, through the interaction with the scaffold protein Abraxas. The BRCA complex contains the ubiquitin protease BRCC36 that removes ubiquitin on histones H2A and H2AX, antagonizing the RNF8/RNF168-dependent ubiquitination.

RNF8, HERC2 and RNF168. Among the many targets of ATM, the histone H2A variant H2AX is phosphorylated on Ser-139. This modification seems to be a recruitment signal for proteins with dedicated phospho-S/T recognition domains such as the FHA [49] or BRCT domain [50]. The RING-type ubiquitin ligase RNF8 [34,51–54] ubiquitinates H2AX and also seems to shift the recruitment mode from being phosphorylation-based to being ubiquitin-based. In spite of that, many reports indicate that phosphorylation of H2AX is not essential for DNA repair [55,56], suggesting that other molecules can orchestrate the assembly of DNA repair complexes.

Noteworthy, DNA damaging complexes rely on protein modularity associated to posttranslational modifications of binding partners. Posttranslational modifications are also reversible, implying as a consequence, the dynamic nature of any kind of protein–protein interactions depending on such modifications.

Large complexes are so built through specific recognition between posttranslational modifications and decoding domains. However, following DDR progression, posttranslational modifications of proteins, intimately involved in DNA repair, can also be edited by specific enzymes thus arresting the repair process and triggering an alternative pathway leading to cell death. Therefore, phosphatases (PPI) and deubiquitylases (DUB) offer additional levels of complexity required for the fine-tuning of DDR pathways in injured cells.

2.1. DNA damage network

In the biological context most protein and gene networks do not have the topological properties of random networks but are rather characterized by a high clustering coefficient and by a degree distribution that is scale-free [57]. If we restrict our analysis to the DDR interactions, most of the proteins (nodes) have only few edges (connections) whereas few proteins (hubs), such as ATM, or p53 [58] have a vast number of connections. However, the assembly of large complexes in the vicinity of the lesions follows a strictly hierarchical process [59] based on domain modularity and localized concentration of factors.

Recently, the 'phosphorylation landscape' of DDR has been expanded through the identification of novel putative substrates of ATM as well as of some ATM independent substrates [60]. These observations underline the vast complexity of the cellular responses in the DDR pathways necessary to maintain genomic integrity and cellular homeostasis. Rapid kinetics for most of the phosphorylation events suggests the existence of similar temporal patterns also for the dephosphorylation response [60]. Shiloh and colleagues have recently explored such kinetics through analysis of system level networks of perturbed cells [60]. Cells were examined after radiomimetic treatment at distinct time points. The analysis of isolated phosphopeptides, through label-free quantitative LC–mass spectrometry, was carried out to follow dynamics of double strand breaks (DSBs)-induced phosphoproteome. They found that the dynamics of the DDR-induced changes are complex and include both phosphorylation and dephosphorylation processes. These events, involving many interconnected proteins (or complexes), indicate a robust and comprehensive cellular response to DNA damage. One important observation regarding the involvement of phosphatases is that they are serving as shutoff signals of DDR-signaling. Moreover, the authors found that 40% of double strand breaks (DSBs)-induced phosphorylation was not ATM-dependent but is potentially induced by several other kinases. This suggests that, although ATM signaling is associated to DSBs, only a fraction of DSBs repair is ATM-dependent [61]. Interestingly, the data from Shiloh and coworkers indicate that the control of DDR events is based on the sustained activity of ATM over an extended time. This mechanism probably serves to counteract the opposing effects mediated by phosphatases. Prolonged ATM activity may be involved in ensuring its retention at the damaged site where ATM acts as a fuel for the signaling cascade.

Ubiquitylation is also an immediate modification underlying the DDR protein–protein networks. Its interplay with phosphorylation is crucial in damage repair and DNA signaling. Histone decoration by ubiquitin-chains has been recently appreciated, fuelled, in part, by the discovery of enzymes responsible for these modifications [28]. Large complexes allow recognition and setting in motion of mechanisms to mark (through ubiquitin-tags) the sites of lesion for an appropriate response [51–54].

3. Ubiquitin-signaling in DDR

Protein modification by a single ubiquitin moiety can have several diverse outcomes, ranging from the control of endocytosis and intracellular trafficking to the regulation of chromatin structure transcription and DNA damage processing [24,62]. However, the complexity of ubiquitin signaling is achieved through its ability to form chains. Polymeric chains can be built on all of ubiquitin's seven Lys residues. Different linkages of ubiquitin moiety or chains adopting distinct geometries ensure the functional complexity of signaling (i.e. Lys-48 chains are linked to the proteasome degradation, while, linear and Lys-63 chains seem to mediate different functions). Both chains can modulate several pathways related to genome stability [63]. Ubiquitin-chains provide recognition sites for complexes assembly and are necessary for signal propagation. Several types of ubiquitin-binding domains (UBDs) have been recently characterized [64–66]. Notably, recognition can be direct or modulated through binding with other domains necessary to gain specificity toward particular geometries of ubiquitin polymers. To date several ubiquitin-modifications and signal decoding are implicated in regulating DNA repair [67].

3.1. Make ubiquitin signals reversible – dynamics through DUBs

Ubiquitin-decoration is achieved through the sequential cascade of activating (E1), conjugating (E2) and ligating (E3) enzymes; such events can occur through the conjugation of single ubiquitin or polyubiquitin chains (homotypic chains, or heterologous, forked or mixed). The vast variety of ubiquitin-signals is recognized and decoded by dedicated ubiquitin-binding domains. In addition, tight control is maintained by the action of DUBs and by the existence of crosstalk between the ubiquitin-network and other posttranslational modifications. In short, high levels of specificity are achieved through (1) specific E2–E3 pairs, (2) recognition of certain ubiquitin branches mediated by individual UBD and eventually, (3) by a presumed relationship between functional outcomes and distinct ubiquitin species [68]. Fine-tuning of ubiquitin-pathways relies on protein complexes, timely regulated in space, mediated by scaffold proteins or chaperones [69,70]. Targeting of E2–E3 pairs in response to specific stresses is mediated by posttranslational modifications, recognition through surrounding domains and adaptors [68]. Ubiquitin-conjugation can mediate nuclear translocation; it can also impact on protein activity, inducing conformational changes with a positive [71] or negative effect [72]. In some circumstances, phosphorylation directly regulates E3 ligase activity [73,74] or indirectly, controls the timing of ubiquitin-attachment and removal by affecting nuclear translocation of deubiquitylating enzymes (DUB) [75].

How the versatility of ubiquitin-complexes at the site of lesion is accomplished? Six classes of UBDs are involved in the response to DNA damage (UBA, UIM, MIU, UEV, UBM, and UBZ [67]. Their recognition occurs through binding of a hydrophobic motif on ubiquitin and of specific regions on the substrate. Such complexes can be modulated by specific proteases (DUBs). DUB activity is induced through binding with substrate; a further regulation is achieved through posttranslational modifications (phosphorylation, ubiquitin or ubiquitin-like modifications) and/or specific binding to accessory molecules that impinges on substrate recognition and/or subcellular localization [25,68]. USP1 auto-deubiquitination is a remarkable example of DUB regulation in DNA repair [76].

DUBs can be distinguished into five distinct classes depending on their domain structure [25]. Their importance in cellular processes is highlighted by recent reports [77,78]. DUBs operate through cleavage of ubiquitin moiety or ubiquitin-linked chains from a substrate. The DUBs activation impinges on (1) recycling of free ubiquitin for cell homeostasis maintenance, (2) rescuing proteins from degradation, and (3) editing the length or type of ubiquitin-modification. Specific E3–DUB (or E2–DUB) pairs are

crucial for the fine-tuning of ubiquitin-conjugation directly affecting enzymatic activation or proteosomal targeting [79]. Large complexes, formed through ubiquitin receptors (UBDs) or by conjugation with small ubiquitin-like modifier (SUMO), in tandem with DUBs are both required for signaling at damaged sites.

Much of the current understanding of DDR is based on the study of ATM and ATR kinases. One of the earliest events is recruitment and activation of the ATM at the damaged DNA sites through the Mre11–Rad50–Nbs1 (MRN) sensor complex. This event clearly illustrates the crosstalk between the ubiquitin-network and posttranslational modifications of DDR. Within minutes after a DSB generation, ATM phosphorylates histone H2AX to become γ-H2AX. γ-H2AX unleashes a cascade of chromatin modulation and DNA repair events through the recruitment of MDC1 (mediator of DNA damage checkpoint 1) [80]. This is followed by accumulation of two closely related RNF ubiquitin ligases, RNF8–RFN168 [26,52,54,81,82] in tandem with the HECT-domain protein HERC2 [83]. Further recruitment of SUMO-ligase PIAS1 and PIAS4 [84,85] then triggers (and amplifies) binding of ubiquitin and SUMO onto histones near the DNA lesions, allowing local recruitment of important repair factors, including 53BP1 and another ubiquitin ligase, BRCA1 [1].

Moyal et al. have recently reported a direct positive effect of ATM on monoubiquitylation of H2B at damaged sites. They observe that the E3 ubiquitin ligase, a heterodimeric complex of the RING-finger–RFN20/RFN40 is phosphorylated by ATM. This event is required for H2B monoubiquitylation, for timely recruitment of components involved in the two major DSB repair pathways (NHEJ and HR) so facilitating DNA repair via both mechanisms [74]. Interestingly RNF20 is also involved in the recruitment of chromatin-remodeling factor SNF2h independently from H2AX [86]. Depletion of RNF20 impairs resection of DNA ends and recruitment of RAD51 and BCRA1. Cells lacking RNF20 or SNF2h or expressing H2BK120R mutant exhibit pronounced defects in homologous recombination repair (HRR) and an enhanced sensitivity to radiation. Interestingly, the function of RNF20 in HRR can be partially bypassed through forced chromatin relaxation. This suggests that RNF20-mediated H2B ubiquitination at DSBs plays a critical role in HHR through chromatin remodeling [86].

Chromatin modulation is a crucial event of the DNA repair cascade. Nonsense mutations in the RNF168 gene impair retention of 53BP1 and BRCA1 at sites of DSB repair [87]. This finding supports the role of the RNF8–RNF168–HERC2–BRCA1 chromatin ubiquitin-ligase complexes [26,85] for genome integrity. Despite considerable efforts, the precise function of BRCA1 in the DNA damage response remains unclear. In addition, BRCA1 seems to promote homologous recombination. BRCA1 has an ubiquitin-ligase activity, it ubiquitylates CtIP a protein involved in DSB resection [88]. The 53BP1 protein promotes other pathways of repair by blocking resection, whereas the 53BP1 sumoylation by PIAS proteins [83,84] may promote its displacement from DSBs, releasing the barrier to resection.

In short, non-degradative ubiquitylation plays a central role in the DNA damage response. RNF8 and RNF168, in tandem with the E2 ubiquitin conjugating enzyme UBC13 catalyze the formation of Lys-63 linked chains at the DSBs sites to promote their faithful repair. By contrast, OTUB1, an ovarian tumor protease acting as a DUB, counteracts RNF8/RNF168-dependent ubiquitin-chains formation at damaged sites [89]. Interestingly, OTUB1 is not involved in the cleavage of polyubiquitin chains but directly targets UBC13 [77]. For this aspect, OTUB1 is an atypical DUB, that prevents ubiquitin ligation, rather than detaching of bound ubiquitin, and in this way inhibits DNA repair. In addition, OTUB1 is targeted by phosphorylation, thus providing another level of control to modulate its affinity for UBC13. Nakada et al. found that inhibition of OTUB1 expression restores the process of homologous recombination in cells in which ATM kinase is inhibited [90]. Thus, OTUB1 depletion can in principle mitigate DNA-repair defects.

Several DUBs have been reported to affect the 'ubiquitin landscape' present at DNA breaks [68]. UCH37/UCHL1 interacts with chromatin-remodeling complex involved in nucleosome sliding (INO80, inositol-requiring 80) [91]. Other DUB, such as BRCC6 (BRCA1–BRCA2 containing complex subunit 36), may act on the RNF8–UBC13 ubiquitin ligase complex deubiquitylating γH2AX [92]. In addition, DUBs involved in DNA damage signaling are USP1 that targets PCNA (proliferating cell nuclear antigen) [76], FANCD2 and FANCI (the Fanconi anemia proteins) [93,94], and USP3 and USP16 that directly deubiquitylate histone H2A [95,96].

3.2. Defying death after DNA damage: does ubiquitin-signaling set threshold?

The experimental results compiled above suggest that the interplay between pair activities of phosphorylation or dephosphorylation (and also ubiquitination or deubiquitination) is required for the fine-tuning of DDR. It may be part of the reason by which the DDR decay in a timely manner, after damage repair, allows a safety path for the cells. The immediate recruitment of factors to DSBs, and the localized concentration of proteins might be particularly important for signaling amplification and to set threshold levels of DNA damage.

DDR depends on the recruitment of the sensors/transducers to the damaged site. Their activation leads cells to a decision point between survival and death. Which are the mechanisms underlying such a decision? Survival of DNA-injured cells depends on removal of the damage. A logical hypothesis is that the amplification of the signaling cascade has the feasibility to drive cells toward death as a default path if not attenuated.

Why an attenuated activation of c-Abl ends in a survival path in female germ cells? c-Abl presumably affects downstream cascades through phosphorylation of several proteins or substrates of enzymes activated/regulated by c-Abl. Pharmacological inhibition of c-Abl could impact on distinct levels of such signaling. A reasonable hypothesis is that c-Abl activation may impinge directly or indirectly on ubiquitin-signaling of DDR. According to this, a recent report provides evidence that Abl regulate foci formation of protein like 53BP1, TopBP1, RAD51 and BRCA1 following DNA damage [14].

3.3. Working hypothesis

Recent findings from Wang et al. indicate that c-Abl may be necessary for the full activation of ATM and ATR and their respective downstream signaling pathways. According to this, c-Abl phosphorylates ATM, thus amplifying ATM activation and signaling. Phosphorylation events mediated by ATM are, in turn, necessary for recruitment of ubiquitin-related enzymes such as RNF8, RNF20–RNF40 and BMI1 (polycomb group proteins) in proximity of DNA breaks. In particular, BMI1 is involved in DNA damage-induced monoubiquitination of H2A. BMI1 interacts with RING1B (RNF2) to form a heterodimer required for PRC1 mediated histone ubiquitination, thus contributing to efficient HR mediated DNA repair [97]. Loss of BMI1 sensitizes cells to ionizing radiation to the same extent as loss of RNF8. In the absence of BMI1, the recruitment to damaged sites of 53BP1, RAP80 and BRCA1 is strongly impaired [98].

In addition, c-Abl directly may impinge (through phosphorylation or its binding) on several proteins and/or enzymes involved in ubiquitin-signaling of DDR. In line with this, c-Abl interacts with BRCA1 a tumor suppressor crucial for cell-cycle arrest and DNA

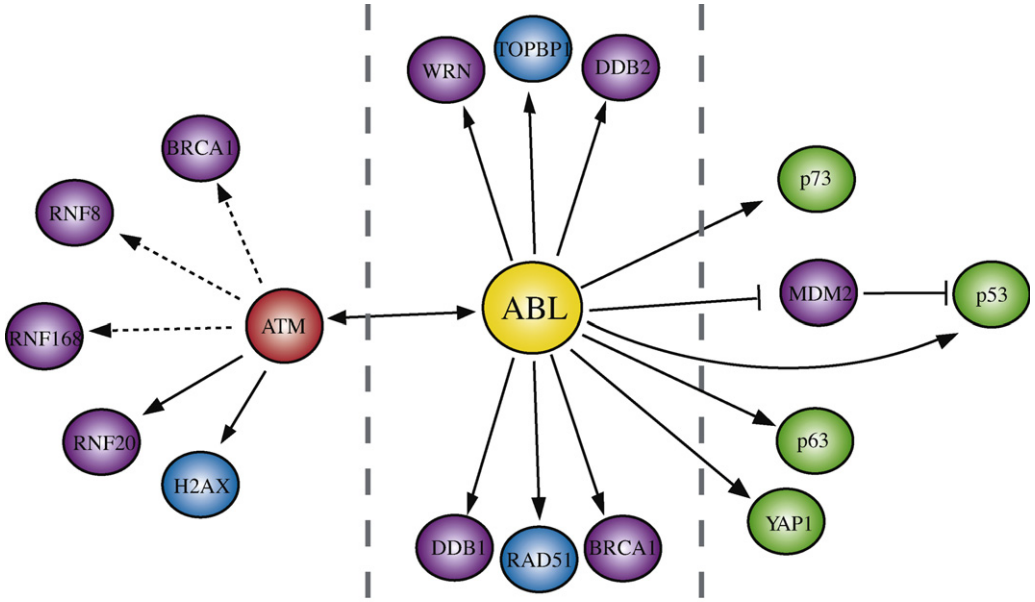

Fig. 3. Model for integrated signaling functions of c-Abl. Abl may regulate double strand breaks repair and/or cell death to damage. The extent of Abl catalytic outcomes seems to shift the balance between life and death. A reasonable hypothesis is that c-Abl presumably affects downstream pathways through phosphorylation of several proteins and/or enzymes involved in ubiquitin-signaling of DDR. Solid line: direct interaction; dashed line: indirect effect; ubiquitin-related proteins are colored in violet; modulators of DNA damage-induced apoptosis are colored in green; DNA repair and DNA signaling proteins are colored in blue.

repair. BRCA1, in complex with another RING-domain BARD1 exhibits ubiquitin-ligase activity. Few targets for this activity have been characterized *in vivo*. The BRCA1/BARD1 can ubiquitylate histones (H2A and H2B) in the context of nucleosome [99]. This suggests that BRCA1 may also affect directly nucleosome structure and dynamics through its ubiquitylation activity. In addition, c-Abl directly phosphorylates ubiquitin-related proteins such as DDB1 [100] (involved in complex with DDB2 in DNA repair through NER mechanism), WRN a helicase containing an UBD domain involved in DNA repair [101], and finally the E3 RING ligase MDM2 [102] (Fig. 3).

MDM2 (along with MDMX) is a part of a multi-component E3-complex that targets p53 for proteasomal degradation [103,104]. Recently, Mayo and colleagues found that multi-site phosphorylation of MDM2 by c-Abl is important for the MDM2–MDMX complex formation [105]. One of the tyrosine residues important for complex formation is proximal to the RING domain of MDM2. This suggests a possible role for this modification in modulating RING domain interactions. Interestingly, RING domain dimerization appears to be a general requirement for the assembly of an active ligase complex [106]. Thus, c-Abl phosphorylation provides a mechanism to regulate ubiquitination by modulating the oligomerization of E3 MDM2–MDMX complexes.

4. Outlook

Several complex cellular responses can be understood only by thinking in terms of a dense web of interactions and feedbacks. Many of the most pressing issues, related to DDR in cells, cannot longer be solved simply by breaking system into parts. Taking few major hubs out of the DNA damage network will simply disassemble it in rather isolated protein–protein connections. Timely series of ubiquitin-modifications and signal decoding are implicated in regulating DNA repair. The current model is that histone ubiquitylation serves as a beacon for the recruitment of effector proteins. Future studies will likely uncover new motifs that recognize single or combinatorial modifications on chromatin. Specific E2–E3 pairs seem to be required for distinct ubiquitin chains, however research is needed to clarify the importance of ubiquitin branching in a physiological context and to identify and characterize more potential DUBs. We need to clarify how different ubiquitin-marks are generated and decoded by UBDs in the cells. We need to know how modifying enzymes are targeted to their site of action and which environmental or metabolic factors affect their activity.

Here, we speculate about some connections occurring between phosphorylation- and ubiquitin-mediated signaling at the damaged sites. Multiple interactions seem to occur between c-Abl (and 'sensor' kinases) with ubiquitin-related proteins involved in DDR. The kinetics of c-Abl activation is certainly an important immediate issue to be addressed. Novel paradigms for DDR may arise from a better understanding of the crosstalk between phosphorylation signals mediated by c-Abl and ubiquitin-related changes on chromatin.

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References

[1] Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature 2009;461:1071–8.
[2] Lavin MF, Kozlov S. ATM activation and DNA damage response. Cell Cycle 2007;6:931–42.
[3] Harper JW, Elledge SJ. The DNA damage response: ten years after. Mol Cell 2007;28:739–45.
[4] Shaul Y, Ben-Yehoyada M. Role of c-Abl in the DNA damage stress response. Cell Res 2005;15:33–5.
[5] Colicelli J. ABL tyrosine kinases: evolution of function, regulation, and specificity. Sci Signal 2010;3:re6.

[6] Gu JJ, Ryu JR, Pendergast AM. Abl tyrosine kinases in T-cell signaling. Immunol Rev 2009;228:170–83.
[7] Sirvent A, Benistant C, Roche S. Cytoplasmic signaling by the c-Abl tyrosine kinase in normal and cancer cells. Biol Cell 2008;100:617–31.
[8] Zhu J, Wang JY. Death by Abl: a matter of location. Curr Top Dev Biol 2004;59:165–92.
[9] Pendergast AM. The Abl family kinases: mechanisms of regulation and signaling. Adv Cancer Res 2002;85:51–100.
[10] Liu ZG, Baskaran R, Lea-Chou ET, Wood LD, Chen Y, Karin M, et al. Three distinct signaling responses by murine fibroblasts to genotoxic stress. Nature 1996;384:273–6.
[11] Kharbanda S, Ren R, Pandey P, Shafman TD, Feller SM, Weichselbaum RR, et al. Activation of the c-Abl tyrosine kinase in the stress response to DNA-damaging agents. Nature 1995;376:785–8.
[12] Meltser V, Ben-Yehoyada M, Shaul Y. c-Abl tyrosine kinase in the DNA damage response: cell death and more. Cell Death Differ 2011;18:2–4.
[13] Gonfloni S. DNA damage stress response in germ cells: role of c-Abl and clinical implications. Oncogene 2010;29:6193–202.
[14] Wang X, Zeng L, Wang J, Chau JF, Lai KP, Jia D, et al. A positive role for c-Abl in ATM and ATR activation in DNA damage response. Cell Death Differ 2011;18:5–15.
[15] Gonfloni S. Modulating c-Abl nuclear activity as a strategy to preserve female fertility. Cell Cycle 2010;9:217–8.
[16] Gonfloni S, Di Tella L, Caldarola S, Cannata SM, Klinger FG, Di Bartolomeo C, et al. Inhibition of the c-Abl-TAp63 pathway protects mouse oocytes from chemotherapy-induced death. Nat Med 2009;15:1179–85.
[17] Woodruff TK. Preserving fertility during cancer treatment. Nat Med 2009;15:1124–5.
[18] Lusser A, Kadonaga JT. Chromatin remodeling by ATP-dependent molecular machines. Bioessays 2003;25:1192–200.
[19] Kouzarides T. Chromatin modifications and their function. Cell 2007;128:693–705.
[20] Marmorstein R. Protein modules that manipulate histone tails for chromatin regulation. Nat Rev Mol Cell Biol 2001;2:422–32.
[21] van Attikum H, Gasser SM. The histone code at DNA breaks: a guide to repair? Nat Rev Mol Cell Biol 2005;6:757–65.
[22] Panier S, Durocher D. Regulatory ubiquitylation in response to DNA double strand breaks. DNA Repair (Amst) 2009;8:436–43.
[23] van Attikum H, Gasser SM. Crosstalk between histone modifications during the DNA damage response. Trends Cell Biol 2009;19:207–17.
[24] Ulrich HD, Walden H. Ubiquitin signaling in DNA replication and repair. Nat Rev Mol Cell Biol 2010;11:479–89.
[25] Atanassov BS, Koutelou E, Dent SY. The role of deubiquitinating enzymes in chromatin regulation. FEBS Lett 2010.
[26] Al-Hakim A, Escribano-Diaz C, Landry MC, O'Donnell L, Panier S, Szilard RK, et al. The ubiquitous role of ubiquitin in the DNA damage response. DNA Repair (Amst) 2010;9:1229–40.
[27] Dianov GL. Regulation of DNA repair by ubiquitination. Biochemistry (Moscow) 2011;76:69–79.
[28] Ramaekers CH, Wouters BG. Regulatory functions of ubiquitin in diverse DNA damage responses. Curr Mol Med 2011;11:152–69.
[29] Lukas J. The interface between the ubiquitin family and the DNA damage response. EMBO Rep 2010;11:907–9.
[30] Shanbhag NM, Rafalska-Metcalf IU, Balane-Bolivar C, Janicki SM, Greenberg RA. ATM-dependent chromatin changes silence transcription in cis to DNA double strand breaks. Cell 2010;141:970–81.
[31] Rich T, Allen RL, Wyllie AH. Defying death after DNA damage. Nature 2000;407:777–83.
[32] Lukas J, Bartek J. DNA repair: new tales of an old tail. Nature 2009;458:581–3.
[33] Boulton SJ. DNA repair: decision at the break point. Nature 2010;465:301–2.
[34] Bennett EJ, Harper JW. DNA damage: ubiquitin marks the spot. Nat Struct Mol Biol 2008;15:20–2.
[35] McHugh PJ, Spanswick VJ, Hartley JA. Repair of DNA interstrand crosslinks: molecular mechanisms and clinical relevance. Lancet Oncol 2001;2:483–90.
[36] Stojic L, Brun R, Jiricny J. Mismatch repair and DNA damage signaling. DNA Repair (Amst) 2004;3:1091–101.
[37] Rastogi RP, Richa, Kumar A, Tyagi MB, Sinha RP. Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. J Nucleic Acids 2010;592980.
[38] Reed SH. Nucleotide excision repair in chromatin: damage removal at the drop of a HAT. DNA Repair (Amst), doi:10.1016/j.dnarep.2011.04.029.
[39] Mladenov E, Iliakis G. Induction and repair of DNA double strand breaks: the increasing spectrum of non-homologous end joining pathways. Mutat Res 2011;711:61–72.
[40] Ozturk S, Demir N. DNA repair mechanisms in mammalian germ cells. Histol Histopathol 2011;26:505–17.
[41] Meltser V, Ben-Yehoyada M, Reuven N, Shaul Y. c-Abl downregulates the slow phase of double-strand break repair. Cell Death Dis 2010;1:e20.
[42] Amrein L, Davidson D, Shawi M, Petruccelli LA, Miller Jr WH, Aloyz R, et al. Dual inhibition of the homologous recombinational repair and the nonhomologous end-joining repair pathways in chronic lymphocytic leukemia therapy. Leuk Res 2011;35(8). doi: 10.1016/j.leukres.2011.01.004.
[43] Sun H, Treco D, Schultes NP, Szostak JW. Double strand breaks at an initiation site for meiotic gene conversion. Nature 1989;338:87–90.
[44] Kharbanda S, Pandey P, Morris PL, Whang Y, Xu Y, Sawant S, et al. Functional role for the c-Abl tyrosine kinase in meiosis I. Oncogene 1998;16:1773–7.
[45] Bartkova J, Horejsi Z, Koed K, Kramer A, Tort F, Zieger K, et al. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature 2005;434:864–70.
[46] Gorgoulis VG, Vassiliou LV, Karakaidos P, Zacharatos P, Kotsinas A, Liloglou T, et al. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 2005;434:907–13.
[47] d'Adda di Fagagna F. Living on a break: cellular senescence as a DNA-damage response. Nat Rev Cancer 2008;8:512–22.
[48] Morita Y, Perez GI, Paris F, Miranda SR, Ehleiter D, Haimovitz-Friedman A, et al. Oocyte apoptosis is suppressed by disruption of the acid sphingomyelinase gene or by sphingosine-1-phosphate therapy. Nat Med 2000;6:1109–14.
[49] Hofmann K, Bucher P. The FHA domain: a putative nuclear signaling domain found in protein kinases and transcription factors. Trends Biochem Sci 1995;20:347–9.
[50] Bork P, Hofmann K, Bucher P, Neuwald AF, Altschul SF, Koonin EV. A superfamily of conserved domains in DNA damage-responsive cell cycle checkpoint proteins. FASEB J 1997;11:68–76.
[51] Wang B, Elledge SJ. UBC13/RNF8 ubiquitin ligases control foci formation of the RAP80/ABRAXAS/BRCA1/BRCC36 complex in response to DNA damage. Proc Natl Acad Sci USA 2007;104:20759–63.
[52] Kolas NK, Chapman JR, Nakada S, Ylanko J, Chahwan R, Sweeney FD, et al. Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. Science 2007;318:1637–40.
[53] Huen MS, Grant R, Manke I, Minn K, Yu X, Yaffe MB, et al. RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. Cell 2007;131:901–14.
[54] Mailand N, Bekker-Jensen S, Faustrup H, Melander F, Bartek J, Lukas C, et al. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. Cell 2007;131:887–900.
[55] Celeste A, Fernandez-Capetillo O, Kruhlak MJ, Pilch DR, Staudt DW, Lee A, et al. Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. Nat Cell Biol 2003;5:675–9.
[56] Yuan J, Adamski R, Chen J. Focus on histone variant H2AX: to be or not to be. FEBS Lett 2010;584:3717–24.
[57] Barabasi AL, Bonabeau E. Scale-free networks. Sci Am 2003;288:60–9.
[58] Collavin L, Lunardi A, Del Sal G. p53-Family proteins and their regulators: hubs and spokes in tumor suppression. Cell Death Differ 2010;17:901–11.
[59] Seebacher J, Gavin AC. SnapShot: protein–protein interaction networks. Cell 2011;144(1000):e1.
[60] Bensimon A, Schmidt A, Ziv Y, Elkon R, Wang SY, Chen DJ, et al. ATM-dependent and -independent dynamics of the nuclear phosphoproteome after DNA damage. Sci Signal 2010;3:rs3.
[61] Riballo E, Kuhne M, Rief N, Doherty A, Smith GC, Recio MJ, et al. A pathway of double strand break rejoining dependent upon ATM, Artemis, and proteins locating to gamma-H2AX foci. Mol Cell 2004;16:715–24.
[62] Messick TE, Greenberg RA. The ubiquitin landscape at DNA double-strand breaks. J Cell Biol 2009;187:319–26.
[63] Chen ZJ, Sun LJ. Nonproteolytic functions of ubiquitin in cell signaling. Mol Cell 2009;33:275–86.
[64] Dikic I, Wakatsuki S, Walters KJ. Ubiquitin-binding domains – from structures to functions. Nat Rev Mol Cell Biol 2009;10:659–71.
[65] Harper JW, Schulman BA. Structural complexity in ubiquitin recognition. Cell 2006;124:1133–6.
[66] Hicke L, Schubert HL, Hill CP. Ubiquitin-binding domains. Nat Rev Mol Cell Biol 2005;6:610–21.
[67] Hofmann K. Ubiquitin-binding domains and their role in the DNA damage response. DNA Repair (Amst) 2009;8:544–56.
[68] Grabbe C, Husnjak K, Dikic I. The spatial and temporal organization of ubiquitin networks. Nat Rev Mol Cell Biol 2011;12:295–307.
[69] Avvakumov N, Nourani A, Cote J. Histone chaperones: modulators of chromatin marks. Mol Cell 2011;41:502–14.
[70] Ransom M, Dennehey BK, Tyler JK. Chaperoning histones during DNA replication and repair. Cell 2010;140:183–95.
[71] Rabut G, Peter M. Function and regulation of protein neddylation. Protein modifications: beyond the usual suspects' review series. EMBO Rep 2008;9:969–76.
[72] Meulmeester E, Melchior F. Cell biology: SUMO. Nature 2008;452:709–11.
[73] Gallagher E, Gao M, Liu YC, Karin M. Activation of the E3 ubiquitin ligase itch through a phosphorylation-induced conformational change. Proc Natl Acad Sci USA 2006;103:1717–22.
[74] Moyal L, Lerenthal Y, Gana-Weisz M, Mass G, So S, Wang SY, et al. Requirement of ATM-dependent monoubiquitylation of histone H2B for timely repair of DNA double strand breaks. Mol Cell 2011;41:529–42.
[75] Pastori V, Sangalli E, Coccetti P, Pozzi C, Nonnis S, Tedeschi G, et al. CK2 and GSK3 phosphorylation on S29 controls wild-type ATXN3 nuclear uptake. Biochim Biophys Acta 2010;1802:583–92.
[76] Huang TT, Nijman SM, Mirchandani KD, Galardy PJ, Cohn MA, Haas W, et al. Regulation of monoubiquitinated PCNA by DUB autocleavage. Nat Cell Biol 2006;8:339–47.
[77] Blackford AN, Stewart GS. When cleavage is not attractive: non-catalytic inhibition of ubiquitin chains at DNA double strand breaks by OTUB1. DNA Repair (Amst) 2010;10:245–9.
[78] Sowa ME, Bennett EJ, Gygi SP, Harper JW. Defining the human deubiquitinating enzyme interaction landscape. Cell 2009;138:389–403.
[79] Wilkinson KD. DUBs at a glance. J Cell Sci 2009;122:2325–9.

[80] Bartek J, Lukas J. DNA damage checkpoints: from initiation to recovery or adaptation. Curr Opin Cell Biol 2007;19:238–45.
[81] Doil C, Mailand N, Bekker-Jensen S, Menard P, Larsen DH, Pepperkok R, et al. RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. Cell 2009;136:435–46.
[82] Ramachandran S, Chahwan R, Nepal RM, Frieder D, Panier S, Roa S, et al. The RNF8/RNF168 ubiquitin ligase cascade facilitates class switch recombination. Proc Natl Acad Sci USA 2010;107:809–14.
[83] Galanty Y, Belotserkovskaya R, Coates J, Polo S, Miller KM, Jackson SP. Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double strand breaks. Nature 2009;462:935–9.
[84] Morris JR, Boutell C, Keppler M, Densham R, Weekes D, Alamshah A, et al. The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. Nature 2009;462:886–90.
[85] Bartek J, Hodny Z. SUMO boosts the DNA damage response barrier against cancer. Cancer Cell 2010;17:9–11.
[86] Nakamura K, Kato A, Kobayashi J, Yanagihara H, Sakamoto S, Oliveira DV, et al. Regulation of homologous recombination by RNF20-dependent H2B ubiquitination. Mol Cell 2011;41:515–28.
[87] Devgan SS, Sanal O, Doil C, Nakamura K, Nahas SA, Pettijohn K, et al. Homozygous deficiency of ubiquitin-ligase ring-finger protein RNF168 mimics the radiosensitivity syndrome of ataxia-telangiectasia. Cell Death Differ 2011.
[88] Yu X, Fu S, Lai M, Baer R, Chen J. BRCA1 ubiquitinates its phosphorylation-dependent binding partner CtIP. Genes Dev 2006;20:1721–6.
[89] Rose A, Schlieker C. DNA repair: blocking ubiquitin transfer. Nature 2010;466:929–30.
[90] Nakada S, Tai I, Panier S, Al-Hakim A, Iemura S, Juang YC, et al. Non-canonical inhibition of DNA damage-dependent ubiquitination by OTUB1. Nature 2010;466:941–6.
[91] Yao T, Song L, Jin J, Cai Y, Takahashi H, Swanson SK, et al. Distinct modes of regulation of the Uch37 deubiquitinating enzyme in the proteasome and in the Ino80 chromatin-remodeling complex. Mol Cell 2008;31:909–17.
[92] Shao G, Lilli DR, Patterson-Fortin J, Coleman KA, Morrissey DE, Greenberg RA. The RAP80–BRCC36 de-ubiquitinating enzyme complex antagonizes RNF8–UBC13-dependent ubiquitination events at DNA double strand breaks. Proc Natl Acad Sci USA 2009;106:3166–71.
[93] Nijman SM, Huang TT, Dirac AM, Brummelkamp TR, Kerkhoven RM, D'Andrea AD, et al. The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. Mol Cell 2005;17:331–9.
[94] Sims AE, Spiteri E, Sims 3rd RJ, Arita AG, Lach FP, Landers T, et al. FANCI is a second monoubiquitinated member of the Fanconi anemia pathway. Nat Struct Mol Biol 2007;14:564–7.
[95] Nicassio F, Corrado N, Vissers JH, Areces LB, Bergink S, Marteijn JA, et al. Human USP3 is a chromatin modifier required for S phase progression and genome stability. Curr Biol 2007;17:1972–7.
[96] Joo HY, Zhai L, Yang C, Nie S, Erdjument-Bromage H, Tempst P, et al. Regulation of cell cycle progression and gene expression by H2A deubiquitination. Nature 2007;449:1068–72.
[97] Ginjala V, Nacerddine K, Kulkarni A, Oza J, Hill SJ, Yao M, et al. BMI1 is recruited to DNA breaks and contributes to DNA damage-induced H2A ubiquitination and repair. Mol Cell Biol 2011;31:1972–82.
[98] Ismail IH, Andrin C, McDonald D, Hendzel MJ. BMI1-mediated histone ubiquitylation promotes DNA double strand break repair. J Cell Biol 2010;191:45–60.
[99] Thakar A, Parvin JD, Zlatanova J. BRCA1/BARD1 E3 ubiquitin ligase can modify histones H2A and H2B in the nucleosome particle. J Biomol Struct Dyn 2010;27:399–406.
[100] Wang H, Zhai L, Xu J, Joo HY, Jackson S, Erdjument-Bromage H, et al. Histone H3 and H4 ubiquitylation by the CUL4-DDB-ROC1 ubiquitin ligase facilitates cellular response to DNA damage. Mol Cell 2006;22:383–94.
[101] Cheng WH, von Kobbe C, Opresko PL, Fields KM, Ren J, Kufe D, et al. Werner syndrome protein phosphorylation by abl tyrosine kinase regulates its activity and distribution. Mol Cell Biol 2003;23:6385–95.
[102] Goldberg Z, Vogt Sionov R, Berger M, Zwang Y, Perets R, Van Etten RA, et al. Tyrosine phosphorylation of MDM2 by c-Abl: implications for p53 regulation. EMBO J 2002;21:3715–27.
[103] Gu J, Kawai H, Nie L, Kitao H, Wiederschain D, Jochemsen AG, et al. Mutual dependence of MDM2 and MDMX in their functional inactivation of p53. J Biol Chem 2002;277:19251–4.
[104] Waning DL, Lehman JA, Batuello CN, Mayo LD. Controlling the MDM2–MDMX-p53 circuit. Pharmaceuticals (Basel) 2010;3:1576–93.
[105] Waning DL, Lehman JA, Batuello CN, Mayo LD. c-Abl phosphorylation of MDM2 facilitates MDM2–MDMX complex formation. J Biol Chem 2011;286:216–22.
[106] Kentsis A, Gordon RE, Borden KL. Control of biochemical reactions through supramolecular RING domain self-assembly. Proc Natl Acad Sci USA 2002;99:15404–9.