

Multifaceted regulation and functions of 53BP1 in NHEJ-mediated DSB repair (Review)

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Abstract. The repair of DNA double-strand breaks (DSBs) is crucial for the preservation of genomic integrity and the maintenance of cellular homeostasis. Non-homologous DNA end joining (NHEJ) is the predominant repair mechanism for any type of DNA DSB during the majority of the cell cycle. NHEJ defects regulate tumor sensitivity to ionizing radiation and anti-neoplastic agents, resulting in immunodeficiencies and developmental abnormalities in malignant cells. p53-binding protein 1 (53BP1) is a key mediator involved in DSB repair, which functions to maintain a balance in the repair pathway choices and in preserving genomic stability. 53BP1 promotes DSB repair via NHEJ and antagonizes DNA end overhang resection. At present, novel lines of evidence have revealed the molecular mechanisms underlying the recruitment of 53BP1 and DNA break-responsive effectors to DSB sites, and the promotion of NHEJ-mediated DSB repair via 53BP1, while preventing homologous recombination. In the present review article, recent advances made in the elucidation of the structural and functional characteristics of 53BP1, the mechanisms of 53BP1 recruitment and interaction with the reshaping of the chromatin architecture around DSB sites, the post-transcriptional modifications of 53BP1, and the up- and downstream pathways of 53BP1 are discussed. The present review article also focuses on the application perspectives, current challenges and future directions of 53BP1 research.

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5. Downstream effectors of 53BP1 in NHEJ repair
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1. Introduction

Every eukaryotic cell contends with various intracellular and extracellular threats during DNA replication and cellular metabolism, such as high-energy radiation, mutagenic chemicals, free radicals and V(D)J recombination, as well as cell type-specific challenges, such as immunoglobulin class-switching recombination (CSR) in B-lymphocytes (1,2). Failure to repair a DNA double-strand break (DSB) or restart replication forks results in cell death, whereas DSB mis-repair and catastrophic genome rearrangements are the major causes of genomic instability and hence, carcinogenesis (3,4). Thus, the fidelity and capacity of DSB repair needs to be clearly elucidated. To date, four conserved and mechanistically distinct pathways have been identified to be involved in the elimination of DSBs from the genome: Homologous recombination (HR), non-homologous end joining (NHEJ), alternative end joining (altEJ) and single-strand annealing (SSA) (5). HR and NHEJ are the two major DNA-repair pathways.

HR is the most accurate DSB repair mechanism and also the default mechanism for replication fork repairs. HR occurs following DSB end resection, which removes a few hundred or more bases from the 5'-terminated strand to yield a 3' single-stranded DNA (ssDNA) tail, and this is achieved via the MRE11-RAD50-NBS1 (MRN) complex (6). The ssDNA invades the template (the adjacent sister chromatid of 3' overhangs) and this is mediated by the recombinase Rad51, whereafter it displaces an intact strand to form a D-loop and produces double Holliday junctions (7). However, since the HR machinery requires an identical DNA template in the homologous sister chromatid for DSB repair, it is most active in the mid-S phase and mid-G2 phase of the cell cycle (8). altEJ was the second method to be identified, and this is mediated by the microhomology of the 3' ssDNA originating from end resection. In altEJ, DNA polymerase θ (Pol θ)-associated helicase activity can displace the ssDNA-binding protein,

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while its polymerase activity can stabilize the joint between the two DNA ends (9). Due to its apparent proclivity for connecting DSBs on different chromosomes, the usage of altEJ for DSB repair has negative ramifications for genomic integrity, resulting in chromosomal translocations and mutagenic rearrangements (10). Third to be discovered was SSA, which is considered to be an obligatorily error-prone pathway. At the cost of deletion of the intervening sequences between the repeats, SSA joins two homologous 3' ssDNA ends (for example, at tandem repeats) through annealing (11). Notably, both altEJ and SSA require DNA end resection, and they are also primarily operational in the S and G2 phases of the cell cycle (12). The error-prone DSB repair pathways of alt-EJ and SSA operate in different biological contexts and contribute to genome rearrangements and oncogenic transformation, but do not serve as main DNA-repair pathways. Alt-NHEJ and SSA are two additional DSB repair mechanisms that primarily serve as backups when c-NHEJ and HR fail (13). In comparison, NHEJ is a relatively simple repair process and remains active throughout the entirety of the cell cycle, but is dominant in G0/G1 and G2 phases of the cell cycle (14). NHEJ takes place substantially at a more rapid rate than HR (several hours), lasting ~30 min and accounting for >75% of repair events, while HR repairs the remaining 25%, according to fluorescent reporter structures integrated into the chromosomes of human cell lines (15). NHEJ repair involves the binding of the ring-shaped Ku70/80 heterodimer to DSB ends and the recruitment of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) to create the DNA-PK complex. DSBs are then ligated by a complex involving DNA ligase IV and its associated factors [e.g., X-ray repair cross complementing protein 4 (XRCC4) and XRCC4-like factor (XLF)] (16,17). Although NHEJ remains active throughout the cell cycle, NHEJ can be inhibited by breast cancer type 1 susceptibility protein (BRCA1) and other HR-related molecules if DSBs contain 5' or 3' overhangs (18). As opposed to HR, altEJ and SSA, which require a 3' ssDNA tail, NHEJ acts first to attempt to repair DSBs and is the only DSB repair pathway active in the G0 and G1 phases (14). Even within the G2 phase, NHEJ also repairs ≥80% of ionizing radiation-induced DSBs (19,20). In general, when the DSB ends are 'clean' (have compatible or blunt ends), NHEJ is rapid, efficient, yet mutagenic and is often accompanied by only short deletions and fewer base changes. As the predominant DSB repair pathway in mammalian cells, NHEJ deficiency can influence tumor sensitivity to ionizing radiation and antineoplastic, and it can also cause immunodeficiencies and other developmental abnormalities, including dwarfism and defective neurogenesis associated with microcephaly (21).

Two key players in the DSB repair process are tumor protein 53-binding protein 1 (53BP1) that promotes NHEJ by antagonizing DNA end overhang resection and BRCA1 that promotes HR by promoting end-resection (22). In response to DSBs, 53BP1 rapidly accumulates on the chromatin surrounding the DNA damage site to form the irradiation-induced foci (IRIF), which is driven by a signaling cascade that originates with the ataxia-telangiectasia mutated (ATM) kinase-mediated phosphorylation of H2A histone family member X (H2AX; known as γ H2AX) (23,24). Similar to ATM deficiency (ATM^{-/-}), defective DNA damage responses (DDRs) following treatment with ionizing radiation occur in 53BP1^{-/-} cells, and 53BP1^{-/-} mice

exhibit growth retardation, immune deficiency, increased radiation sensitivity and an increased risk of developing cancers (25). For several decades, 53BP1 has been described as a regulator and scaffold for DSB signaling, which functions by recruiting other responsive proteins to DNA damage sites to facilitate the NHEJ repair process. Therefore, the identification of 53BP1 binding and the proteins it interacts with has become an increasingly studied topic in an attempt to uncover the biological functions of 53BP1-dependent NHEJ repair.

In the present review article, the structure, functional characteristics and post-transcriptional modifications (PTMs) of 53BP1 in the process of response to DSBs are discussed. Progress on the identification of 53BP1 assembly and recruitment to DSB sites, with a particular focus on the interactions of 53BP1 and the reshaping of the chromatin architecture around DSB sites is reviewed. The role of upstream factors in regulating 53BP1 recruitment, and the mechanisms through which 53BP1 interacts with the downstream responsive effectors involved in the NHEJ signaling pathways is also discussed. The present review also sheds light on the challenges that remain to be overcome and the potential roles of 53BP1 in cancer treatment and CRISPR/Cas9-induced HR repair, providing a theoretical basis for the further study of 53BP1.

2. The structural characteristics of 53BP1

Human 53BP1 has 1,972 amino acids, a mass of ≥200 kDa, and is encoded by the TP53BP1 gene that is located on human chromosome 15q15-12 (26,27). As a large scaffolding protein that mediates the interactions with modified histones and several effector proteins, 53BP1 consists of multiple interaction surfaces for the DSB-response. Pivotal structural regions of 53BP1 include the N-terminal region (1-1,220 aa), minimal focus forming region (1,220-1,711 aa) and the C-terminal region (1,712-1,972 aa) (28). The 53BP1 N-terminal region contains 28 amino-terminal Ser/Thr-Gln sites that are involved in the interactions with the Pax transactivation domain-interacting protein (PTIP) and RAP1-interacting factor 1 (RIF1) (29). The ability of 53BP1 to form IRIF is attributable to its minimal focus forming region. This region includes two dynein light chain (LC8) binding domains that bind to dynein light chain 1 to promote 53BP1 oligomerization and recruitment (30-32), an oligomerization domain that mediates 53BP1 dimer and multimer formation and recruitment to a DSB (33,34), a glycine-arginine-rich (GAR) motif that is methylated by the protein arginine N-methyltransferase 1 (PRMT1) to enhance DNA-binding function (34,35), two tandem Tudor domains that bind to H4K20me2 (36,37) and a ubiquitylation-dependent recruitment (UDR) motif that interacts with H2AK15ub (38). The 53BP1 C-terminal region contains two BRCA1 carboxyl-terminal (BRCT) domains that interact with p53 and γ H2AX, which is important for DSB repair in heterochromatin (39,40). Overall, all interaction domains of 53BP1 are indispensable for DSB repair in heterochromatin; however, the contribution of these domains varies when the context of DSB repair is altered (Fig. 1).

3. The 53BP1-mediated NHEJ pathway

Interplay between 53BP1 and other primary factors involved in NHEJ repair. Canonical DNA (c-)NHEJ is the major

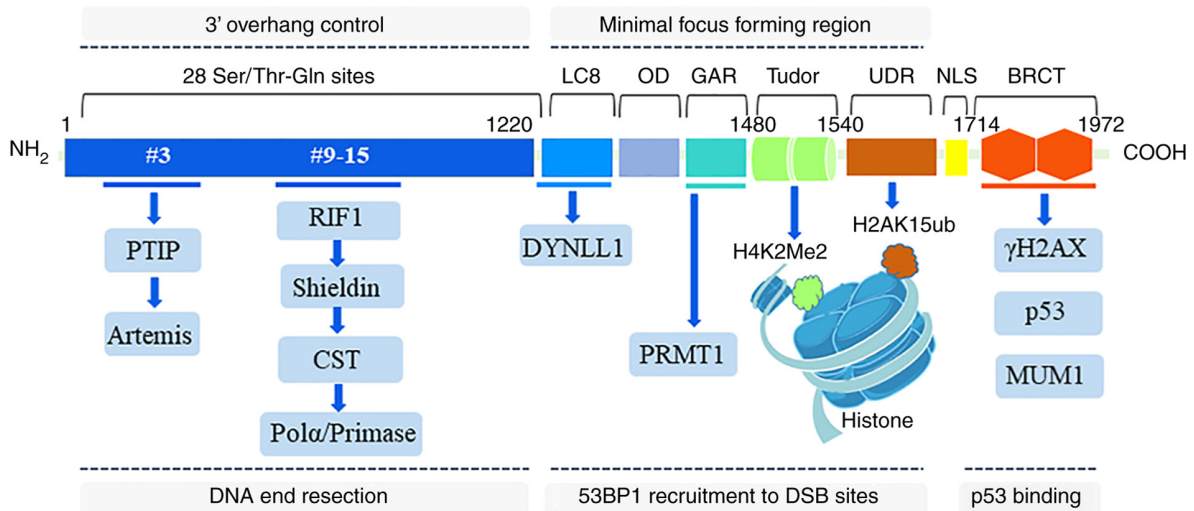


Figure 1. Domain structures and functions of 53BP1. Recruitment of 53BP1 to DSB sites requires the minimal focus forming region, comprising the OD, the GAR motif, the tandem Tudor domain, the UDR motif, and the LC8 binding domain. The N-terminal S-T/Q phosphorylation sites mediate interactions with PTIP and RIF1/Shieldin/CST/Polα/Primase axis, which control DNA end resection. The C-terminal includes two BRCT domains connected in series, which are 53BP1 interacting with other proteins in a phosphorylation-independent pathway, such as p53 and MUM1 (or EXPAND1). 53BP1; p53-binding protein 1; DSB, double-strand break; OD, oligomerization domain; GAR, glycine-arginine-rich; UDR, ubiquitin-dependent recognition; LC8, dynein light chain; PTIP, Pax transactivation domain-interacting protein; BRCT, breast cancer type 1 susceptibility protein carboxyl-terminal; RIF1, RAP1-interacting factor 1; MUM1, mutated melanoma-associated antigen 1; CST, CTC1-STN1-TEN1; Polα, polymerase-α; DYNLL1, dynein light chain 1; PRMT1, protein arginine N-methyltransferase 1; H2AX, H2A histone family member X.

DSB repair pathway in mammalian cells due to its ability to function in all phases of the cell cycle. c-NHEJ is a rapid kinetics-based repair process involving the binding of the Ku heterodimer (Ku70/Ku80) to dsDNA ends, the recruitment of the DNA-PKcs to create the DNA-PK complex, and the DSB end ligation by XRCC4, XLF and DNA ligase IV (LIG4) (41,42). Concomitant with DNA-PK binding to DSB sites, the MRN (MRE11, RAD50 and NBS1) complex is also located in the same region, and recruits ATM, which phosphorylates it (43). ATM amplifies the damage signal continuously via phosphorylation of the histone H2A variant (H2AX; the Ser139 phosphorylated state is termed γH2AX) (44). γH2AX is located at DSB sites and recruits the mediator of DNA damage checkpoint protein 1 (MDC1) through a protein interaction network, and then E3 ubiquitin ligase ring finger protein (RNF)8 and RNF168 are recruited by MDC1 (45,46). RNF8 and RNF168 cooperate with E2 ubiquitin-conjugating enzyme to ubiquitinate chromatin around DSB sites. The histone H2A, serving as a key substrate of RNF168, is ubiquitinated at Lys13 and Lys15 (H2AK13ub/15ub) (47). RNF8/RNF168-dependent ubiquitination can produce a specific region on chromatin to allow ubiquitin-dependent DSB-responding proteins (such as 53BP1) to gather and generate IRIF (48,49). 53BP1 binds to residues of H2AK15ub and H4K20me2 to form 53BP1 foci via its UDR motif and Tudor domain, respectively (50). Although methylation transferase may not be the primary driving force for the selective recruitment of 53BP1, the space-time exposure of ubiquitin-regulated H4K20me2 modification is a vital factor mediating the accurate position of 53BP1 (51). The lethal 3 malignant brain tumor-like protein 1 (L3MBTL1) and Jumonji domain-containing protein 2A (JMJD2A or KDM4A) competitively bind to H4K20me2; thus this molecular marker is 'buried' under physiological conditions. Following the occurrence of a DSB, RNF8/NF168-mediated ubiquitination

modification can rapidly degrade these competitive proteins and promote the stable binding of the 53BP1 Tudor domain with H4K20Me2 (52,53). Additionally, point mutations of the UDR motif (I1617A, L1619A, N1621A, L1622A and R1627A) hinder 53BP1 recruitment by inhibiting the binding of 53BP1 to H2AK15ub; however, it does not affect the binding of 53BP1 to H4K20me2 (38). This suggests that RNF168-mediated H4K20me2 competitive protein degradation and H2A ubiquitin modification are mutually independent for 53BP1 recruitment. In a phosphorylation-independent pathway, 53BP1 serves as a scaffold protein inducing mutated melanoma-associated antigen 1 (MUM1 or EXPAND1) to anchor at DSB sites through its BRCT domains (54). Disrupting the nuclear localization of MUM1 leads to a decrease in DNA damage repair efficiency. As the primary downstream molecules, RIF1 and PTIP interact with 53BP1 N-terminal Ser/Thr-Gln sites in an ATM-dependent phosphorylated manner (55,56) (Fig. 2A).

53BP1 reshapes the chromatin architecture around the DSB sites to promote NHEJ repair. The tridimensional organization of chromatin in the nuclear space controls 53BP1 foci accumulation, and the formation of 53BP1 foci may in turn affect chromatin organization in the vicinity of DSBs (Fig. 2B). Xie *et al* (57) found that following DNA damage induced by camptothecin, microorchidia family CW-type zinc finger protein 2 (MORC2), an ATPase-dependent chromatin remodeling enzyme, can form a homodimer through its C-terminal coiled-coil (CC) domain. The homodimer is required for nucleosome destabilization after DNA damage by promoting the recruitment of the DNA repair proteins, BRCA1, 53BP1 and Rad51, to sites of DNA damage. This suggests that the decondensation of the highly compacted chromatin architecture is essential for efficient DNA repair. Using single molecule localization microscopy (STORM),

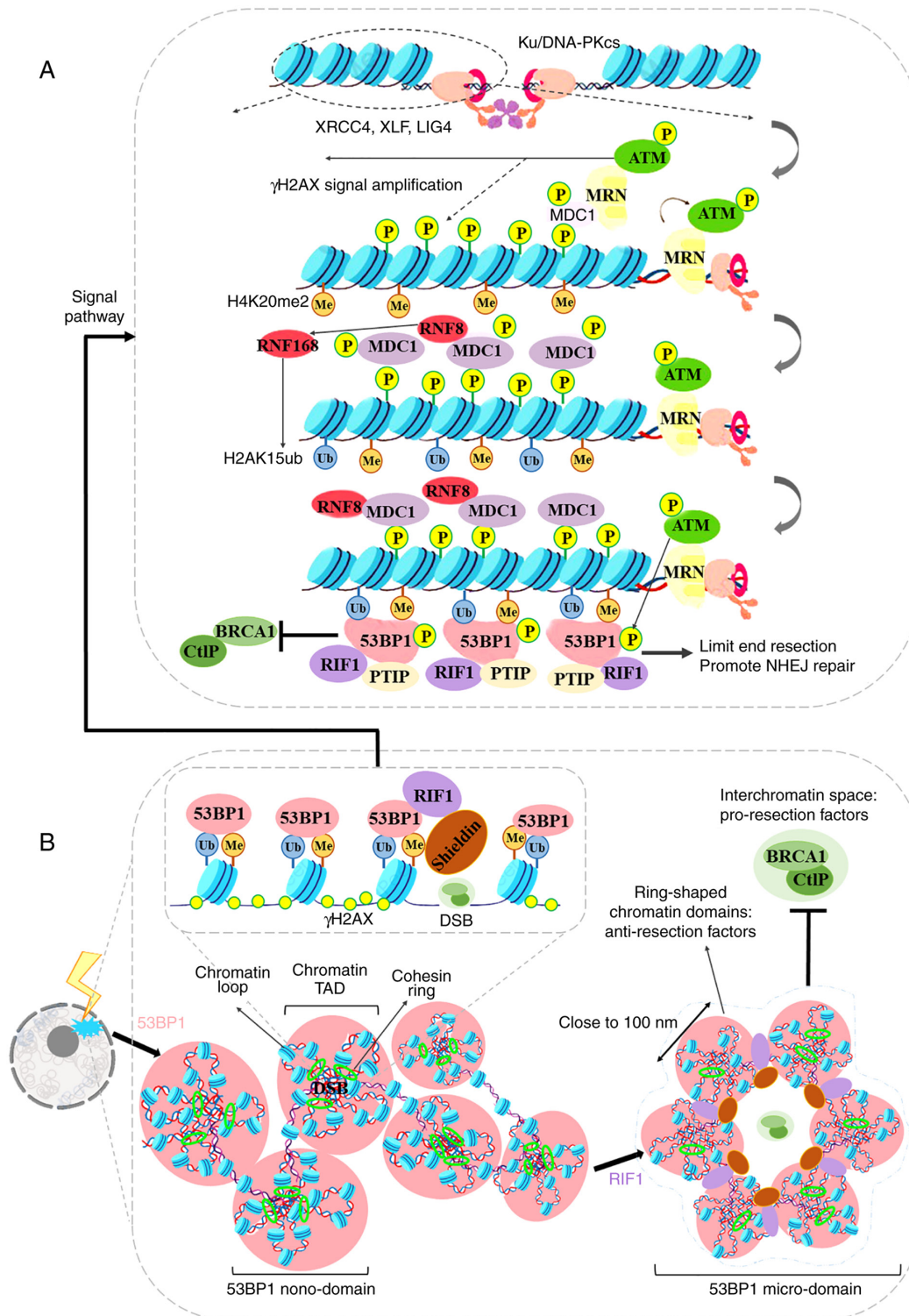


Figure 2. Graphic representation of 53BP1 recruitment and its nano-foci formation around DSB sites (DSBs). (A) DSB formation triggers a range of protein modifications that orchestrate the cellular response and DNA repair. DNA-PKcs, Ku70/Ku80, XRCC4 and etc. bind to DSBs, followed ligate end by one after another recruiting or activating MRN complex, ATM, γ H2AX and MDC1. This provides a positive feedback loop for DSB signal amplification. MDC1 recruits RNF8, which cooperates with RNF168 to catalyze histone H2A ubiquitylation at DSBs. H2AK15ub, together with H4K20me2, mediates 53BP1 recruitment at DSBs. In its ATM-phosphorylated form, 53BP1 interacts with RIF1 and PTIP, which promote NHEJ repair. (B) 3D reorganization of 53BP1 foci and chromatin architecture. 53BP1 binds to histone modifications on damaged chromatin at the vicinity of the DSB and recruits RIF1, which elicits the assembly of the Shieldin complex. Shieldin complex protects broken DNA ends from nucleolytic degradation by resection factors. The spreading of 53BP1 on chromatin occurs over megabases around the DSB and is shaped by chromatin topology with the formation of distinct 53BP1 nanodomains (close to 100 nm) corresponding to chromatin TADs. RIF1 and Cohesin complex lead to the 'loop extrusion' and promote the circularization of 53BP1 nanodomains into one ring-like micro-domain. This ring-like structure can limit the recruitment of BRCA1/CtIP and prevent excessive cleavage of DNA breaks. DSB, double-strand break; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; XRCC4, X-ray repair cross complementing protein 4; RIF1, replication timing regulatory factor 1; TAD, topologically associated domain; XLF, XRCC4-like factor; LIG4, DNA ligase IV; H2AX, H2A histone family member X; ATM, ataxia-telangiectasia mutated; MRN, MRE11-RAD50-NBS1; MDC1, mediator of DNA damage checkpoint protein 1; RNF, ring finger protein; 53BP1, p53-binding protein 1; BRCA1, breast cancer type 1 susceptibility protein; CtIP, C-terminal binding protein (CtBP)-interacting protein; PTIP, Pax transactivation domain-interacting protein; NHEJ, non-homologous DNA end joining.

Wu *et al* (58) observed that the nuclear chromatin was relaxed from a 200–400 nm thick irregular frame and remodeled to a disperse sub-100 nm structure following X-ray irradiation. The relaxed nuclear chromatin is a more feasible portion for the recruitment of DSB repair factors (γ H2AX, MDC1 and 53BP1) that were distributed as microscale-colocalized and nanoscale interlaced substructures (58). Notably, Ochs *et al* (59), using 3D-SIM and 2D-stimulated emission depletion super-resolution microscopy techniques, demonstrated that the 53BP1 and RIF1 proteins can form an autonomous functional module, which can stabilize the chromatin topological structure of DNA fragmentation sites. When DNA damage occurs, 4–7 53BP1 sub-domains form ring structures (with a uniform spherical body) in the DNA fragments. The diameter of the 53BP1 sub-domain is ~100 nm, and the center distance of the two 53BP1 sub-domains is close to 140 nm, which facilitates the reciprocal association between the chromatin topology and the formation of 53BP1 foci in response to DSB. Further research (59) demonstrated that the chromatin recruitment of 53BP1 foci occurs over megabases around the DSB and corresponds to chromatin topologically associated domains (TADs). Subsequently, RIF1 and the Cohesin complex [Shieldin/CTC1-STN1-TEN1 (CST)/polymerase- α (Pol α)] are recruited to the boundary of the TAD structure, and the alternating distribution of 53BP1 and RIF1 stabilizes several adjacent TAD structures into an ordered ring arrangement. This ring-like structure can limit the recruitment of BRCA1 and prevent excessive cleavage of DNA breaks. Recently, Arnould *et al* (60) verified the hypothesis that chromatin high-dimensional structure regulates DSB repair, and proposed that ‘loop extrusion’ may be the mechanism through which the DNA repair center is formed. Following the occurrence of a DSB, ATM and the Cohesin complex mediate roadblock for unilateral loop extrusion, in which ATM phosphorylates H2AX constitutively. Divergent one-sided loop extrusion and the bidirectional spreading of phosphorylated H2AX induce the assembly of the full DDR reaction focus. Notably, although RIF1 organizes 53BP1 foci and accumulates at the boundaries between 53BP1 nano-domains, RIF1 does not colocalize with these domains (60,61).

The generation of 53BP1 foci surrounding DNA lesions is required to recruit downstream effectors. The time frame and mechanisms through which the spatial and temporal confinement of protein assemblies at DNA damaged sites is achieved requires further investigation. 53BP1 dimers, a dimerization mediated by the 53BP1 oligomerization domain, relocate from the nucleoplasm to sites of DSBs (33). At these sites, the consecutive recognition of H2AK15ub and dH4K20me leads to the assembly of 53BP1 oligomers and promotes the formation of mature 53BP1 foci structures (62). Using state-of-the-art microscopy, Kilic *et al* (63) observed that the 53BP1 foci exhibit the hallmarks of phase-separated compartments and exhibit droplet-like behavior. Phase-separated proteins self-organize into liquid-like droplets, allowing NHEJ-interrelated molecules to become concentrated, while excluding NHEJ-irrelevant molecules (64). The droplet-like 53BP1 foci is highly sensitive to changes in osmotic pressure, temperature, salt concentration and to the disruption of hydrophobic interactions, suggesting that the assembly of 53BP1 is reversibly abolished (63). The liquid-like nature of 53BP1

assemblies verifies previous observed results that demonstrated that 53BP1 undergoes phase separation and forms a spatiotemporally spherical shape (65,66). Pessina *et al* (67) proposed that DNA damage-induced transcriptional promoters drive molecular crowding off DDR proteins and RNA synthesis, which stimulates the phase separation of 53BP1 in the shape of foci. Therefore, it is possible that the phase separation of 53BP1 foci integrates the localized DNA damage recognition and the assembly of repair. However, the forming speed of droplet-like 53BP1 foci and the fidelity of DSB repair is dependent on the complexity of the lesion. 53BP1 has been shown to be recruited in a few seconds to complex DSB sites using live cell imaging combined with heavy ion trackers (68). In almost half of the isolated DSB sites, the recruitment of 53BP1 is delayed ~5 min (68). Following neocarzinostatin treatment, 53BP1 foci is formed in ~60 min and observed to co-localize with γ H2AX at the sites of DSBs (~80% of 53BP1 foci contain exactly one DSB) that are accompanied by the higher chromatin compaction (69).

4. Upstream regulators of 53BP1 in NHEJ repair

As aforementioned, 53BP1 is recruited to the DSB sites to coordinate the chromatin architecture around DSB sites and to promote NHEJ repair. Therefore, the upstream molecules that regulate recruitment and functions of 53BP1 in DNA repair deserve further investigation.

Tudor-interacting repair regulator (TIRR) regulates 53BP1 recruitment to chromatin. The DNA ends are marked with histones H4K20me₂, which is a specific binding target for the Tudor domain of 53BP1. TIRR (or NUDT16L1), a member of the family of the nucleoside diphosphate-linked moiety X (Nudix) hydrolases, was first identified as an upstream molecule that inhibits this unique binding in 2017 by Drané *et al* (70) and Zhang *et al* (71). Drané *et al* (70) demonstrated that TIRR directly binds the tandem Tudor domain of 53BP1 and masks its H4K20me₂ binding motif, and TIRR overexpression in the cells with low expression of BRCA1 abrogated the development of resistance to poly(ADP-Ribose) polymerase (PARP) inhibitors (PARPis), which may be related to the loss of 53BP1 function. However, upon DNA damage, ATM phosphorylates 53BP1 and recruits RIF1, thus inducing the dissociation of the 53BP1-TIRR complex from chromatin. Thus, the major function of TIRR is to serve as an off switch in the absence of DNA damage, maintaining tandem Tudor domain in an inactive state and keeping 53BP1 away from chromatin (Fig. 3A).

The recently reported crystal structures of TIRR in complex with 53BP1 Tudor domain, together with supporting binding assays using ubiquitinated modification and demethylated modification nucleosomes, reveals that TIRR occludes the methyl-lysine-binding site of Tudor domain (72–74). Guided by X-ray crystallography, Botuyan *et al* (72) revealed that a TIRR arginine (Arg107) residue could mask the histone methyllysine-binding surface of 53BP1. They also found that a mutation of a phenylalanine residue (F1553R) in 53BP1 abolished the interaction with TIRR, but preserved interaction with H4K20me₂, which indicates that the two binding activities of the 53BP1 Tudor domain could be functionally separated and independently explored by mutagenesis. After analyzing the

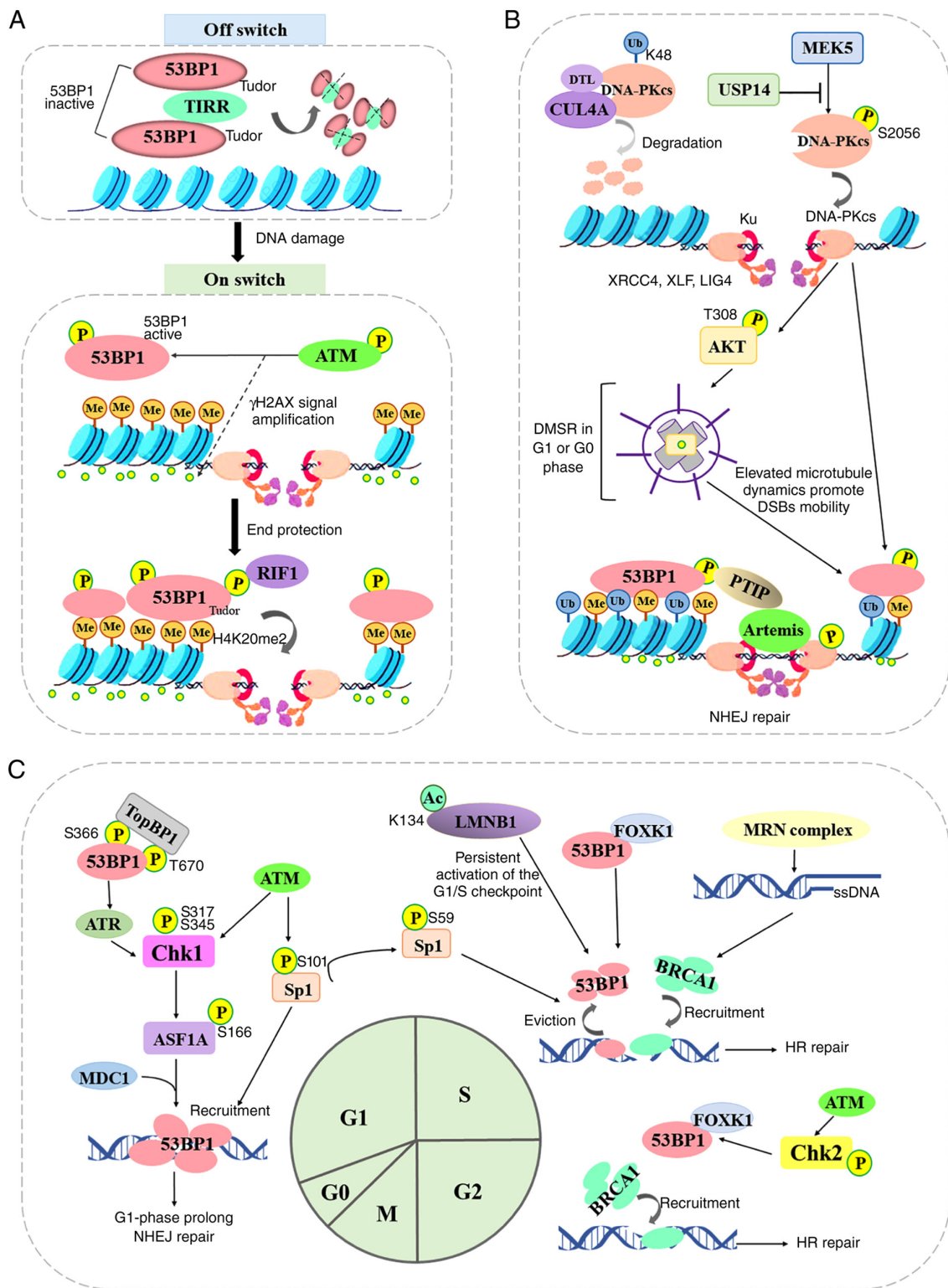


Figure 3. Upstream regulators of 53BP1 in NHEJ repair. (A) In a stress-free environment, TIRR inhibits the histone binding function of 53BP1 by binding to its Tudor domain, which is known as the 'off switch'. However, upon DNA damage, 53BP1 is recruited to chromatin and promotes DSB NHEJ repair, which is known as the 'on switch'. (B) At DSB ends, the assembly of phosphorylated DNA-PKcs serves as a platform to recruit Artemis, 53BP1 and other NHEJ factors. Post-transcriptional modification of DNA-PKcs affects its ability to promote NHEJ repair. The autophosphorylation or MEK5-dependent phosphorylation of DNA-PKcs contributes to 53BP1 recruitment, and induces DSB-induced microtubule dynamics stress response. The CRL4ADTL-induced ubiquitination degradation of DNA-PKcs inhibits the NHEJ repair. (C) The cell cycle phase is an important determinant of the repair pathway selection at DSB sites. In the G1 phase, the phosphorylation of Chk1 (S317, S345), regulated by ATM and ATR, induces the formation of 53BP1 foci following DNA damage. In the S/G2 phase, the recruitment of 53BP1 is inhibited by the phosphorylation of Chk1 (S59), FOXK1, BRCA1 and acetylated LMNB1 (K134). DSB, double-strand break; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; 53BP1, p53-binding protein 1; NHEJ, non-homologous DNA end joining; Chk1, checkpoint kinase 1; ATM, ataxia-telangiectasia mutated; 53BP1, p53-binding protein 1; BRCA1, breast cancer type 1 susceptibility protein; LMNB1, lamin B1; TIRR, Tudor-interacting repair regulator; CUL4A, cullin 4A; USP14, ubiquitin-specific protease 14; XRCC4, X-ray repair cross complementing protein 4; XLF, XRCC4-like factor; LIG4, DNA ligase IV; DMSR, DNA induced DSB-induced microtubule dynamics stress response; PTIP, Pax transactivation domain-interacting protein; TopBP1, topoisomerase II β binding protein 1; ASF1A, anti-silencing function 1A histone chaperone; MDC1, mediator of DNA damage checkpoint protein 1; HR, homologous recombination; FOXK1, forkhead box K1.

protein structure of 53BP1 Tudor and TIRR, Dai *et al* (74) revealed that the TIRR amino-terminal region (residues 10-24) combined with the TIRR L8-loop could prevent the methylation reader joining surface (centered around Arg107) in the Tudor domain of 53BP1, which inhibits 53BP1 recruitment to nucleosomes bearing H4K20me₂. Structural comparisons identified a TIRR histidine (H106 is absent from the TIRR homolog NUDT16) that is essential for 53BP1 Tudor binding. Wang *et al* (73) demonstrated that three loops (α 1- β 1 loop, N-terminus loop and β 4- β 5 loop) from TIRR interact with the 53BP1 Tudor domain and mask the methylated lysine-binding pocket in tandem Tudor domain. Additionally, TIRR inhibited the complex formation between the Tudor domain of 53BP1 and a dimethylated form of p53 (K382me₂), which inhibited transcriptional activation of the p53 target genes (75). Overall, these studies elucidate the mechanisms by which TIRR recognizes the 53BP1 Tudor domain and functions as a cellular inhibitor of the histone methyl-lysine readers.

DNA-PK regulates 53BP1 recruitment to DSB sites. At DSB ends, the assembly of DNA-PK, a nuclear serine/threonine protein kinase composed of a large catalytic subunit (DNA-PKcs) and a heterodimeric DNA-targeting subunit Ku, serves as a platform to recruit Artemis, DNA ligase IV and NHEJ factors (such as 53BP1 and γ H2AX), all of which are involved in end-processing and ligation (76). Although the autophosphorylation of DNA-PKcs occurs at numerous Ser/Thr residues throughout the kinase, and this mediates NHEJ, certain molecules were confirmed to function as a potential phosphorylase regulator (77,78). Mitogen-activated protein kinase kinase 5 (MAPKK or MEK5) was found to promote phosphorylation of the catalytic subunit of DNA-PK at serine 2,056 in response to ionizing radiation or etoposide treatment by Broustas *et al* (77). This revealed a convergence between MEK5 upstream signaling and DNA repair by NHEJ in conferring resistance to genotoxic stress in advanced prostate cancer (77). Conversely, Sharma and Almasan (78) identified that ubiquitin-specific protease (USP)14, a proteasomal deubiquitinase, decreased the IRIF formation of 53BP1 and pS2056-DNA-PKcs, ultimately inhibiting NHEJ repair, promoting HR repair, and suppressing the radiosensitization of non-small cell lung cancer cells. Feng *et al* (79) demonstrated that the ubiquitin ligase Cullin 4A binds to the DNA-PKcs protein in the NHEJ repair pathway for nuclear degradation through its substrate receptor DTL. CRL4A^{DTL} is recruited to DSB sites and promotes the ubiquitination of DNA-PKcs at K48 in the nucleus, inhibiting the NHEJ repair pathway to increase cell genomic instability. Similarly, as previously demonstrated, when cisplatin resistance developed, DNA-PKcs activity and the formation of 53BP1 foci was reduced, which antagonized cisplatin cytotoxicity for germ cell tumor cells (80). Additionally, Ma *et al* (81) found that the activation of the DNA-PK-AKT cascade facilitated interphase centrosome maturation and induced DSB-induced microtubule dynamics stress response (DMSR), thus promoting DSB mobility and 53BP1-dependent NHEJ repair. DMSR occurs in G1 or G0 cells and lasts around 6 h, providing an aggregated time for 53BP1 and its partners. Although the mechanism by which DNA-PK promotes 53BP1 recruitment to DSB sites remains

unclear, DNA-PK may serve as a potential upstream regulatory molecule for 53BP1 (Fig. 3B).

53BP1 upstream regulators associated with the cell cycle phase. The cell cycle phase is a critical determinant of the choice of repair pathway at DSB sites (Fig. 3C). BRCA1-mediated HR repair is restricted to the S and G2 phases of the cell cycle when a sister chromatid is present, while 53BP1-mediated NHEJ repair is the dominant process in the G1 phase. The checkpoint kinase 1 (Chk1), activated by ATM kinase on DNA breaks in the G1 phase, phosphorylates the histone chaperone, anti-silencing function 1A histone chaperone (ASF1A) at Ser166 (82). The phosphorylation of ASF1A interacts with the repair protein MDC1 and thus enhances its downstream 53BP1 recruitment. Similarly, topoisomerase II β binding protein 1 (TopBP1), a multi-domain 'scaffold' protein, has been revealed to control the DNA damage checkpoint regulating S-phase entry by binding to 53BP1 (83). The BRCT domains of TopBP1 bind to conserved phosphorylation sites (Ser366, Thr670) in the N-terminus of 53BP1, which promotes the recruitment of TopBP1, ATR and Chk1 to 53BP1 damage foci, but does not affect the formation of 53BP1 or ATM foci following DNA damage (83,84). Chk1 is phosphorylated by ATR on Ser317 and Ser345 in a DNA damage-dependent manner, thus prolonging the G1 phase and inducing NHEJ repair by coordinating cell cycle progression with DSB repair (84). Moreover, Ha *et al* (85) found that DSB sites in S/G2 cells can be processed by the Ku heterodimers and the MRN complex. When a DSB site is bound by Ku heterodimers, the break is then destined for 53BP1-mediated NHEJ. While DSB sites are bound by an MRN complex, the ends are resected and ssDNA is generated, leading to the activation of the ATR/Chk1/APC^{Cdh1} axis, and eventually the destruction of deubiquitinating enzyme USP1 and the recruitment of BRCA1. Beishline *et al* (86) found that the transcription factor Sp1, phosphorylated on serine 101 (pSp1) by ATM, was recruited to DSBs 7.5 min following ionizing radiation-induced damage and remained at the DSB site for at least 8 h. The same research group researched further and revealed that Sp1 localized to DSBs in the G1 phase and was necessary for the recruitment of 53BP1 to promote NHEJ repair, while the phosphorylation of Sp1-S59 in the early S phase evicted Sp1 and 53BP1 from the DSB site to allow BRCA1 binding (87). The forkhead box K1 (FOXK1) associates with 53BP1 to negatively regulate 53BP1 function by inhibiting 53BP1 localization to DSB sites (88). The FOXK1-53BP1 interaction is enhanced upon DNA damage during the S phase in an ATM/CHK2-dependent manner, which reduces the association of 53BP1 with its downstream factors RIF1 and PTIP. The acetylation of lamin B1 (LMNB1) at K134 negatively regulates canonical NHEJ repair by impairing the recruitment of 53BP1 to DSB sites, and induces the persistent activation of the G1/S checkpoint (89). Thus, given the apparent switching effects of these regulators in integration of the cell cycle and DSB repair pathway choice to favor NHEJ, a more complete understanding of the function of these is required to validate the aforementioned findings.

Notably, similar to how H4K20me₂ promotes NHEJ repair by presenting a binding site for the 53BP1 protein, H4K20me₃ interactions with 53BP1 have been shown to be markedly pronounced at DNA lesions in the G1 phase (90). Together,

H4K20me3 and H3K9me3 represent epigenetic markers that are important for the function of the 53BP1 recruitment in NHEJ repair, while the levels of these histone markers are reduced in the very late S and G2 phases when PCNA was recruited to locally micro-irradiated chromatin (90). Moreover, Nakamura *et al* (91) reported that the ankyrin repeat domain of BRCA1-associated RING domain protein 1 (BARD1) promoted BRCA1 recruitment to DSB sites in the S and G2 phases by recognizing and reading histone H4 unmethylated at lysine 20 (H4K20me0). The BARD1 recognition of H4K20me0 is required for HR repair and resistance to PARPis, and opposes 53BP1 function and NHEJ repair.

Upstream molecules that regulate the post-transcriptional modification of 53BP1. It has been shown that 53BP1 protein levels do not significantly change in a DSB response, and that the expression of 53BP1 remains basically unaltered throughout the entirety of the cell cycle (92,93). Therefore, 53BP1 is regulated by multiple PTMs (Table I). The first PTMs are phosphorylation and dephosphorylation. There are 28 ATM-regulated phosphorylation sites at the N-terminal phospho-SQ/TQ domain of 53BP1 (29,94). The interaction between PTIP and 53BP1 is primarily dependent on the third phosphorylation site (S25), which plays a role in pathological injury repair selection and telomere fusion (56). Interactions between RIF1 and 53BP1 are dependent on the phosphorylation sites 9-15 (T302, S437, S452, S523, S543, S580 and S625), which govern the processing of DNA ends by recruiting Shieldin (55). Additionally, the phosphorylation of 53BP1 is also involved in its recruitment and cell cycle regulation: i) The vaccinia-related kinase 1 stably phosphorylates 53BP1 at Ser25/29 without ATM, and is involved in the formation of γ H2AX, NBS1 and 53BP1 foci induced in NHEJ repair, and the entry of the cell cycle into the G2/M phase (95,96). ii) The AMP-activated protein kinase directly binds to 53BP1 and phosphorylates it at Ser1317, and promotes 53BP1 recruitment, thus maintaining genomic stability and diversity of the immune repertoire (97). iii) The glycogen synthesis kinase 3 β was revealed to translocate from the cytoplasm to the nucleus after exposure to ionizing radiation, where it induced DSB repair in the nuclei of glioblastoma cells via the phosphorylation of 53BP1 at Ser166 (98). Moreover, the dephosphorylation of 53BP1 plays a noteworthy role in DSB repair pathway choice: i) The serine/threonine-protein phosphatase 4 catalytic subunit C (PP4C)/PP4CR3 β complex dephosphorylates 53BP1 at T1609/T1618, and provides the structural basis for the normal enrichment of 53BP1 in the G1 phase for NHEJ repair (99). ii) Both BRCA1 and PP4C can promote the dephosphorylation of 53BP1 at T543 and the release of the 53BP1-RIF1 complex from DSB sites to direct repair toward HR (100). iii) The protein phosphatase 2C δ (referred to as WIP1) decreases the 53BP1 positioning after IR by mediating 53BP1 dephosphorylation at Thr543 and inhibiting 53BP1 interaction with RIF1 (101).

Secondly, 53BP1 is also regulated by ubiquitination. RNF168 modifies 53BP1 through the addition of a chain of ubiquitin-polypeptides. Lysine 1268 of 53BP1 is important for this ubiquitin modification, while the loss of this modification impairs 53BP1 recruitment to sites of DNA damage (47,102). Additionally, the UDR motifs of 53BP1 can recognize and bind to H2AK15ub (H2A monoubiquitination by RNF168), which is

crucial for recruiting 53BP1 to promote NHEJ repair. However, the E3 ligase RNF168-mediated 53BP1 ubiquitination and recruitment can be attenuated by lipolytic inhibitor G0/G1 switch gene 2 (103), ring finger protein 126 (RNF126) (104), ubiquitin-editing enzyme A20/TNFAIP3 (105) and the phosphorylation of H2AK15ub at Thr12 (referred to as H2AK15pUbT12) (106). Conversely, RNF169, an uncharacterized E3 ubiquitin ligase paralogous to RNF168, accumulates in DSB repair foci by recognizing RNF168-catalyzed ubiquitylation products and acting as a molecular rheostat to limit 53BP1 deposition at DSBs (107,108). Hu *et al* (109) found that RNF169 induces 53BP1 disengagement from H2AK15ub-H4K20me2-53BP1 complex. RNF169 bridges ubiquitin and histone surfaces, stabilizing a pre-existing ubiquitin orientation in H2AK15ub-H4K20me2-53BP1 complex to form a high-affinity complex (109). This conformational selection mechanism contrasts with the low-affinity binding mode of 53BP1, and it avails 53BP1 displacement.

Thirdly, 53BP1 is regulated by methylation/acetylation. PRMT1, a protein that catalyzes substrates to produce mono-methylation or symmetric demethylated arginine, methylates the GAR motif of 53BP1 to facilitate 53BP1 oligomerization and recruitment (34,35). Similarly, PRMT5, a homologous protein of PRMT1, plays a parallel role to that of PRMT1 (110). Wild-type PRMT5 maintains 53BP1 stability and promotes NHEJ repair by methylating 53BP1 GAR motif, while pY324 (phosphorylated by Src kinase) of PRMT5 inhibits its activity during the DNA damage process and blocks NHEJ repair (110). However, PRMT5 methylates RUVBL1 at R205, a cofactor of the TIP60 complex, which promotes TIP60-dependent histone H4K16 acetylation and subsequently facilitates 53BP1 displacement from DSB sites (111). Unlike methylation, recognition or modification by acetylation appears to induce DSB repair towards the HR pathway. As previously mentioned, the UDR motif mediates the selective aggregation of 53BP1 by recognizing H2AK15Ub. Through a histone reader domain for H4K20me1/2, the MBT domain-containing protein 1 (MBTD1) allows TIP60 complex to associate with DSB sites and acetylate H2AK15 (112,113). This acetylation blocks H2AK15 ubiquitylation that was regulated by RNF168, and inhibits 53BP1 recruitment through competitive bivalent binding. Additionally, nuclear ATP-citrate lyase phosphorylation facilitates TIP60-dependent histone acetylation at DSB sites, impairing 53BP1 localization and enabling BRCA1 recruitment (114,115). Notably, the acetylation of 53BP1 itself inhibits NHEJ and promotes HR by negatively regulating its recruitment to DSB sites (116). Mechanistically, acetyltransferase CBP acetylates the UDR motif of 53BP1 at K1626/1628, thus disrupting the interaction between 53BP1 and H2AK15ub, subsequently blocking the recruitment of 53BP1 and its downstream factors PTIP and RIF1.

Finally, ADP-ribosylation can signal for ubiquitination and promote the degradation of ADP-ribosylated proteins (117,118). RNF146 contains a RING domain that is an E3 ubiquitin ligase and a WWE domain that is a PAR-binding domain, and it functions as an E3 ubiquitin ligase for ADP-ribosylated 53BP1 (119,120). As the amount of DNA damage increases, the C terminus (1043-1972aa) of 53BP1 is ADP-ribosylated by PARP1, and ADP-ribosylated 53BP1 is targeted by RNF146, leading to 53BP1 ubiquitination and degradation (121). NUDT16, member of Nudix proteins that is characterized by

Table I. Post-transcriptional modifications of 53BP1 in NHEJ repair.

Post-transcriptional modification	Catalytic molecule	Catalytic site(s) of 53BP1	Effects	(Refs.)
Phosphorylation	ATM	S25	Interacts with PTIP to promote DSB end protection	(56)
	ATM	T302, S437, S452, S523, S543, S580, S625	Interacts with RIF1 to inhibit DNA end resection	(55)
	VRK1 (without ATM)	S25, S29	Induces the formation of 53BP1 foci	(95,96)
	AMPK	S1317	Promotes 53BP1 recruitment to DSB sites	(97)
	GSK3β	S166	Promotes 53BP1 recruitment to DSB sites and induces DNA repair	(98)
Dephosphorylation	PP4C	T1609, T1618	Promotes the normal enrichment of 53BP1 in G1 phase	(99)
	BRCA1 and PPP4C	T543	Induces the release of RIF1 from 53BP1/RIF1 complex	(100)
	WIP1	T543	Decreases 53BP1 positioning after IR by parting 53BP1/RIF1 complex	(101)
Ubiquitylation	RNF168	K1268	Promotes 53BP1 recruitment to DSB sites	(47,102)
	RNF169	Not stated	Induces 53BP1 disengagement from the H2AK15ub-H4K20me2-53BP1 complex	(109)
Methylation	PRMT1	GAR motif	Facilitates 53BP1 oligomerization and recruitment to DSB sites	(34,35)
	PRMT5	GAR motif	Maintains 53BP1 stability and promotes NHEJ repair	(110)
Acetylation	Acetyltransferase	K1626, K1628 (UDR motif)	Disrupts the interaction of 53BP1 and H2AK15ub, subsequently blocking 53BP1 recruitment	(116)
Poly-ADP ribosylation	PARP1	C terminus (1043-1972aa)	Leads to 53BP1 ubiquitination and degradation by targeting RNF146	(121)
	NUDT16	Not stated (Tudor domain)	Removes protein ADP- ribosylation of 53BP1	(122)

ATM, ataxia-telangiectasia mutated; PTIP, Pax transactivation domain-interacting protein; DSB, double-strand break; RIF1, RAP1-interacting factor 1; VRK1, vaccinia-related kinase 1; 53BP1, p53-binding protein 1; AMPK, AMP-activated protein kinase; GSK3β, glycogen synthesis kinase 3β; PP4C, phosphatase 4 catalytic subunit C; BRCA1, breast cancer type 1 susceptibility protein; WIP1, protein phosphatase 2Cδ; RNF, ring finger protein; PRMT1, protein arginine N-methyltransferase 1; NHEJ, non-homologous end joining; UDR, ubiquitylation-dependent recruitment; PARP1, poly (ADP-Ribose) polymerase 1; NUDT16L1, Tudor-interacting repair regulator (TIRR).

a highly conserved 23-amino acid Nudix motif, exhibits the hydrolase activity that removes the protein ADP-ribosylation of 53BP1 (122), and inhibits 53BP1 ubiquitination and degradation, stabilizing 53BP1 protein and allowing its recruitment to DSB sites (121). Together, the PTM status of 53BP1 plays key roles in its recruitment to DSB sites, and reveals how specific 53BP1 modification and recognition modulate the selection of DNA repair pathways.

Other factors involved in 53BP1 recruitment. There are other factors that contribute to the regulation of 53BP1 recruitment and NHEJ repair. The nuclear basket of nuclear pore complexes contains three nucleoporins Nup153, Nup50 and Tpr, and they play key roles in DSB repair by promoting the nuclear import of 53BP1. Nup153 is required for the proper nuclear import of 53BP1 and SENP1-dependent sumoylation of 53BP1, which promotes the recruitment of 53BP1 to DNA repair foci (123,124). DROSHA, a miRNA biogenesis enzyme, is required within minutes of a break occurring to control the

recruitment of NHEJ repair factors in a DROSHA-dependent manner (125). DROSHA is recruited to DSB sites without neither H2AX, nor ATM or DNA-PK kinase activities, and interacts with RAD50 to promote its recruitment (126). Indeed, DROSHA knockdown and MRN complex inactivation (mirin treatment) increase the association of downstream HR factors, such as RAD51 to DNA ends and reduce NHEJ (125,126). Tripartite motif-containing protein 29 (TRIM29) is required for the efficient recruitment of 53BP1 to facilitate the NHEJ pathway and thereby suppress the HR pathway in response to DSB (127). The knockdown of histone lysine demethylase PHF2 inhibits the resolution of 53BP1 foci, the localization of C-terminal binding protein (CtBP)-interacting protein (CtIP) and subsequent NHEJ repair (128). TNF receptor-associated death domain (TRADD), an essential mediator of TNF receptor signaling, facilitates NHEJ repair by recruiting 53BP1 and the Ku70/80 complex (129). In contrast to the depletion of the ubiquitin ligase HUWE1 increasing RAD51 levels to partially restore HR, the depletion of histone acetyltransferase

KAT5 rewires DSB repair by promoting 53BP1 binding to DSBs (130). KAT5 depletion can promote PARPi sensitivity via the induction of imprecise NHEJ repair in BRCA2-deficient cells. The chromodomain helicase DNA-binding protein 1 (CHD1), a common genomic mutation found in human prostate cancers associated with genomic instability, disrupts 53BP1 stability and decreases error-prone NHEJ repair for DSB repair (131). PARP2 limits the accumulation of the resection barrier factor 53BP1 at DSB sites independently of its PAR synthesis activity (132). PARP2 induces DSBs towards resection-dependent repair pathways, which includes HR repair, SSA and altEJ, rather than NHEJ repair.

5. Downstream effectors of 53BP1 in NHEJ repair

The current research consensus is that BRCA1- and 53BP1-dependent pathways compete with each other during the early stages of DSB repair, particularly for DNA end resection. In the G1 phase, 53BP1 is recruited to the DSB site where it forms a protein complex that antagonizes BRCA1-mediated terminal modification (a single stranded homologous arm of ~200 nt), thereby protecting the terminal from excessive removal and determining the manner of cell repair (133). Hence, it is crucial to determine the effector molecules of 53BP1, and it is beneficial for researchers to fully elucidate the effects of 53BP1 chromatin recruitment in DNA damage.

53BP1 interacts with the RIF1/Shieldin/CST axis in NHEJ repair. 53BP1 phosphorylation, catalyzed by ATM on >25 sites that are concentrated in the N-terminal half of the protein, leads to the activation of the DNA repair function of 53BP1 and promotes its interaction with two proteins, PTIP and RIF1. These two proteins are involved in limiting end resection at DSB sites independently of each other (134). NHEJ repair is abolished in 53BP1^{-/-} cells and in cells expressing 53BP1^{28A} (an allele harboring alanine substitutions in all 28 N-terminal phosphorylation sites), while exerting a considerably milder defect in RIF1^{-/-} cells (135). Moreover, similar to the effect of 53BP1 ablation, the conditional ablation of mouse RIF1 (not PTIP) specifically in B-cells results in a profound defect in the function of 53BP1 in several NHEJ-driven processes, such as immunoglobulin CSR (55,136). Both processes of CSR and DSB end ligation involve Ku70/80, DNA-PKcs, LIG4 and XRCC4/XLF of NHEJ repair molecules (137). The 53BP1-RIF1 complex has indications for processing short overhangs, and ssDNA longer than 20-30 nt is characteristic of resection (16). Concerning the mechanism by which the 53BP1-RIF1 complex limits the formation of ssDNA at DNA breaks, there are two main models.

In the first model, 53BP1 uses the loading of Shieldin onto the ssDNA to protect the 5' end from resection. The Shieldin complex is composed of REV7 plus SHLD3 (RINN1 or CTC-534A2.2), SHLD2 (RINN2 or FAM35A) and SHLD1 (RINN3 or C20ORF196), and is recruited to DSBs via the ATM-RNF8-RNF168-53BP1-RIF1 axis, thus promoting NHEJ repair of intrachromosomal breaks, CSR and the fusion of unprotected telomeres (138,139). For the sake of clarity, the SHLD1/2/3 nomenclature will be used herein. Shieldin localizes to DSB sites in a 53BP1- and RIF1-dependent manner, and its SHLD3 and REV7 subunits associate with

the SHLD2 N-terminus to form the 53BP1-RIF1 complex localization module, while its SHLD1 subunit associates with the SHLD2 C-terminus to form the ssDNA-binding module (140). REV7 binds to SHLD2/3 in the crystal structure of the SHLD3-REV7-SHLD2 ternary complex by adopting two conformations with different topologies, closed (C-REV7) and open (O-REV7) states (141). Therein, SHLD2 forms a β sheet sandwich with O-REV7 and SHLD3 to promote NHEJ repair (141), while the conserved FXPWFP motif of SHLD3 binds to C-REV7 and blocks REV7 binding to REV1, which excludes Shieldin from the REV1/Pol ζ translesion synthesis complex (141). Additionally, The C-terminal half of SHLD2 is predicted at a high level of confidence to form three tandem OB-folds to function as a ssDNA binding domain (142). The OB-folds are similar to those found in RPA1 (subunit of replication protein A) and CTC1 (one of CTC1-STN1-TEN1 complex), and may provide a binding site for these ssDNA-binding complexes (143,144). Hereby, the decision point of the 53BP1-RIF1 complex in NHEJ repair revolves around Shieldin (Fig. 3A). In order to ensure that the 53BP1-RIF1-Shieldin complex induces 5' ends to produce sufficient resection to antagonize BRCA1-mediated HR repair, the binding between Shieldin and ssDNA is worthy of further study. The initiation of end resection occurs in a two-step process: Firstly, the MRN resection complex induces endonuclease generated nicks on the 5'-terminated strands on either side of the DSB site with the aid of CtIP (145,146). These nicks are then expanded through the 3'-5' exonuclease activity of MRN and the 5'-3' exonuclease activity of exonuclease 1 (EXO1) or DNA2-BLM (147,148). The resulting large tracts of ssDNA are bound by RPA, which is then replaced by RAD51 to initiate extensive degradation of the 5' strands that are required for HR repair. Although the SHLD2/SKLD1 complex binds to oligonucleotides of 60-10 nt *in vitro* (149), the SHLD2/SKLD1 complex does not completely inhibit BRCA1. Thus, these biochemical characterizations of Shieldin presented above leave some unresolved questions: One involves the mechanisms through which Shieldin prevents end-resection prior to the initiation of resection by binding to ssDNA. The other involves the mechanisms through which Shieldin interrupts EXO1 or DNA2-BLM following the initiation of resection by binding to ssDNA (Fig. 4A).

In the second model, Shieldin functions in recruiting CST/Pol α /Primase at resected ends, rather than blocking end-resection nucleases per se, or by directly inducing resection. The CST complex binds with high affinity to ssDNA and dsDNA junctions, potentially allowing the complex to protect 5' ends from EXO1 and block access of the BLM and WRN helicases (150). The CST complex may function as downstream molecules of 53BP1/RIF1 to protect DSBs from end resection, which confers PARPi resistance in BRCA1-deficient cells (151). 53BP1/RIF1/Shieldin/CST complex binding at a DSB site requires a 3' overhang (for CST, in the range of 10-18 nt) (150). As an accessory factor of Pol α -primase, CST interacts with Shieldin and localizes with Pol α to DSB sites in a 53BP1- and Shieldin-dependent manner (152). However, EXO1 and DNA2-BLM can generate long ssDNA tracts, while Pol α has limited ability and usually synthesizes 20-25 nt overhangs (153). Therefore, the Shieldin/CST/Pol α /Primase fill-in reaction is predicted to leave a considerable 3' overhang that may be as long as 60 nt. During telomere replication, CST-induced

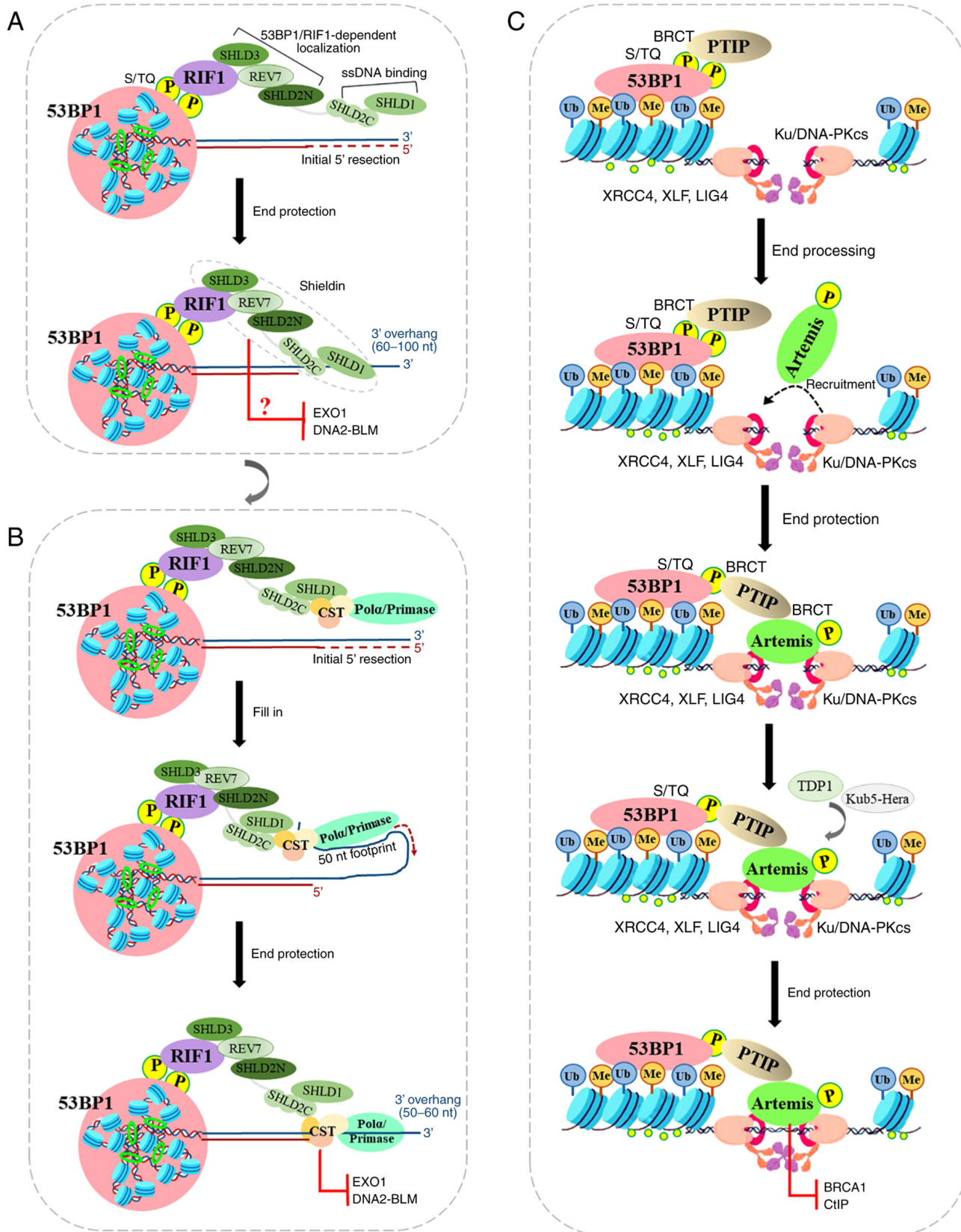


Figure 4. Two main downstream pathways of 53BP1. (A) DSB end protection by the ssDNA-binding Shieldin complex (REV7, SHLD3, SHLD2 and SHLD1) limits resection by EXO1 and DNA2-BLM. (B) Shieldin recruits CST and Polα/Primase, promoting the fill in reaction to counteract the DSBs end resection and leave a considerable 3' overhang (50–60 nt). The initial 5' end resection also occurs to allow ssDNA binding by Shieldin and CST. The CST/Polα/Primase-mediated fill in reaction helps to control the DSB repair by 53BP1, RIF1 and Shieldin. (C) Sequential phosphorylation events on multiple Ku/DNA-PKcs amino acid clusters favors the initial processing of DNA ends by Artemis. Artemis binds to 53BP1 to promote NHEJ, and consequently to prevent end resection and RAD51-dependent HR repair. DSB, double-strand break; ssDNA, 3' single-stranded DNA; CST, CTC1-STN1-TEN1; Polα, polymerase-α; 53BP1; p53-binding protein 1; NHEJ, non-homologous DNA end joining; RIF1, replication timing regulatory factor 1; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; PTIP, Pax transactivation domain-interacting protein; XRCC4, X-ray repair cross complementing protein 4; XLF, XRCC4-like factor; LIG4, DNA ligase IV; BRCT, breast cancer type 1 susceptibility protein carboxyl-terminal; CtIP, C-terminal binding protein (CtBP)-interacting protein.

fill-in reactions allows for retention of overhangs of at least 50 nt (154). The RIF1/Shieldin/CST axis has the ability to protect 5' ends from further resection, while 53BP1 action is predicted to carry a 3' overhang. These results suggest that CST/Pol α /Primase-mediated fill-in reactions help to control the repair of DSB by 53BP1, RIF1 and Shieldin (Fig. 4B).

The PTIP/Artemis axis mediates 53BP1-dependent DNA repair. PTIP is another critical factor acting as a downstream effector of 53BP1, and it antagonizes BRCA1 function in DNA repair by cooperating with RIF1. PTIP recruitment to DSB sites depend on phosphorylated 53BP1^{8A} (the first eight amino-terminal ATM sites), while PTFP depletion provides additional or sustained end resection that is required for rescuing HR repair in BRCA1-deficient cells (56). PTIP, a large nuclear protein containing six BRCT (BRCA1 C-Terminal) domains, regulates gene transcription as part of the MLL3-MLL4 methyltransferase complex that catalyzes H3K4me3 (155). PTIP interacts with phosphorylated Ser 25 of 53BP1 through its tandem BRCT domains (156,157) (Fig. 4C).

PTIP promotes NHEJ repair by recruiting proteins required by NHEJ, Artemis, to sites of DNA damage (158). PTIP interacts with Artemis through its second BRCT domain, while Artemis interacts with PTIP through its damage-dependent phosphorylation of six S/T sites (T656) at the very C-terminal end (158). Artemis, a nuclease with exo- and endonuclease activity, cleaves a hairpin intermediate during V(D)J recombination during DSB end processing (42,159). Artemis nuclease activity is dependent on DNA-PKcs autophosphorylation, suggesting that DNA-PK may remodel the end to allow Artemis cleavage (160). Ku70/80 protein binds to DSB end and promotes Artemis recruitment, and DNA-PKcs also phosphorylates Artemis. Artemis separates DNA-PKcs from end-joining complex (Ku70/80, DNA ligase IV, XRCC4, XLF, and PAXX) by interacting with XRCC4 (161). Therefore, the endonuclease activity of Artemis permits it to trim DSB ends to promote NHEJ, and consequently to prevent end resection and RAD51-dependent HR repair (Fig. 4C).

Similar to Artemis nuclease, tyrosyl-DNA phosphodiesterase (TDP1) is capable of resolving protruding 3'-phosphoglycolate termini of DSB sites to promote the C-NHEJ pathway (162). Artemis deficiency results in a fraction of unrepaired DSBs in 53BP1 foci, while TDP1 deficiency tends to promote DSB end mis-joining. TDP1 and Artemis perform different but interrelated functions in the repair of terminally blocked DSBs. Additionally, Kub5-Hera, the human homolog of the yeast transcription termination factor Rtt103, forms novel complexes with DSB repair factors (Ku70/Ku86, Artemis, and others) and terminate transcription (RNA polymerase II) at DSB sites (163). In Kub5-Hera-deficient cells that are hypersensitive to cytotoxic agents-induced DSBs, Artemis induces γ -H2AX and 53BP1 repair-related foci regression. 53BP1 promotes toxic end-joining events (alt-NHEJ and c-NHEJ) via the retention of Artemis at DSB sites, while BRCA2 antagonizes 53BP1, RIF1, and Artemis-dependent NHEJ repair to prevent gross genomic instability in a RAD51-independent manner (164). Thus, although these studies highlight the importance of the 53BP1/PTIP/Artemis axis at DSB repair, Artemis-related downregulation requires further research (Fig. 4C).

Other downstream effectors of the 53BP1/RIF1 axis. As described above, RIF1 negatively regulates resection through the effector Shieldin to prevent further resection and HR repair. Isobe *et al* (165) found that RIF1 immediately inhibited the accumulation of CtIP at DSB sites following damage, suggesting that RIF1 has another effector in addition to Shieldin. They found that protein phosphatase 1 localized to DSB sites in a RIF1-dependent manner, and suppressed downstream CtIP accumulation and limited MRN complex-mediated resection (165). Indeed, Cockayne syndrome (CS) is a DNA repair impaired syndrome characterized by a broad mutation of CS protein B (CSB), which is considered another RIF1 effector (166). Batenburg *et al* (167) found that CSB, a member of the switch/sucrose non-fermentable (SWI2/SNF2) superfamily, was phosphorylated by ATM (at S10) and cyclin A-CDK2 (at S158). In the DNA DSB repair pathway choice in the S/G2 phases, CSB interacts with RIF1 via its winged helix domain (WHD) and is recruited to FokI-induced DSB sites in the S phase, limiting RIF1 and its effector REV7, and evicting histones, but promoting BRCA1-mediated HR repair (167). Further research has found that the UV-induced disengagement of the C-terminal region of CSB from the ATPase domain requires two conserved amino acids (W1486 and L1488), and it contributes to the hydrophobic core formation of WHD at its C-terminus (168). The dissociation of the CSB domain interactions is a necessary step in repairing DNA damage. Following RIF1 eviction, CSB interacts with the BRCT domain of BRCA1 and this interaction is regulated by CDK-dependent phosphorylation of CSB at S1276 in late S/G2 phase, mediating the interaction of CSB with HR repair-related proteins consisting of BRCA1, the MRN complex and CtIP (169).

Similar to CSB, the suppressor of cancer cell invasion (SCAI) interacts with the tumor suppressing SWI/SNF chromatin remodeling complex to promote changes in gene expression (170). Hansen *et al* (171) initially demonstrated that SCAI is a mediator of 53BP1-dependent repair of heterochromatin-associated DSBs and facilitates ATM kinase signaling. SCAI undergoes prominent enrichment at DSB sites through 53BP1-dependent recruitment to DSB-surrounding chromatin, while SCAI deficiency results in reduced NHEJ repair capacity. SCAI was recently shown to stimulate HR repair through an interaction with 53BP1 phosphorylated at S/TQ sites in the S/G2 phases (172). SCAI inhibits and evicts RIF1 at DSB sites via binding to 53BP1, thus facilitating BRCA1-mediated HR repair (172). Inversely, LMO2 (also known as RBTN2, Rhombotin-2, or Ttg-2) inhibits BRCA1 recruitment to DSBs by interacting with 53BP1 during repair, promoting error-prone NHEJ repair and increasing tumor cells' sensitivity to PARPis in the G1 phase (173). Collectively, these molecules are physiologically important components of both the NHEJ- and HR-mediated pathways, in potentiating the DSB repair choice via modulation of the downstream signaling of the 53BP1 axis.

6. The challenges in targeting 53BP1 and its potential roles in several diseases and in genome editing

53BP1 inhibits the formation of 3' overhangs at DSB sites and alters DSB chromatin dynamics; however, its selective advantage remains an enigma. For this reason, 53BP1 may

contribute to the response to DSBs, but may also be potentially detrimental for cells with multiple DSBs.

53BP1-mediated NHEJ repair in resistance to treatment and prognosis of cancer treatment. 53BP1 not only affects resistance to cancer treatments, such as chemotherapeutic agents, PARPs and radiation, but is also a predictor of outcomes after undergoing treatment. Studies have demonstrated that low levels of 53BP1 prolong the overall survival of patients with non-small lung cancer cell undergoing treatment with platinum to 19.3 months (high levels of 53BP1 to 8.2 months) (174). However, in germ cell tumors, cisplatin-resistant cell lines have a NHEJ-less phenotype characterized by a reduced basal expression of 53BP1 and DNA-PKcs (80). Similarly, low levels of 53BP1 have an inferior response to treatment with high-dose alkylating agents in breast cancer (175), while 53BP1 is upregulated in temozolomide-resistant glioblastoma cells (176). 53BP1^{-/-} leads to 5-fluorouracil resistance in colorectal cancer cells by inhibiting the ATM-CHK2-P53 pathway (177). It is hypothesized that the reason for the ambiguous role of 53BP1 in cancer chemotherapy resistance may be due to the fact that it is often studied in isolation without taking the role of the ATM-CHK2-P53 pathway and DNA repair into consideration. As previously demonstrated, a 53BP1^{-/-} genotype increased resistance to PARPs in BRCA1-deficient mice by promoting the re-emergence of HR repair. BRCA1-deficient cancers prevent error-prone NHEJ-induced excessive genomic alterations by downregulating RNF168 ubiquitin signaling (178). The concept of BRCA1^{-/-}-affected HR repair is not an 'all-or-nothing' concept. When the inhibition of RNF168-ub-H2AX signaling is not sufficient to activate 53BP1 recruitment, PALB2, a partner and localizer of BRCA2, potentially stimulates the DNA strand-invasion activity of RAD51 to prompt residual HR repair (178). In this process, 53BP1 binds to the nucleosome acidic patch region via its UDR domain to block the interaction between PALB2 chromatin-associated motif (ChAM) and the nucleosome at the site of the DSB (179). It was previously demonstrated that olaparib co-treatment with DNA synthesis-inhibiting agents significantly increased 53BP1/γH2AX co-localization in anticancer drug-treated cells to attenuate the toxicity of treatments (180). In BRCA1/53BP1-deficient cells, RAD51 foci are formed at resected DSBs in a PALB2/BRCA2-dependent manner, and thereby induce HR repair (179). As regards sensitivity to PARPs, it is worth mentioning that targeting the upstream signaling of 53BP1 is also an effective target.

The rapid and error-prone DSB repair of NHEJ in cancer radiation therapy is considered to be the primary factor involved in radiation resistance. Ward *et al* (25) demonstrated that 53BP1-deficient mice were hypersensitive to radiation due to defects in NHEJ. Mu *et al* (181) found that the reduction in 53BP1 phosphorylation levels (not the levels of 53BP1 protein) induced the radiosensitization of glioblastoma cells by inhibiting NHEJ repair. All ionizing radiation therapy, whether it is multifraction radiotherapy (MFR) or single-dose radiotherapy activates different DNA repair mechanisms (182). Compared with an equivalent single dose of irradiation, both cancer cells and normal fibroblasts exhibit an enhanced survival following MFR, and this effect is entirely dependent on 53BP1/RIF1-mediated NHEJ repair (183). These results are of

clinical significance as they can guide the selection of the most effective ionizing radiation regimen by analyzing the expression status of the 53BP1-regulated NHEJ repair in tumors. However, although the mechanisms through which the 53BP1-mediated promotion of cancer cell recovery and survival can reduce patient outcome are understood, little is known regarding the DNA repair method that occurs between different radiation fractions. Roobol *et al* (184) monitored the accumulation of the endogenous 53BP1 and replication protein A using live-cell microscopy and found that low linear-energy-transfer (LET) X-ray-induced 53BP1 foci were rapidly and more dynamically resolved (184). Low-LET X-ray irradiation triggers NHEJ repair, while high-LET α-particles induce multiple replication protein A foci at closely interspaced DSB sites, thus promoting HR-prone repair (184). Nevertheless, the γH2AX and 53BP1 foci size have been shown to increase with LET, suggesting that the delay in repair kinetics was due to the occurrence of more complex damage (185). These findings appear to suggest that the biological effects of NHEJ or HR repair choices may be significantly influenced by the dose, as well as the type of radiation exposure. Therefore, current knowledge regarding the importance of 53BP1-mediated NHEJ repair in cancer therapy is at its early stages, and further studies focusing on the selective advantage of NHEJ-prone repair are required.

Function of 53BP1 in aging and telomere fusion. In human mammary epithelial cells from older individuals, the decreased activity of the primary DSB repair pathways, which play crucial roles in maintaining genome integrity, was found by Anglada *et al* (186). The deficient recruitment of 53BP1 to DSB sites in G1 cells from aged donors reveals a positive association between age-associated DNA repair defects and the aging process. As the expression levels of γH2AX and 53BP1 are promoted, Li *et al* (187) found a protective function of 53BP1-mediated NHEJ repair in premature ovarian failure. In addition to DSB repair modulation, 53BP1 maintains heterochromatin integrity and genomic stability through liquid-liquid phase separation (LLPS) with the heterochromatin protein HP1α in a mutually dependent manner (188). The LLPS of 53BP1 rescues heterochromatin de-repression and protects cells against stress-induced DNA damage and senescence. If senescence is bypassed, cells undergo crisis through the loss of checkpoints and this results in mass cell death, concomitant with further telomere shortening and spontaneous telomere fusions. Based on this, the auxo-actions of 53BP1-dependent NHEJ repair in telomere fusions cannot be ignored. Telomeres are protected by the six-subunit shelterin complex [telomeric repeat binding factor (TRF)1, TRF2, protection of telomeres 1 (POT1), TERF1 interacting nuclear factor 2 (TIN2), TINT1 and Rap1], which suppresses DNA damage signaling, DNA repair, and 5' end hyper-resection. In telomeres lacking TRF2, telomere fusion boosts are due to several separable effects of 53BP1 promote: A promotion of mobility of unprotected telomeres (189), the effects of oligomerization and synapsis involving telomere clustering (135), and the recruitment of the RIF1/Shieldin/CST axis, which is involved in counteracting 5' end resection. When telomeres are lost due to aging-associated erosion, breakage, or failed replication, the telomere fusions serve as a cell's final attempt to protect exposed chromosomal ends. However, inappropriate end-to-end chromosomal

rearrangements and telomere fusions promote genomic instability and carcinogenesis.

Function of 53BP1 in neurodevelopment and hyperproliferative diseases. Although 53BP1 is most well-known for its regulation of DNA damage repair mechanisms, it was initially discovered via its binding to p53. During the differentiation of human embryonic stem cells into neurons or into cortical organoids, a transcriptional co-regulatory effect of 53BP1 and UTX, a chromatin modifier, promotes human neurogenesis by upregulating key neurodevelopmental genes (190). Additionally, the activation of a 53BP1-USP28-p53 mitotic surveillance pathway facilitates centrosome defect-induced neural progenitor cell (NPC) depletion and microcephaly during development of the brain (191). In a p53-dependent pathway underlying primary microcephaly, a delay of spindle assembly caused by centrosome gene mutations triggers the activation of the 53BP1-USP28-p53 pathway, while 53BP1 deletion restores NPC proliferation and brain size (192). In another p53-dependent pathway, mutations in genes required for DNA repair or genomic stability induce the accumulation of DNA lesions that trigger DNA damage signaling in NPCs to activate p53 (192). Thus, the role of 53BP1 as a regulator of DNA damage repair deserves further study. In the developing epidermis, the activation of the 53BP1-USP28-p53 pathway induced by genetically ablating centrosomes also cause a thinner epidermis and hair follicle arrest (193). These studies provide insight into 53BP1-related neurodevelopment and hyperproliferative diseases that may recapitulate developmental programs.

53BP1 inhibition increases CRISPR-Cas9 genome-editing efficiency. Precise genomic editing based on programming nucleases, such as the CRISPR/CRISPR-Cas system, are controlled by HR repair and limited by the competing error-prone NHEJ repair (194,195). As a critical regulator of the method of repair between NHEJ and HR, 53BP1 deficiency induces an increase in BRCA1-mediated HR repair, which suggests that the inhibition of 53BP1 may be a promising tool to manipulate repair method and promote genome-editing efficiency. Recently, Canny *et al* (196) and Sun *et al* (197) screened out inhibitors of 53BP1, inhibitor 53 (i53) and DP308, and they targeted the tandem Tudor domain of 53BP1. i53 blocked the interaction between 53BP1 and H4K20Me2 at DSB sites and improved gene targeting and chromosomal gene conversion by up to 5.6-fold. Paulsen *et al* (198) found that the ectopic expression of the dominant-negative murine form of 53BP1 (mdn53BP1) competitively antagonized 53BP1 recruitment to DSB sites and improved Cas9-mediated HR repair activity. Similarly, RAD18, a DNA damage response factor on Cas9-induced HDR, competitively binds H2AK15ub with greater affinity than 53BP1, thereby inhibiting 53BP1 recruitment to DSB sites (199). Additionally, researchers fused Cas9 nucleases and DN1S, a dominant-negative mutant of 53BP1, and this fusion improved HR repair frequency, reaching 86% in K562 cells, and almost 70% in leukocyte adhesion deficiency (LAD) patient-derived immortalized B lymphocytes (200). Therefore, the inhibition of 53BP1 improves the efficiencies of CRISPR-Cas9-mediated precise gene correction/insertion, significantly reducing undesirable NHEJ repairs at the nuclease cleavage site.

7. Conclusion and future perspectives

There are several open-ended questions remaining in this field. First, ~80% of ionizing radiation- or drug-induced DSBs are repaired by the NHEJ pathway, even in the G2 phase. However, in the face of the different causes of DSBs, it would be beneficial to determine the reasons why 53BP1-mediated NHEJ is beneficial or harmful. The nucleolytic, polymerization and ligation steps of NHEJ are flexible, as numerous different structural and chemical DNA end configurations can be ligated at DSB sites. Based on the present review, the mechanical or biochemical environment of chromatin, cell cycle phases and PTMs of 53BP1 may explain the synergistic effects of these ligated complexes. Second, the recruitment of 53BP1 on chromatin around the DSB form 53BP1 nano-domains that are shaped by chromatin topology. However, it remains unknown as to whether the regulation of 53BP1 recruitment by histone molecular markers (such as H2AK15Ub and H4K20Me2) with binding specificity and epigenetic modification enzymes (such as MMSET and KAT5) are implemented in parallel, or whether they actually regulate different stages of 53BP1 nanodomain formation. Third, 53BP1, in conjunction with RIF1 and PTIP, promotes the restraints of end resection to antagonize HR repair, and consequently promotes NHEJ repair. As the cell cycle progresses, 53BP1 gradually loses its dominant role in binding with its helper complex. However, the mechanisms through which 53BP1 and HR-related proteins, such as BRCA1 achieve a dynamic balance in damaged chromatin remain unknown. Fourth, telomere protection in mammals is mediated by TRF2, which binds chromosomal ends and ensures genomic integrity through inhibiting NHEJ repair, which triggers chromosome fusion end connection (201,202). 53BP1 disturbs telomere stability, possibly through interaction with the TRF2 Shelterin component, and induces telomere dysfunction and the aging process (186). In future studies, these questions regarding 53BP1 function need be addressed to obtain a more complete and accurate understanding of DSB repair and improve the clinical options available to patients of several diseases.

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LC conceived and designed the review article. TL wrote the manuscript, and prepared the table and figures. SD and ZP reviewed and edited the manuscript. All authors have read and approved the manuscript, and agree to be accountable for all

aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved. Data authentication is not applicable.

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Competing interests

The authors declare that they have no competing interests.

References

1. Aparicio T, Baer R and Gautier J: DNA double-strand break repair pathway choice and cancer. *DNA Repair (Amst)* 19: 169-175, 2014.
2. Jackson SP and Bartek J: The DNA-damage response in human biology and disease. *Nature* 461: 1071-1078, 2009.
3. Alt FW and Schwer B: DNA double-strand breaks as drivers of neural genomic change, function, and disease. *DNA Repair (Amst)* 71: 158-163, 2018.
4. Gorthi A and Bishop AJR: Ewing sarcoma fusion oncogene: At the crossroads of transcription and DNA damage response. *Mol Cell Oncol* 5: e1465014, 2018.
5. Daley JM, Niu H, Miller AS and Sung P: Biochemical mechanism of DSB end resection and its regulation. *DNA Repair (Amst)* 32: 66-74, 2015.
6. Heyer WD, Ehmsen KT and Liu J: Regulation of homologous recombination in eukaryotes. *Annu Rev Genet* 44: 113-139, 2010.
7. Matos J and West SC: Holliday junction resolution: Regulation in space and time. *DNA Repair (Amst)* 19: 176-181, 2014.
8. Ira G, Pellicioli A, Balijja A, Wang X, Fiorani S, Carotenuto W, Liberi G, Bressan D, Wan L, Hollingsworth NM, *et al*: DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. *Nature* 431: 1011-1017, 2004.
9. Roerink SF, van Schendel R and Tijsterman M: Polymerase theta-mediated end joining of replication-associated DNA breaks in *C. Elegans*. *Genome Res* 24: 954-962, 2014.
10. Zhang Y and Jasin M: An essential role for CtIP in chromosomal translocation formation through an alternative end-joining pathway. *Nat Struct Mol Biol* 18: 80-84, 2011.
11. Paques F and Haber JE: Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* 63: 349-404, 1999.
12. Scully R, Panday A, Elango R and Willis NA: DNA double-strand break repair-pathway choice in somatic mammalian cells. *Nat Rev Mol Cell Biol* 20: 698-714, 2019.
13. Mimori T and Hardin JA: Mechanism of interaction between Ku protein and DNA. *J Biol Chem* 261: 10375-10379, 1986.
14. Ceccaldi R, Rondinelli B and D'Andrea AD: Repair pathway choices and consequences at the double-strand break. *Trends Cell Biol* 26: 52-64, 2016.
15. Mao Z, Bozzella M, Seluanov A and Gorbunova V: Comparison of nonhomologous end joining and homologous recombination in human cells. *DNA Repair (Amst)* 7: 1765-1771, 2008.
16. Pannunzio NR, Watanabe G and Lieber MR: Nonhomologous DNA end-joining for repair of DNA double-strand breaks. *J Biol Chem* 293: 10512-10523, 2018.
17. Soutoglou E, Dorn JF, Sengupta K, Jasin M, Nussenzweig A, Ried T, Danuser G and Misteli T: Positional stability of single double-strand breaks in mammalian cells. *Nat Cell Biol* 9: 675-682, 2007.
18. Tarsounas M and Sung P: The antitumorigenic roles of BRCA1-BARD1 in DNA repair and replication. *Nat Rev Mol Cell Biol* 21: 284-299, 2020.
19. Beucher A, Birraux J, Tchouandong L, Barton O, Shibata A, Conrad S, Goodarzi AA, Krempler A, Jeggo PA and Löbrich M: ATM and Artemis promote homologous recombination of radiation-induced DNA double-strand breaks in G2. *EMBO J* 28: 3413-3427, 2009.
20. Karanam K, Kafri R, Loewer A and Lahav G: 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: 320-329, 2012.
21. Zhao B, Rothenberg E, Ramsden DA and Lieber MR: The molecular basis and disease relevance of non-homologous DNA end joining. *Nat Rev Mol Cell Biol* 21: 765-781, 2020.
22. Hustedt N and Durocher D: The control of DNA repair by the cell cycle. *Nat Cell Biol* 19: 1-9, 2016.
23. Morales JC, Xia Z, Lu T, Aldrich MB, Wang B, Rosales C, Kellems RE, Hittelman WN, Elledge SJ and Carpenter PB: Role for the BRCA1 C-terminal repeats (BRCT) protein 53BP1 in maintaining genomic stability. *J Biol Chem* 278: 14971-14977, 2003.
24. Rappold I, Iwabuchi K, Date T and Chen J: Tumor suppressor p53 binding protein 1 (53BP1) is involved in DNA damage-signaling pathways. *J Cell Biol* 153: 613-620, 2001.
25. Ward IM, Minn K, van Deursen J and Chen J: p53 Binding protein 53BP1 is required for DNA damage responses and tumor suppression in mice. *Mol Cell Biol* 23: 2556-2563, 2003.
26. Adams MM and Carpenter PB: Tying the loose ends together in DNA double strand break repair with 53BP1. *Cell Div* 1: 19, 2006.
27. Panier S and Boulton SJ: Double-strand break repair: 53BP1 comes into focus. *Nat Rev Mol Cell Biol* 15: 7-18, 2014.
28. von Morgen P, Lidak T, Horejsi Z and Macurek L: Nuclear localisation of 53BP1 is regulated by phosphorylation of the nuclear localisation signal. *Biol Cell* 110: 137-146, 2018.
29. Mirman Z and de Lange T: 53BP1: A DSB escort. *Genes Dev* 34: 7-23, 2020.
30. He YJ, Meghani K, Caron MC, Yang C, Ronato DA, Bian J, Sharma A, Moore J, Niraj J, Detappe A, *et al*: DYNLL1 binds to MRE11 to limit DNA end resection in BRCA1-deficient cells. *Nature* 563: 522-526, 2018.
31. Becker JR, Cuella-Martin R, Barazas M, Liu R, Oliveira C, Oliver AW, Bilham K, Holt AB, Blackford AN, Heierhorst J, *et al*: The ASCIZ-DYNLL1 axis promotes 53BP1-dependent non-homologous end joining and PARP inhibitor sensitivity. *Nat Commun* 9: 5406, 2018.
32. West KL, Kelliher JL, Xu Z, An L, Reed MR, Eoff RL, Wang J, Huen MSY and Leung JWC: LC8/DYNLL1 is a 53BP1 effector and regulates checkpoint activation. *Nucleic Acids Res* 47: 6236-6249, 2019.
33. Zgheib O, Pataky K, Brugger J and Halazonetis TD: An oligomerized 53BP1 Tudor domain suffices for recognition of DNA double-strand breaks. *Mol Cell Biol* 29: 1050-1058, 2009.
34. Adams MM, Wang B, Xia Z, Morales JC, Lu X, Donehower LA, Bochar DA, Elledge SJ and Carpenter PB: 53BP1 oligomerization is independent of its methylation by PRMT1. *Cell Cycle* 4: 1854-1861, 2005.
35. Boisvert FM, Rhie A, Richard S and Doherty AJ: The GAR motif of 53BP1 is arginine methylated by PRMT1 and is necessary for 53BP1 DNA binding activity. *Cell Cycle* 4: 1834-1841, 2005.
36. Botuyan MV, Lee J, Ward IM, Kim JE, Thompson JR, Chen J and Mer G: Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in DNA repair. *Cell* 127: 1361-1373, 2006.
37. Pellegrino S, Michelena J, Teloni F, Imhof R and Altmeyer M: Replication-coupled dilution of H4K20me2 guides 53BP1 to pre-replicative chromatin. *Cell Rep* 19: 1819-1831, 2017.
38. Fradet-Turcotte A, Canny MD, Escobedo-Diaz C, Orthwein A, Leung CC, Huang H, Landry MC, Kitevska-LeBlanc J, Noordermeer SM, Sicheri F and Durocher D: 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. *Nature* 499: 50-54, 2013.
39. Derbyshire DJ, Basu BP, Serpell LC, Joo WS, Date T, Iwabuchi K and Doherty AJ: Crystal structure of human 53BP1 BRCT domains bound to p53 tumour suppressor. *EMBO J* 21: 3863-3872, 2002.
40. Cuella-Martin R, Oliveira C, Lockstone HE, Snellenberg S, Grolmusova N and Chapman JR: 53BP1 Integrates DNA repair and p53-dependent cell fate decisions via distinct mechanisms. *Mol Cell* 64: 51-64, 2016.
41. Riballo E, Kühne M, Rief N, Doherty A, Smith GC, Recio MJ, Reis C, Dahm K, Fricke A, Krempler A, *et al*: A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to gamma-H2AX foci. *Mol Cell* 16: 715-724, 2004.
42. Chang HHY, Pannunzio NR, Adachi N and Lieber MR: Non-homologous DNA end joining and alternative pathways to double-strand break repair. *Nat Rev Mol Cell Biol* 18: 495-506, 2017.

43. Uziel T, Lerenthal Y, Moyal L, Andegeko Y, Mittelman L and Shiloh Y: Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J* 22: 5612-5621, 2003.
44. Panier S and Durocher D: Push back to respond better: Regulatory inhibition of the DNA double-strand break response. *Nat Rev Mol Cell Biol* 14: 661-672, 2013.
45. Stewart GS, Panier S, Townsend K, Al-Hakim AK, Kolas NK, Miller ES, Nakada S, Ylanko J, Olivarius S, Mendez M, *et al*: The RIDDLE syndrome protein mediates a ubiquitin-dependent signaling cascade at sites of DNA damage. *Cell* 136: 420-434, 2009.
46. Stewart GS, Wang B, Bignell CR, Taylor AM and Elledge SJ: MDC1 is a mediator of the mammalian DNA damage checkpoint. *Nature* 421: 961-966, 2003.
47. Mattioli F, Vissers JH, van Dijk WJ, Ikpa P, Citterio E, Vermeulen W, Martijn JA and Sixma TK: RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. *Cell* 150: 1182-1195, 2012.
48. Gudjonsson T, Altmeyer M, Savic V, Toledo L, Dinant C, Grøfte M, Bartkova J, Poulsen M, Oka Y, Bekker-Jensen S, *et al*: TRIP12 and UBR5 suppress spreading of chromatin ubiquitylation at damaged chromosomes. *Cell* 150: 697-709, 2012.
49. Bekker-Jensen S, Rendtlew Danielsen J, Fugger K, Gromova I, Nerstedt A, Lukas C, Bartek J, Lukas J and Mailand N: HERC2 coordinates ubiquitin-dependent assembly of DNA repair factors on damaged chromosomes. *Nat Cell Biol* 12: 80-86, 1-12, 2010.
50. Shibata A and Jeggo PA: Roles for 53BP1 in the repair of radiation-induced DNA double strand breaks. *DNA Repair (Amst)* 93: 102915, 2020.
51. Pesavento JJ, Yang H, Kelleher NL and Mizzen CA: Certain and progressive methylation of histone H4 at lysine 20 during the cell cycle. *Mol Cell Biol* 28: 468-486, 2008.
52. Mallette FA, Mattioli F, Cui G, Young LC, Hendzel MJ, Mer G, Sixma TK and Richard S: RNF8- and RNF168-dependent degradation of KDM4A/JMJD2A triggers 53BP1 recruitment to DNA damage sites. *EMBO J* 31: 1865-1878, 2012.
53. Min J, Allali-Hassani A, Nady N, Qi C, Ouyang H, Liu Y, MacKenzie F, Vedadi M and Arrowsmith CH: L3MBTL1 recognition of mono- and dimethylated histones. *Nat Struct Mol Biol* 14: 1229-1230, 2007.
54. Huen MS, Huang J, Leung JW, Sy SM, Leung KM, Ching YP, Tsao SW and Chen J: Regulation of chromatin architecture by the PWWP domain-containing DNA damage-responsive factor EXPAND1/MUM1. *Mol Cell* 37: 854-864, 2010.
55. Chapman JR, Barral P, Vannier JB, Borel V, Steger M, Tomas-Loba A, Sartori A, Adams IR, Batista FD and Boulton SJ: RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. *Mol Cell* 49: 858-871, 2013.
56. Callen E, Di Virgilio M, Kruhlak MJ, Nieto-Soler M, Wong N, Chen HT, Faryabi RB, Polato F, Santos M, Starnes LM, *et al*: 53BP1 mediates productive and mutagenic DNA repair through distinct phosphoprotein interactions. *Cell* 153: 1266-1280, 2013.
57. Xie HY, Zhang TM, Hu SY, Shao ZM and Li DQ: Dimerization of MORC2 through its C-terminal coiled-coil domain enhances chromatin dynamics and promotes DNA repair. *Cell Commun Signal* 17: 160, 2019.
58. Wu R, Liu W, Sun Y, Shen C, Guo J, Zhao J, Mao G, Li Y and Du G: Nanoscale insight into chromatin remodeling and DNA repair complex in HeLa cells after ionizing radiation. *DNA Repair (Amst)* 96: 102974, 2020.
59. Ochs F, Karemore G, Miron E, Brown J, Sedlackova H, Rask MB, Lampe M, Buckle V, Schermelleh L, Lukas J and Lukas C: Stabilization of chromatin topology safeguards genome integrity. *Nature* 574: 571-574, 2019.
60. Arnould C, Rocher V, Finoux AL, Clouaire T, Li K, Zhou F, Caron P, Mangeot PE, Ricci EP, Mourad R, *et al*: Loop extrusion as a mechanism for formation of DNA damage repair foci. *Nature* 590: 660-665, 2021.
61. Caron P and Polo SE: Reshaping chromatin architecture around DNA breaks. *Trends Biochem Sci* 45: 177-179, 2020.
62. Lou J, Priest DG, Solano A, Kerjovan A and Hinde E: Spatiotemporal dynamics of 53BP1 dimer recruitment to a DNA double strand break. *Nat Commun* 11: 5776, 2020.
63. Kilic S, Lezaja A, Gatti M, Bianco E, Michelenia J, Imhof R and Altmeyer M: Phase separation of 53BP1 determines liquid-like behavior of DNA repair compartments. *EMBO J* 38: e101379, 2019.
64. Banani SF, Lee HO, Hyman AA and Rosen MK: Biomolecular condensates: Organizers of cellular biochemistry. *Nat Rev Mol Cell Biol* 18: 285-298, 2017.
65. Shin Y, Berry J, Pannucci N, Haataja MP, Toettcher JE and Brangwynne CP: Spatiotemporal control of intracellular phase transitions using light-activated optoDroplets. *Cell* 168: 159-171, e14, 2017.
66. Piccinno R, Minneker V and Roukos V: 53BP1-DNA repair enters a new liquid phase. *EMBO J* 38: e102871, 2019.
67. Pessina F, Giavazzi F, Yin Y, Gioia U, Vitelli V, Galbiati A, Barozzi S, Garre M, Oldani A, Flaus A, *et al*: Functional transcription promoters at DNA double-strand breaks mediate RNA-driven phase separation of damage-response factors. *Nat Cell Biol* 21: 1286-1299, 2019.
68. Jakob B, Dubiak-Szepietowska M, Janiel E, Schmidt A, Durante M and Taucher-Scholz G: Differential repair protein recruitment at sites of clustered and isolated DNA double-strand breaks produced by high-energy heavy ions. *Sci Rep* 10: 1443, 2020.
69. Eaton JA and Zidovska A: Structural and dynamical signatures of local DNA damage in live cells. *Biophys J* 118: 2168-2180, 2020.
70. Drané P, Brault ME, Cui G, Meghani K, Chaubey S, Detappe A, Parnandi N, He Y, Zheng XF, Botuyan MV, *et al*: TIRR regulates 53BP1 by masking its histone methyl-lysine binding function. *Nature* 543: 211-216, 2017.
71. Zhang A, Peng B, Huang P, Chen J and Gong Z: The p53-binding protein 1-Tudor-interacting repair regulator complex participates in the DNA damage response. *J Biol Chem* 292: 6461-6467, 2017.
72. Botuyan MV, Cui G, Drané P, Oliveira C, Detappe A, Brault ME, Parnandi N, Chaubey S, Thompson JR, Bragantini B, *et al*: Mechanism of 53BP1 activity regulation by RNA-binding TIRR and a designer protein. *Nat Struct Mol Biol* 25: 591-600, 2018.
73. Wang J, Yuan Z, Cui Y, Xie R, Yang G, Kassab MA, Wang M, Ma Y, Wu C, Yu X and Liu X: Molecular basis for the inhibition of the methyl-lysine binding function of 53BP1 by TIRR. *Nat Commun* 9: 2689, 2018.
74. Dai Y, Zhang A, Shan S, Gong Z and Zhou Z: Structural basis for recognition of 53BP1 tandem Tudor domain by TIRR. *Nat Commun* 9: 2123, 2018.
75. Parnandi N, Rendo V, Cui G, Botuyan MV, Remisova M, Nguyen H, Drané P, Beroukhim R, Altmeyer M, Mer G and Chowdhury D: TIRR inhibits the 53BP1-p53 complex to alter cell-fate programs. *Mol Cell* 81: 2583-2595.e6, 2021.
76. Shibata A and Jeggo PA: Roles for the DNA-PK complex and 53BP1 in protecting ends from resection during DNA double-strand break repair. *J Radiat Res* 61: 718-726, 2020.
77. Broustas CG, Duval AJ, Chaudhary KR, Friedman RA, Virk RK and Lieberman HB: Targeting MEK5 impairs nonhomologous end-joining repair and sensitizes prostate cancer to DNA damaging agents. *Oncogene* 39: 2467-2477, 2020.
78. Sharma A and Almasan A: USP14 regulates DNA damage response and is a target for radiosensitization in non-small cell lung cancer. *Int J Mol Sci* 21: 6383, 2020.
79. Feng M, Wang Y, Bi L, Zhang P, Wang H, Zhao Z, Mao JH and Wei G: CRL4^{DTL} degrades DNA-PKcs to modulate NHEJ repair and induce genomic instability and subsequent malignant transformation. *Oncogene* 40: 2096-2111, 2021.
80. Caggiano C, Cavallo F, Giannattasio T, Cappelletti G, Rossi P, Grimaldi P, Feldman DR, Jasim M and Barchi M: Testicular germ cell tumors acquire cisplatin resistance by rebalancing the usage of DNA repair pathways. *Cancers (Basel)* 13: 787, 2021.
81. Ma S, Rong Z, Liu C, Qin X, Zhang X and Chen Q: DNA damage promotes microtubule dynamics through a DNA-PK-AKT axis for enhanced repair. *J Cell Biol* 220: e201911025, 2021.
82. Lee KY and Dutta A: Chk1 promotes non-homologous end joining in G1 through direct phosphorylation of ASF1A. *Cell Rep* 34: 108680, 2021.
83. Wardlaw CP, Carr AM and Oliver AW: TopBP1: A BRCT-scaffold protein functioning in multiple cellular pathways. *DNA Repair (Amst)* 22: 165-174, 2014.
84. Bigot N, Day M, Baldock RA, Watts FZ, Oliver AW and Pearl LH: Phosphorylation-mediated interactions with TOPBP1 couple 53BP1 and 9-1-1 to control the G1 DNA damage checkpoint. *Elife* 8: e44353, 2019.
85. Ha K, Ma C, Lin H, Tang L, Lian Z, Zhao F, Li JM, Zhen B, Pei H, Han S, *et al*: The anaphase promoting complex impacts repair choice by protecting ubiquitin signalling at DNA damage sites. *Nat Commun* 8: 15751, 2017.
86. Beishline K, Kelly CM, Olofsson BA, Koduri S, Emrich J, Greenberg RA and Azizkhan-Clifford J: Spl facilitates DNA double-strand break repair through a nontranscriptional mechanism. *Mol Cell Biol* 32: 3790-3799, 2012.

87. Swift ML, Beishline K, Flashner S and Azizkhan-Clifford J: DSB repair pathway choice is regulated by recruitment of 53BP1 through cell cycle-dependent regulation of Sp1. *Cell Rep* 34: 108840, 2021.
88. Tang M, Feng X, Pei G, Srivastava M, Wang C, Chen Z, Li S, Zhang H, Zhao Z, Li X and Chen J: FOXK1 participates in DNA damage response by controlling 53BP1 function. *Cell Rep* 32: 108018, 2020.
89. Murray-Nerger LA, Justice JL, Rekapalli P, Hutton JE and Cristea IM: Lamin B1 acetylation slows the G1 to S cell cycle transition through inhibition of DNA repair. *Nucleic Acids Res* 49: 2044-2064, 2021.
90. Svobodová Kovaříková A, Legartová S, Krejčí J and Bártová E: H3K9me3 and H4K20me3 represent the epigenetic landscape for 53BP1 binding to DNA lesions. *Aging (Albany NY)* 10: 2585-2605, 2018.
91. Nakamura K, Saredi G, Becker JR, Foster BM, Nguyen NV, Beyer TE, Cesa LC, Faull PA, Lukauskas S, Frimurer T, *et al*: H4K20me0 recognition by BRCA1-BARD1 directs homologous recombination to sister chromatids. *Nat Cell Biol* 21: 311-318, 2019.
92. Harding SM and Bristow RG: Discordance between phosphorylation and recruitment of 53BP1 in response to DNA double-strand breaks. *Cell Cycle* 11: 1432-1444, 2012.
93. Feng L, Li N, Li Y, Wang J, Gao M, Wang W and Chen J: Cell cycle-dependent inhibition of 53BP1 signaling by BRCA1. *Cell Discov* 1: 15019, 2015.
94. Bothmer A, Robbiani DF, Di Virgilio M, Bunting SF, Klein IA, Feldhahn N, Barlow J, Chen HT, Bosque D, Callen E, *et al*: Regulation of DNA end joining, resection, and immunoglobulin class switch recombination by 53BP1. *Mol Cell* 42: 319-329, 2011.
95. Sanz-Garcia M, Monsalve DM, Sevilla A and Lazo PA: Vaccinia-related kinase 1 (VRK1) is an upstream nucleosomal kinase required for the assembly of 53BP1 foci in response to ionizing radiation-induced DNA damage. *J Biol Chem* 287: 23757-23768, 2012.
96. Campillo-Marcos I, Garcia-Gonzalez R, Navarro-Carrasco E and Lazo PA: The human VRK1 chromatin kinase in cancer biology. *Cancer Lett* 503: 117-128, 2021.
97. Jiang Y, Dong Y, Luo Y, Jiang S, Meng FL, Tan M, Li J and Zang Y: AMPK-mediated phosphorylation on 53BP1 promotes c-NHEJ. *Cell Rep* 34: 108713, 2021.
98. Yang Y, Lei T, Du S, Tong R, Wang H, Yang J, Huang J, Sun M, Wang Y and Dong Z: Nuclear GSK3 β induces DNA double-strand break repair by phosphorylating 53BP1 in glioblastoma. *Int J Oncol* 52: 709-720, 2018.
99. Lee DH, Acharya SS, Kwon M, Drane P, Guan Y, Adelmant G, Kalev P, Shah J, Pellman D, Marto JA and Chowdhury D: Dephosphorylation enables the recruitment of 53BP1 to double-strand DNA breaks. *Mol Cell* 54: 512-525, 2014.
100. Isono M, Niimi A, Oike T, Hagiwara Y, Sato H, Sekine R, Yoshida Y, Isobe SY, Obuse C, Nishi R, *et al*: BRCA1 directs the repair pathway to homologous recombination by promoting 53BP1 dephosphorylation. *Cell Rep* 18: 520-532, 2017.
101. Burdova K, Storchova R, Palek M and Macurek L: WIP1 promotes homologous recombination and modulates sensitivity to PARP inhibitors. *Cells* 8: 1258, 2019.
102. Bohgaki M, Bohgaki T, El Ghamrasni S, Srikumar T, Maire G, Panier S, Fradet-Turcotte A, Stewart GS, Raught B, Hakem A and Hakem R: RNF168 ubiquitylates 53BP1 and controls its response to DNA double-strand breaks. *Proc Natl Acad Sci USA* 110: 20982-20987, 2013.
103. Wang Y, Hou Y, Zhang W, Alvarez AA, Bai Y, Hu B, Cheng SY, Yang K, Li Y and Feng H: Lipolytic inhibitor GOS2 modulates glioma stem-like cell radiation response. *J Exp Clin Cancer Res* 38: 147, 2019.
104. Lee NS, Chang HR, Kim S, Ji JH, Lee J, Lee HJ, Seo Y, Kang M, Han JS, Myung K, *et al*: Ring finger protein 126 (RNF126) suppresses ionizing radiation-induced p53-binding protein 1 (53BP1) focus formation. *J Biol Chem* 293: 588-598, 2018.
105. Yang C, Zang W, Tang Z, Ji Y, Xu R, Yang Y, Luo A, Hu B, Zhang Z, Liu Z and Zheng X: A20/TNFAIP3 regulates the DNA damage response and mediates tumor cell resistance to DNA-damaging therapy. *Cancer Res* 78: 1069-1082, 2018.
106. Walser F, Mulder MPC, Bragantini B, Burger S, Gubser T, Gatti M, Botuyan MV, Villa A, Altmeyer M, Neri D, *et al*: Ubiquitin phosphorylation at Thr12 modulates the DNA damage response. *Mol Cell* 80: 423-436.e9, 2020.
107. Poulsen M, Lukas C, Lukas J, Bekker-Jensen S and Mailand N: Human RNF169 is a negative regulator of the ubiquitin-dependent response to DNA double-strand breaks. *J Cell Biol* 197: 189-199, 2012.
108. An L, Dong C, Li J, Chen J, Yuan J, Huang J, Chan KM, Yu CH and Huen MSY: RNF169 limits 53BP1 deposition at DSBs to stimulate single-strand annealing repair. *Proc Natl Acad Sci USA* 115: E8286-E8295, 2018.
109. Hu Q, Botuyan MV, Cui G, Zhao D and Mer G: Mechanisms of ubiquitin-nucleosome recognition and regulation of 53BP1 chromatin recruitment by RNF168/169 and RAD18. *Mol Cell* 66: 473-487.e9, 2017.
110. Hwang JW, Kim SN, Myung N, Song D, Han G, Bae GU, Bedford MT and Kim YK: PRMT5 promotes DNA repair through methylation of 53BP1 and is regulated by Src-mediated phosphorylation. *Commun Biol* 3: 428, 2020.
111. Clarke TL, Sanchez-Bailon MP, Chiang K, Reynolds JJ, Herrero-Ruiz J, Bandejas TM, Matias PM, Maslen SL, Skehel JM, Stewart GS and Davies CC: PRMT5-dependent methylation of the TIP60 coactivator RUVBL1 is a key regulator of homologous recombination. *Mol Cell* 65: 900-916.e7, 2017.
112. Zhang H, Devoucoux M, Song X, Li L, Ayaz G, Cheng H, Tempel W, Dong C, Loppnau P, Côté J and Min J: Structural basis for EPC1-mediated recruitment of MBTD1 into the NuA4/TIP60 acetyltransferase complex. *Cell Rep* 30: 3996-4002.e4, 2020.
113. Jacquet K, Fradet-Turcotte A, Avvakumov N, Lambert JP, Roques C, Pandita RK, Paquet E, Herst P, Gingras AC, Pandita TK, *et al*: The TIP60 complex regulates bivalent chromatin recognition by 53BP1 through direct H4K20me binding and H2AK15 acetylation. *Mol Cell* 62: 409-421, 2016.
114. Sivanand S, Rhoades S, Jiang Q, Lee JV, Benci J, Zhang J, Yuan S, Viney I, Zhao S, Carrer A, *et al*: Nuclear acetyl-CoA production by ACLY promotes homologous recombination. *Mol Cell* 67: 252-265.e6, 2017.
115. Chen Z, Zhong J, Ren X, Liu W, Wu D, Chen C, Huang H, Huang X, Liu Y and Liu J: Involvement of a novel regulatory cascade consisting of SET-H3K18ac/H3K27ac-53BP1 in Cr(VI)-induced malignant transformation of 16HBE cells. *Toxicol Lett* 339: 70-77, 2021.
116. Guo X, Bai Y, Zhao M, Zhou M, Shen Q, Yun CH, Zhang H, Zhu WG and Wang J: Acetylation of 53BP1 dictates the DNA double strand break repair pathway. *Nucleic Acids Res* 46: 689-703, 2018.
117. Li N, Zhang Y, Han X, Liang K, Wang J, Feng L, Wang W, Songyang Z, Lin C, Yang L, *et al*: Poly-ADP ribosylation of PTEN by Tankyrases promotes PTEN degradation and tumor growth. *Genes Dev* 29: 157-170, 2015.
118. Guettler S, LaRose J, Petsalaki E, Gish G, Scotter A, Pawson T, Rottapel R and Sicheri F: Structural basis and sequence rules for substrate recognition by Tankyrases explain the basis for cherubism disease. *Cell* 147: 1340-1354, 2011.
119. DaRosa PA, Wang Z, Jiang X, Pruneda JN, Cong F, Klevit RE and Xu W: Allosteric activation of the RNF146 ubiquitin ligase by a poly(ADP-ribosyl)ation signal. *Nature* 517: 223-226, 2015.
120. Zhang Y, Liu S, Mickanin C, Feng Y, Charlat O, Michaud GA, Schirle M, Shi X, Hild M, Bauer A, *et al*: RNF146 is a poly(ADP-ribose)-directed E3 ligase that regulates axin degradation and Wnt signalling. *Nat Cell Biol* 13: 623-629, 2011.
121. Zhang F, Lou L, Peng B, Song X, Reizes O, Almasan A and Gong Z: Nudix hydrolase NUDT16 regulates 53BP1 protein by Reversing 53BP1 ADP-ribosylation. *Cancer Res* 80: 999-1010, 2020.
122. Palazzo L, Thomas B, Jemth AS, Colby T, Leidecker O, Feijs KL, Zaja R, Loseva O, Puigvert JC, Matic I, *et al*: Processing of protein ADP-ribosylation by Nudix hydrolases. *Biochem J* 468: 293-301, 2015.
123. Mackay DR, Howa AC, Werner TL and Ullman KS: Nup153 and Nup50 promote recruitment of 53BP1 to DNA repair foci by antagonizing BRCA1-dependent events. *J Cell Sci* 130: 3347-3359, 2017.
124. Duheron V, Nilles N, Pecenko S, Martinelli V and Fahrenkrog B: Localisation of Nup153 and SENP1 to nuclear pore complexes is required for 53BP1-mediated DNA double-strand break repair. *J Cell Sci* 130: 2306-2316, 2017.
125. Lu WT, Hawley BR, Skalka GL, Baldock RA, Smith EM, Bader AS, Malewicz M, Watts FZ, Wilczynska A and Bushell M: Drosha drives the formation of DNA:RNA hybrids around DNA break sites to facilitate DNA repair. *Nat Commun* 9: 532, 2018.

126. Cabrini M, Roncador M, Galbiati A, Cipolla L, Maffia A, Iannelli F, Sabbioneda S, d'Adda di Fagnana F and Francia S: DROSHA is recruited to DNA damage sites by the MRN complex to promote non-homologous end joining. *J Cell Sci* 134: jcs249706, 2021.
127. Wikinayadhaanee R, Lerksuthirat T, Stitthantrakul W, Chitphuk S, Sura T and Dejsuphong D: TRIM29 is required for efficient recruitment of 53BP1 in response to DNA double-strand breaks in vertebrate cells. *FEBS Open Bio* 10: 2055-2071, 2020.
128. Alonso-de Vega I, Paz-Cabrera MC, Rother MB, Wiegant WW, Checa-Rodriguez C, Hernandez-Fernaund JR, Huertas P, Freire R, van Attikum H and Smits VAI: PHF2 regulates homology-directed DNA repair by controlling the resection of DNA double strand breaks. *Nucleic Acids Res* 48: 4915-4927, 2020.
129. Koo GB, Ji JH, Cho H, Morgan MJ and Kim YS: Nuclear TRADD prevents DNA damage-mediated death by facilitating non-homologous end-joining repair. *Sci Rep* 7: 3332, 2017.
130. Clements KE, Schleicher EM, Thakar T, Hale A, Dhooonmoon A, Tolman NJ, Sharma A, Liang X, Imamura Kawasawa Y, Nicolae CM, *et al.*: Identification of regulators of poly-ADP-ribose polymerase inhibitor response through complementary CRISPR knockout and activation screens. *Nat Commun* 11: 6118, 2020.
131. Shenoy TR, Boysen G, Wang MY, Xu QZ, Guo W, Koh FM, Wang C, Zhang LZ, Wang Y, Gil V, *et al.*: CHD1 loss sensitizes prostate cancer to DNA damaging therapy by promoting error-prone double-strand break repair. *Ann Oncol* 28: 1495-1507, 2017.
132. Fouquin A, Guirouilh-Barbat J, Lopez B, Hall J, Amor-Gu  ret M and Pennaneach V: PARP2 controls double-strand break repair pathway choice by limiting 53BP1 accumulation at DNA damage sites and promoting end-resection. *Nucleic Acids Res* 45: 12325-12339, 2017.
133. Zimmermann M and de Lange T: 53BP1: Pro choice in DNA repair. *Trends Cell Biol* 24: 108-117, 2014.
134. Escribano-Diaz C and Durocher D: DNA repair pathway choice-a PTIP of the hat to 53BP1. *EMBO Rep* 14: 665-666, 2013.
135. Lottersberger F, Bothmer A, Robbiani DF, Nussenzweig MC and de Lange T: Role of 53BP1 oligomerization in regulating double-strand break repair. *Proc Natl Acad Sci USA* 110: 2146-2151, 2013.
136. Manis JP, Morales JC, Xia Z, Kutok JL, Alt FW and Carpenter PB: 53BP1 links DNA damage-response pathways to immunoglobulin heavy chain class-switch recombination. *Nat Immunol* 5: 481-487, 2004.
137. Methot SP and Di Noia JM: Molecular mechanisms of somatic hypermutation and class switch recombination. *Adv Immunol* 133: 37-87, 2017.
138. Gupta R, Somyajit K, Narita T, Maskey E, Stanlie A, Kremer M, Typas D, Lammers M, Mailand N, Nussenzweig A, *et al.*: DNA repair network analysis reveals shieldin as a key regulator of NHEJ and PARP inhibitor sensitivity. *Cell* 173: 972-988.e23, 2018.
139. Ghezraoui H, Oliveira C, Becker JR, Bilham K, Moralli D, Anzilotti C, Fischer R, Deobagkar-Lele M, Sanchiz-Calvo M, Fueyo-Marcos E, *et al.*: 53BP1 cooperation with the REV7-shieldin complex underpins DNA structure-specific NHEJ. *Nature* 560: 122-127, 2018.
140. Noordermeer SM, Adam S, Setiaputra D, Barazas M, Pettitt SJ, Ling AK, Olivieri M,   lvarez-Quil  n A, Moatti N, Zimmermann M, *et al.*: The shieldin complex mediates 53BP1-dependent DNA repair. *Nature* 560: 117-121, 2018.
141. Liang L, Feng J, Zuo P, Yang J, Lu Y and Yin Y: Molecular basis for assembly of the shieldin complex and its implications for NHEJ. *Nat Commun* 11: 1972, 2020.
142. Bochkarev A and Bochkareva E: From RPA to BRCA2: Lessons from single-stranded DNA binding by the OB-fold. *Curr Opin Struct Biol* 14: 36-42, 2004.
143. Findlay S, Heath J, Luo VM, Malina A, Morin T, Coulombe Y, Djerir B, Li Z, Samiei A, Simo-Cheyue E, *et al.*: SHLD2/FAM35A co-operates with REV7 to coordinate DNA double-strand break repair pathway choice. *EMBO J* 37: e100158, 2018.
144. Gao S, Feng S, Ning S, Liu J, Zhao H, Xu Y, Shang J, Li K, Li Q, Guo R and Xu D: An OB-fold complex controls the repair pathways for DNA double-strand breaks. *Nat Commun* 9: 3925, 2018.
145. Cannavo E and Cejka P: Sae2 promotes dsDNA endonuclease activity within Mre11-Rad50-Xrs2 to resect DNA breaks. *Nature* 514: 122-125, 2014.
146. Anand R, Ranjha L, Cannavo E and Cejka P: Phosphorylated CtIP functions as a co-factor of the MRE11-RAD50-NBS1 endonuclease in DNA end resection. *Mol Cell* 64: 940-950, 2016.
147. Nimonkar AV, Genschel J, Kinoshita E, Polaczek P, Campbell JL, Wyman C, Modrich P and Kowalczykowski SC: BLM-DNA2-RPA-MRN and EXO1-BLM-RPA-MRN constitute two DNA end resection machineries for human DNA break repair. *Genes Dev* 25: 350-362, 2011.
148. Gravel S, Chapman JR, Magill C and Jackson SP: DNA helicases Sgs1 and BLM promote DNA double-strand break resection. *Genes Dev* 22: 2767-2772, 2008.
149. Dev H, Chiang TW, Lescale C, de Krijger I, Martin AG, Pilger D, Coates J, Szczaniecka-Clift M, Wei W, Ostermaier M, *et al.*: Shieldin complex promotes DNA end-joining and counters homologous recombination in BRCA1-null cells. *Nat Cell Biol* 20: 954-965, 2018.
150. Bhattacharjee A, Wang Y, Diao J and Price CM: Dynamic DNA binding, junction recognition and G4 melting activity underlie the telomeric and genome-wide roles of human CST. *Nucleic Acids Res* 45: 12311-12324, 2017.
151. Barazas M, Annunziato S, Pettitt SJ, de Krijger I, Ghezraoui H, Roobol SJ, Lutz C, Frankum J, Song FF, Brough R, *et al.*: The CST complex mediates end protection at double-strand breaks and promotes PARP inhibitor sensitivity in BRCA1-deficient cells. *Cell Rep* 23: 2107-2118, 2018.
152. Mirman Z, Lottersberger F, Takai H, Kibe T, Gong Y, Takai K, Bianchi A, Zimmermann M, Durocher D and de Lange T: 53BP1-RIF1-shieldin counteracts DSB resection through CST- and Pola-dependent fill-in. *Nature* 560: 112-116, 2018.
153. Pellegrini L: The Pol α -primase complex. *Subcell Biochem* 62: 157-169, 2012.
154. Sfeir A and Symington LS: Microhomology-mediated end joining: A back-up survival mechanism or dedicated pathway? *Trends Biochem Sci* 40: 701-714, 2015.
155. Mu  oz IM and Rouse J: Control of histone methylation and genome stability by PTIP. *EMBO Rep* 10: 239-245, 2009.
156. Munoz IM, Jowsey PA, Toth R and Rouse J: Phospho-epitope binding by the BRCT domains of hPTIP controls multiple aspects of the cellular response to DNA damage. *Nucleic Acids Res* 35: 5312-5322, 2007.
157. Callen E, Faryabi RB, Luckey M, Hao B, Daniel JA, Yang W, Sun HW, Dressler G, Peng W, Chi H, *et al.*: The DNA damage- and transcription-associated protein paxipl controls thymocyte development and emigration. *Immunity* 37: 971-985, 2012.
158. Wang J, Aroumougame A, Lobrich M, Li Y, Chen D, Chen J and Gong Z: PTIP associates with Artemis to dictate DNA repair pathway choice. *Genes Dev* 28: 2693-2698, 2014.
159. Ma Y, Pannicke U, Schwarz K and Lieber MR: Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D) J recombination. *Cell* 108: 781-794, 2002.
160. Goodarzi AA, Yu Y, Riballo E, Douglas P, Walker SA, Ye R, H  rer C, Marchetti C, Morrice N, Jeggo PA and Lees-Miller SP: DNA-PK autophosphorylation facilitates Artemis endonuclease activity. *EMBO J* 25: 3880-3889, 2006.
161. Park SJ, Gavrilova O, Brown AL, Soto JE, Bremner S, Kim J, Xu X, Yang S, Um JH, Koch LG, *et al.*: DNA-PK promotes the mitochondrial, metabolic, and physical decline that occurs during aging. *Cell Metab* 26: 447, 2017.
162. Kawale AS, Akopiants K, Valerie K, Ruis B, Hendrickson EA, Huang SN, Pommier Y and Povirk LF: TDP1 suppresses mis-joining of radiomimetic DNA double-strand breaks and cooperates with Artemis to promote optimal nonhomologous end joining. *Nucleic Acids Res* 46: 8926-8939, 2018.
163. Morales JC, Richard P, Rommel A, Fattah FJ, Motea EA, Patidar PL, Xiao L, Leskov K, Wu SY, Hittelman WN, *et al.*: Kub5-Hera, the human Rtt103 homolog, plays dual functional roles in transcription termination and DNA repair. *Nucleic Acids Res* 42: 4996-5006, 2014.
164. Han J, Ruan C, Huen MSY, Wang J, Xie A, Fu C, Liu T and Huang J: BRCA2 antagonizes classical and alternative nonhomologous end-joining to prevent gross genomic instability. *Nat Commun* 8: 1470, 2017.
165. Isobe SY, Hiraga SI, Nagao K, Sasanuma H, Donaldson AD and Obuse C: Protein phosphatase 1 acts as a RIF1 effector to suppress DSB resection prior to Shieldin action. *Cell Rep* 36: 109383, 2021.
166. Spyropoulou Z, Papaspyropoulos A, Lagopati N, Myrianthopoulos V, Georgakilas AG, Foustieri M, Kotsinas A and Gorgoulis VG: Cockayne syndrome group B (CSB): The regulatory framework governing the multifunctional protein and its plausible role in cancer. *Cells* 10: 866, 2021.

167. Batenburg NL, Walker JR, Noordermeer SM, Moatti N, Durocher D and Zhu XD: ATM and CDK2 control chromatin remodeler CSB to inhibit RIF1 in DSB repair pathway choice. *Nat Commun* 8: 1921, 2017.
168. Batenburg NL, Qin J, Walker JR and Zhu XD: Efficient UV repair requires disengagement of the CSB winged helix domain from the CSB ATPase domain. *DNA Repair (Amst)* 68: 58-67, 2018.
169. Batenburg NL, Walker JR, Coulombe Y, Sherker A, Masson JY and Zhu XD: CSB interacts with BRCA1 in late S/G2 to promote MRN- and CtIP-mediated DNA end resection. *Nucleic Acids Res* 47: 10678-10692, 2019.
170. Kreßner C, Nollau P, Grosse R and Brandt DT: Functional interaction of SCAI with the SWI/SNF complex for transcription and tumor cell invasion. *PLoS One* 8: e69947, 2013.
171. Hansen RK, Mund A, Poulsen SL, Sandoval M, Klement K, Tsouroula K, Tollenaere MA, Räsche M, Soria R, Offermanns S, *et al*: SCAI promotes DNA double-strand break repair in distinct chromosomal contexts. *Nat Cell Biol* 18: 1357-1366, 2016.
172. Isobe SY, Nagao K, Nozaki N, Kimura H and Obuse C: Inhibition of RIF1 by SCAI allows BRCA1-mediated repair. *Cell Rep* 20: 297-307, 2017.
173. Parvin S, Ramirez-Labrada A, Aumann S, Lu X, Weich N, Santiago G, Cortizas EM, Sharabi E, Zhang Y, Sanchez-Garcia I, *et al*: LMO2 confers synthetic lethality to PARP inhibition in DLBCL. *Cancer Cell* 36: 237-249.e6, 2019.
174. Bonanno L, Costa C, Majem M, Sanchez JJ, Gimenez-Capitan A, Rodriguez I, Vergnenegre A, Massuti B, Favaretto A, Ruge M, *et al*: The predictive value of 53BP1 and BRCA1 mRNA expression in advanced non-small-cell lung cancer patients treated with first-line platinum-based chemotherapy. *Oncotarget* 4: 1572-1581, 2013.
175. Schouten PC, Vollebergh MA, Opdam M, Jonkers M, Loden M, Wesseling J, Hauptmann M and Linn SC: High XIST and low 53BP1 expression predict poor outcome after high-dose alkylating chemotherapy in patients with a BRCA1-like breast cancer. *Mol Cancer Ther* 15: 190-198, 2016.
176. Zhang T, Chai J and Chi L: Induction Of XLF And 53BP1 expression is associated with temozolomide resistance in glioblastoma cells. *Onco Targets Ther* 12: 10139-10151, 2019.
177. Yao J, Huang A, Zheng X, Liu T, Lin Z, Zhang S, Yang Q, Zhang T and Ma H: 53BP1 loss induces chemoresistance of colorectal cancer cells to 5-fluorouracil by inhibiting the ATM-CHK2-P53 pathway. *J Cancer Res Clin Oncol* 143: 419-431, 2017.
178. Kraiss JJ, Wang Y, Bernhardt AJ, Clausen E, Miller JA, Cai KQ, Scott CL and Johnson N: RNF168-mediated ubiquitin signaling inhibits the viability of BRCA1-null cancers. *Cancer Res* 80: 2848-2860, 2020.
179. Belotserkovskaya R, Raga Gil E, Lawrence N, Butler R, Clifford G, Wilson MD and Jackson SP: PALB2 chromatin recruitment restores homologous recombination in BRCA1-deficient cells depleted of 53BP1. *Nat Commun* 11: 819, 2020.
180. Miyamoto K, Minegaki T, Hirano S, Hayashi I, Tsujimoto M and Nishiguchi K: Olaparib potentiates anticancer drug cytotoxicity via 53BP1 in oesophageal squamous cell carcinoma cells. *Anticancer Res* 40: 813-823, 2020.
181. Mu F, Liu T, Zheng H, Xie X, Lei T, He X, Du S, Tong R and Wang Y: Mangiferin induces radiosensitization in glioblastoma cells by inhibiting nonhomologous end joining. *Oncol Rep* 40: 3663-3673, 2018.
182. Pustovalova M, Alhaddad L, Smetanina N, Chigasova A, Blokhina T, Chuprov-Netochin R, Osipov AN and Leonov S: The p53-53BP1-related survival of A549 and H1299 human lung cancer cells after multifractionated radiotherapy demonstrated different response to additional acute X-ray exposure. *Int J Mol Sci* 21: 3342, 2020.
183. Eke I, Zong D, Aryankalayil MJ, Sandfort V, Bylicky MA, Rath BH, Graves EE, Nussenzweig A and Coleman CN: 53BP1/RIF1 signaling promotes cell survival after multifractionated radiotherapy. *Nucleic Acids Res* 48: 1314-1326, 2020.
184. Roobol SJ, van den Bent I, van Cappellen WA, Abraham TE, Paul MW, Kanaar R, Houtsmuller AB, van Gent DC and Essers J: Comparison of high- and low-LET radiation-induced DNA double-strand break processing in living cells. *Int J Mol Sci* 21: 6602, 2020.
185. Oizumi T, Ohno R, Yamabe S, Funayama T and Nakamura AJ: Repair kinetics of DNA double strand breaks induced by simulated space radiation. *Life (Basel)* 10: 341, 2020.
186. Anglada T, Genescà A and Martin M: Age-associated deficient recruitment of 53BP1 in G1 cells directs DNA double-strand break repair to BRCA1/CtIP-mediated DNA-end resection. *Aging (Albany NY)* 12: 24872-24893, 2020.
187. Li N, Wang J, Wang X, Sun J and Li Z: Icaritin exerts a protective effect against d-galactose induced premature ovarian failure via promoting DNA damage repair. *Biomed Pharmacother* 118: 109218, 2019.
188. Zhang L, Geng X, Wang F, Tang J, Ichida Y, Sharma A, Jin S, Chen M, Tang M, Pozo FM, *et al*: 53BP1 regulates heterochromatin through liquid phase separation. *Nat Commun* 13: 360, 2022.
189. Dimitrova N, Chen YC, Spector DL and de Lange T: 53BP1 promotes non-homologous end joining of telomeres by increasing chromatin mobility. *Nature* 456: 524-528, 2008.
190. Yang X, Xu B, Mulvey B, Evans M, Jordan S, Wang YD, Pagala V, Peng J, Fan Y, Patel A and Peng JC: Differentiation of human pluripotent stem cells into neurons or cortical organoids requires transcriptional co-regulation by UTX and 53BP1. *Nat Neurosci* 22: 362-373, 2019.
191. Lambrus BG, Daggubati V, Uetake Y, Scott PM, Clutario KM, Sluder G and Holland AJ: A USP28-53BP1-p53-p21 signaling axis arrests growth after centrosome loss or prolonged mitosis. *J Cell Biol* 214: 143-153, 2016.
192. Phan TP, Maryniak AL, Boatwright CA, Lee J, Atkins A, Tijhuis A, Spierings DC, Bazzi H, Foijer F, Jordan PW, *et al*: Centrosome defects cause microcephaly by activating the 53BP1-USP28-TP53 mitotic surveillance pathway. *EMBO J* 40: e106118, 2021.
193. Damen M, Wirtz L, Soroka E, Khatif H, Kukut C, Simons BD and Bazzi H: High proliferation and delamination during skin epidermal stratification. *Nat Commun* 12: 3227, 2021.
194. Turocy J, Adashi EY and Egli D: Heritable human genome editing: Research progress, ethical considerations, and hurdles to clinical practice. *Cell* 184: 1561-1574, 2021.
195. Yang H, Ren S, Yu S, Pan H, Li T, Ge S, Zhang J and Xia N: Methods favoring homology-directed repair choice in response to CRISPR/Cas9 induced-double strand breaks. *Int J Mol Sci* 21: 6461, 2020.
196. Canny MD, Moatti N, Wan LCK, Fradet-Turcotte A, Krasner D, Mateos-Gomez PA, Zimmermann M, Orthwein A, Juang YC, Zhang W, *et al*: Inhibition of 53BP1 favors homology-dependent DNA repair and increases CRISPR-Cas9 genome-editing efficiency. *Nat Biotechnol* 36: 95-102, 2018.
197. Sun Y, Lu H, Fang X, Xiao S, Yang F, Chen Y, Wang H, Li X, Lu J, Lin H, *et al*: Discovery of a novel 53BP1 inhibitor through AlphaScreen-based high-throughput screening. *Bioorg Med Chem* 34: 116054, 2021.
198. Paulsen BS, Mandal PK, Frock RL, Boyraz B, Yadav R, Upadhyayula S, Gutierrez-Martinez P, Ebina W, Fasth A, Kirchhausen T, *et al*: Ectopic expression of RAD52 and dn53BP1 improves homology-directed repair during CRISPR-Cas9 genome editing. *Nat Biomed Eng* 1: 878-888, 2017.
199. Nambiar TS, Billon P, Didenhofen G, Hayward SB, Taghialatela A, Cai K, Huang JW, Leuzzi G, Cuella-Martin R, Palacios A, *et al*: Stimulation of CRISPR-mediated homology-directed repair by an engineered RAD18 variant. *Nat Commun* 10: 3395, 2019.
200. Jayavaradhan R, Pillis DM, Goodman M, Zhang F, Zhang Y, Andreassen PR and Malik P: CRISPR-Cas9 fusion to dominant-negative 53BP1 enhances HDR and inhibits NHEJ specifically at Cas9 target sites. *Nat Commun* 10: 2866, 2019.
201. Markiewicz-Potoczny M, Lobanova A, Loeb AM, Kirak O, Olbrich T, Ruiz S and Lazzarini Denchi E: TRF2-mediated telomere protection is dispensable in pluripotent stem cells. *Nature* 589: 110-115, 2021.
202. Vančevska A, Ahmed W, Pfeiffer V, Feretzaki M, Boulton SJ and Lingner J: SMCHD1 promotes ATM-dependent DNA damage signaling and repair of uncapped telomeres. *EMBO J* 39: e102668, 2020.



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