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53BP1: A guardian for centrosomal integrity

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1. ABSTRACT

53BP1 is known as a mediator in DNA damage response and a regulator of DNA double-stranded breaks (DSBs) repair. 53BP1 was recently reported to be a centrosomal protein and a binding partner of mitotic polo-like kinase 1 (Plk1). The stability of 53BP1, in response to DSBs, is regulated by its phosphorylation, deubiquitination, and ubiquitination. During mitosis, 53BP1 is stabilized by phosphorylation at S380, a putative binding region with polo-box domain of Plk1, and deubiquitination by ubiquitin-specific protease 7 (USP7). In the absence of DSBs, 53BP1 is abundant in the nucleoplasm; DSB formation results in its rapid localization to the damaged chromatin. Mitotic 53BP1 is also localized at the centrosome and spindle pole. 53BP1 depletion induces mitotic defects such as disorientation of spindle poles attributed to extra centrosomes or mispositioning of centrosomes, leading to phenotypes similar to those in USP7-deficient cells. Here, we discuss how 53BP1 controls the centrosomal integrity through its interaction with USP7 and centromere protein F by regulation of its stability and its physiology in response to DNA damage.

2. INTRODUCTION

Centrosome, the main microtubule-organizing center, is composed of a pair of mother centriole and daughter centriole. It regulates the bipolar spindle formation in mitosis and forms the basal body of the primary cilia in senescence (1). The integrity of the centrosome in terms of its structure, function, and number is subject to tight control in cells. In cell cycle, the centrosome is duplicated in S phase, matured in G2-M transition, and separated in mitosis. The exact process of centrosome duplication and maturation is essential for the accurate formation of spindle poles (2). Tight regulation of centrosome integrity is critical for equal chromosomal segregation and transfer of genetic material (Figure 1) (2). Centrosome amplification by overduplication, abortion of cell division, and cell fusion induces extra centrosomes in cells (Figure 1) (3, 4). Extra centrosomes disturb normal cell division or induce multipolar spindle formation, leading to mitotic catastrophe. For cell survival, extra centrosomes are clustered to form pseudo-bipolar spindle, leading to successful cell division (4). However, cells still display supernumerary centrosome with aneuploidy, which leads to chromosomal instability (Figure 1). The regulation of centrosomal integrity is critical in

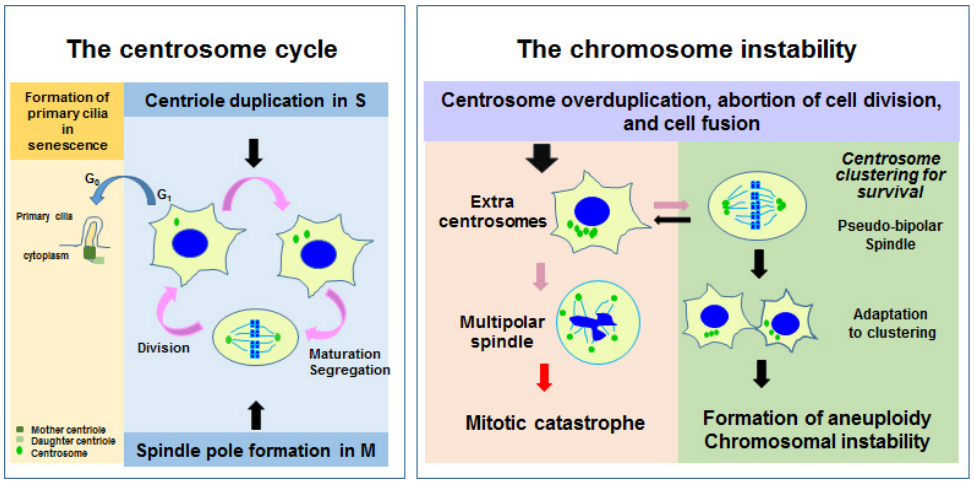

Figure 1. The centrosomal cycle and chromosomal instability due to centrosomal abnormalities. (Left panel) The centrosome consists of a pair of mother centriole and daughter centriole. In senescence, the centrosome provides the basal body for the primary cilia. In the cell cycle, the centrosome duplicates during DNA replication. Duplicated centrosomes mature in G2 phase and separate from each other. Centrosomes form two spindle poles in mitosis, distributing one centrosome to each daughter cell after cell division. (Right panel) The centrosome can be amplified by over-duplication in S phase, abortion of cell division, and cell fusion. Extra centrosomes will disturb normal cell division or induce multipolar spindle formation, leading to mitotic catastrophe. Mitotic catastrophe is avoided in cancer cells through centrosome clustering. Extra centrosomes may be clustered to form pseudo-bipolar spindle, enabling successful cell division. After cell division, cancer cells still exhibit supernumerary centrosome with aneuploidy, which causes chromosomal instability.

understanding cancer biology for developing novel cancer therapies.

Using a yeast two-hybrid screening, 53BP1 was found as one of the tumor suppressor protein p53-interacting proteins. 53BP1 has a tandem repeat of BRCA1 C-terminal (BRCT) domain and it is responsible for the interaction with p53 (Figure 2). BRCT repeats are frequently found in DNA damage response proteins including BRCA1 and BRCA2 (5–9). 53BP1 is a large protein of 1972 amino acids that regulates the cellular response to the DNA double-stranded break (DSB), although it has no enzymatic activity (Figure 2). 53BP1 exhibits sites that interact with DNA damage signaling proteins; it functions to amplify the ataxia telangiectasia mutated (ATM) kinase activity and promote checkpoint signaling in response to DNA damage (9–11). Our recent study reported that the phosphorylation of 53BP1 at serine residue 380 increased its stability through the interaction with ubiquitin-specific protease 7 (USP7) (5). Here we discuss how 53BP1 controls the centrosomal integrity through its interaction with USP7 and centromere protein F (CENPF) by regulation of its stability, as well as its physiology and functions in the DNA damage response.

3. FUNCTIONS OF 53BP1

3.1. Functions of 53BP1 in response to DNA damage

The cellular response to DNA DSBs is critical for the maintenance of genome integrity and proper functioning of cells. The failure to manage DSBs can lead to cell death and gross chromosomal rearrangements, including translocations, deletions, and amplifications. Mammalian cells have well conserved DSB response signaling pathways that coordinate cell cycle checkpoint activation and DSB repair machineries (12). The early stage of DSB response signaling is characterized by the activation of the ATM kinase through autophosphorylation and monomerization (12, 13), leading to the phosphorylation of a series of mediator proteins such as histone 2A variant H2A.X (known as gamma-H2A.X), mediator of DNA damage checkpoint protein 1 (MDC1), checkpoint kinase 2 (Chk2), and 53BP1 (12, 14–18). 53BP1 was first identified as a binding partner of the tumor suppressor protein p53 (6, 19). Now, numerous studies demonstrate that 53BP1 acts not only as a mediator of DSB response signaling (9–12) but also as a regulator of DSB repair pathway (20–22). The ATM-mediated phosphorylation of 53BP1 results in its rapid localization to the damaged chromatin upon DSB formation. Within chromatin it interacts with other DSB response proteins, including EXPAND1/MUM1, RAP1-interacting factor 1 (RIF1), and Pax2 transactivation domain interaction protein (PTIP) (23–25) and provides a platform for the recruitment of other DSB signaling and repair proteins. Recent evidence shows that 53BP1 localization to the damaged chromatin requires a series of chromatin ubiquitination, including that of histone H2A Lys13 and Lys15 (H2AK13/K15). Ubiquitination of histone is carried out by an E3 ubiquitin ligase RNF168 and mediated by gamma-H2A.X, MDC1, and RNF8 (16, 26). 53BP1 has an ubiquitination-dependent recruitment (UDR) motif that recognizes and binds to ubiquitinated H2A (27) (Figure 2). At least two other domains of 53BP1 have crucial roles in its stable localization to DNA

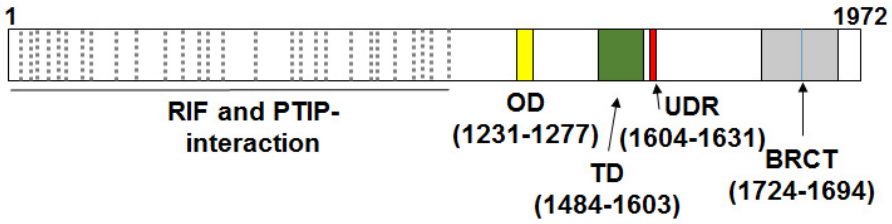

Figure 2. Schematic representation of 53BP1 domain. 53BP1 has an N-terminal domain with 28 SQ/TQ-motifs that can be phosphorylated by ATM/ATR. The N-terminal domain interacts with RIF1 and PTIP. In addition, serine 380 of 53BP1 can be phosphorylated by Cdk1 and interacted with Plk1 during mitosis. Oligomerization domain (OD) mediates homodimerization of 53BP1 in response to DNA damage. Tudor domain (TD) recognizes and binds to methylated H4K20 and acetylated H4K16 interferes the interaction between 53BP1 and methylated H4K20. Ubiquitin-dependent recruitment (UDR) is responsible for the interaction with ubiquitinated H2A in response to DNA damage. BRCT domain mediates the interaction with p53 and EXPAND1

lesions. One is the tandem Tudor domain that can recognize and bind to dimethylated histone H4 Lys20 and the other is the oligomerization motif that enables the protein to form dimers on DNA lesions (28–32) (Figure 2). In addition, 53BP1 is thought to select a proper repair pathway during DSB repair (20–22). Nonhomologous end-joining (NHEJ) and homologous recombination (HR) are the two major DSB repair pathways. 53BP1 seems to promote NHEJ-dependent DSB repair pathway by antagonizing BRCA1 and inhibiting DSB end recession by CtIP (CtBP-interacting protein) required for HR repair (Figure 3), although the exact mechanism is unclear (33, 34). RIF1, PTIP, and REV7 have been identified as downstream effectors of 53BP1 promoting NHEJ pathway (25, 35). There are other factors that are important for DSB repair pathway choice, including histone modifications, cell cycle stages, and chromatin organization. 53BP1 can mediate the crosstalk among DSB response proteins, cell cycle checkpoint proteins, and epigenetic regulators for proper DSB repair.

3.2. Functions of 53BP1 in mitosis

Although 53BP1 has been established well as a mediator in DNA damage response and a decision maker in repair pathway, its mitotic function is relatively unexplored. Accumulating evidence suggests that 53BP1 regulates mitotic progression. Mitotic cells synchronized with nocodazole or double-thymidine block exhibited higher levels of 53BP1 expression and phosphorylation (36–38). As a mediator of DNA damage response, 53BP1 is involved in the regulation of spindle checkpoint through its interaction with kinetochore-associated proteins such as centromere protein E, suggestive of its role in the metaphase checkpoint (36). In addition, the highly expressed 53BP1 is phosphorylated by Cdk1 and Plk1 in mitotic cells synchronized with double thymidine block and the interaction between 53BP1 and Plk1 during mitosis is required for proper inactivation of the DNA damage checkpoint (38). Thus, 53BP1 is suggested to exhibit a role as a common molecular component between DNA damage response machinery and mitotic checkpoint signaling (36, 38). Recent studies revealed that the recruitment of 53BP1 to DNA lesions was inhibited during mitosis in response to DSBs, although other factors involved in DSBs, including gamma-H2AX, MDC1, and MRN showed normal accumulation at DNA lesions (39, 40). The protein levels of 53BP1 were low in the presence of mitotic damage induced by polo-like kinase 1 (Plk1) depletion, although ATM signaling was activated (5, 41). Mitotic cells showed attenuated DNA damage signaling mediated by 53BP1; however, the phosphorylation of Chk2 at G1 resulted in the activation of the attenuated DNA damage response at interphase (40). Improper activation of DSB repair in mitotic cells may contribute to fusion of mitotic telomeres (42). As a result of fusogenic potentials of mitotic telomeres, mitotic kinases inhibit the recruitment of 53BP1 and RNF8 to DSB-chromatin (42). As mitotic telomeres might be prone to deprotection and fusions, mitotic cells must suppress DSB repair, which induces the inactivation of 53BP1 and RNF8 despite DNA damage during mitosis (42, 43).

Although most studies about 53BP1 function were performed in presence of DNA damage, a recent study explored the cellular physiology of 53BP1 in the absence of DNA damage. In this study, 53BP1 stabilization was mediated by the interaction between USP7 and the polo-box domain (PBD) of Plk1. 53BP1 regulated centrosome positioning through its interaction with CENPF involved in proper kinetochore attachments (5). PBD of Plk1 serves as the binding domain for potential substrates of Plk1 (44–46). The interaction between 53BP1 and Plk1 can be occurred in the centrosome. Depletion of 53BP1 induces extra centrosomes as a consequence of cytokinetic failure associated with the downregulation of CENPF and p53. The complex of 53BP1-USP7-CENPF increases the stability of CENPF, which maintains the centrosome integrity (5). Thus, in the absence of DNA damage during mitosis, 53BP1 is thought to regulate centrosome integrity. In addition, the function of 53BP1 in mitotic error induced by centrosome loss was reported recently (53–55). Centrosome loss and prolonged mitosis induced 53BP1/USP28-mediated stabilization of p53, leading to the activation of p53 signaling pathway for cell cycle arrest (53–55). This

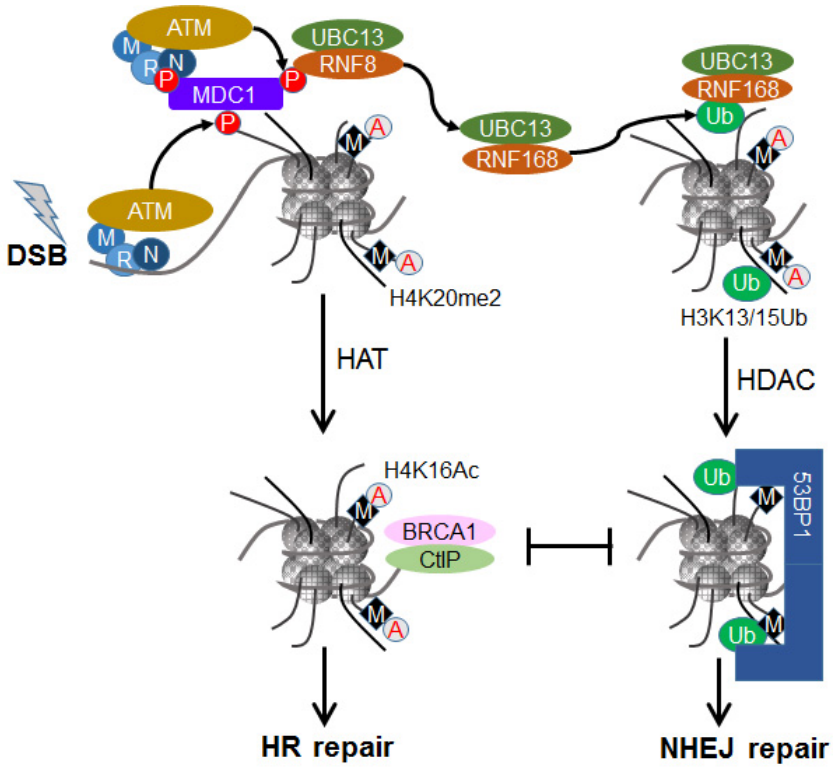

Figure 3. Localization of 53BP1 at DSB and its role in DSB repair pathway. Upon DSB formation, ATM rapidly localizes to the DSB site and phosphorylates Ser 139 of histone H2A.X, followed by recruitment of MDC1. The homologous recombination (HR) repair is characterized by the acetylation at histone H4K16. Acetylation is mediated by a histone acetyltransferase that prevents the binding of 53BP1 to H4K20me2 and recruits BRCA1 and CtIP to initiate HR repair. For the nonhomologous end-joining (NHEJ) repair, phosphorylated MDC1 recruits RNF8 that can ubiquitinate unknown chromatin protein and RNF168 to the damaged chromatin. RNF168 ubiquitinates H3K13/15. H3K13/15 ubiquitination promotes the binding of 53BP1 to the damaged chromatin. HDAC1/2 deacetylate H4K16 to promote the binding of 53BP1 to H4K20me2. 53BP1 prevents BRCA1 and CtIP binding to DSB site and promotes NHEJ repair.

combined function of 53BP1 and USP28 is independent of their previously characterized role in DNA damage response and promoting mitotic efficiency (53–55). These studies suggested that 53BP1 functions for the stabilization of p53 in response to centrosome loss, although the signaling may be different with previously characterized DNA damage response.

4. REGULATION OF 53BP1 STABILITY

4.1. The stability of 53BP1 in DNA damage

In the absence of DSBs, 53BP1 is an abundant protein in the nucleoplasm. The formation of DSB is characterized by rapid recruitment of 53BP1 at the damaged chromatin site in the presence of the ubiquitinated histone H2A (27). Ubiquitination of H2A is mediated by RNF168 that is recruited to the site of DSBs by RNF8. RNF8 and/or RNF168 may also promote 53BP1 degradation in the nucleoplasm but not chromatin (56) (Figure 4A). In addition, RNF8 and RNF168 facilitate the focal localization of 53BP1 to promote recruitment of other factors involved in DSB response and repair of the damaged chromatin (56). It is unclear how RNF8 and RNF168 specifically ubiquitinate nucleoplasmic 53BP1 but not chromatin-bound 53BP1. It is possible that RNF8 and RNF168 interact with different E2 ubiquitin-conjugating enzymes at different sites. RAD6 is an E2 ubiquitin-conjugating enzyme thought to interact with RNF168 and ubiquitinate histone H2A and other chromatin proteins at the site of DSB (57). Another E2 ubiquitin-conjugating enzyme is UbcH7 that mediates replicative stress-induced ubiquitination and degradation of 53BP1 and promotes BRCA1-mediated homologous recombination repair (58). Therefore, RNF8 and RNF168 may regulate DSB response signaling and 53BP1 stability through their interaction with at least two different E2 ubiquitin-conjugating enzymes.

In addition to ubiquitination, the degradation of 53BP1 requires phosphorylation by ATM and ATM- and Rad3-related (ATR) kinases (58). 53BP1 bears a phosphodegron sequence that triggers the ubiquitination and degradation of 53BP1 upon phosphorylation in the nucleoplasm but not chromatin. In addition, a deubiquitination enzyme USP28 copurified with 53BP1 by tandem affinity purification is thought to be potentially involved in stabilizing 53BP1

at the damaged chromatin (59). Using short hairpin RNAs, USP28 was shown to stabilize 53BP1 and Chk2 in response to DSBs. Furthermore, the activity of USP28 can also be regulated by ATM. USP28 displays two SQ motifs (Ser 67 and Ser 714) as potential ATM/ATR phosphorylation sites; gamma-irradiation induced phosphorylation at both sites (59). Taken together, the stability of 53BP1 can be modulated in response to DSB by at least three following events: ATM-mediated phosphorylation of 53BP1 and other effector proteins, including USP28; deubiquitination of 53BP1 at damaged chromatin by USP28 deubiquitinase; and selective ubiquitination of 53BP1 in the nucleoplasm by RNF8 and RNF168 E3 ubiquitin ligases through their interaction with UbcH7 E2 ubiquitin-conjugating enzyme.

4.2. The stability of 53BP1 in mitosis

As discussed above, the expression of 53BP1 varies at different stages of cell cycle, with high-level expression observed in mitosis (36–38). At the same time, 53BP1 turnover is regulated by its phosphorylation and ubiquitination (5). Inhibition of Plk1 kinase activity with Plk1-specific inhibitor or Plk1-targeting shRNA results in increased degradation of the dephosphorylated 53BP1, while its activity may be restored following treatment with MG132 protease inhibitor. 53BP1 turnover is regulated by ubiquitination in an ubiquitination assay, which is restored by phosphorylation of S380, a PBD-binding site of Plk1 and priming phosphorylation site by Cdk1 in mitosis (38). Treatment with a Cdk1 inhibitor roscovitine reduced 53BP1 levels, indicative of the importance of Cdk1 (as a priming kinase) and Plk1 in regulation of 53BP1 stability.

Liquid chromatography tandem-mass spectrometry (LC-MS/MS) analysis revealed the interaction between 53BP1 and USP28 or USP7 in nocodazole-treated mitotic cells (5, 59). USP28 and USP7 deubiquitinating enzymes remove ubiquitin from specific substrates to prevent their degradation (60). The interaction of USP28 or USP7 with phosphomimic mutant of 53BP1 at S380 during mitosis was verified by immunoprecipitation (5). The interaction of USP7 with the phosphomimic mutant of 53BP1 was higher than that with dephosphomimic mutant 53BP1. USP28 exhibited no such difference. Inhibition of USP7 activity with specific inhibitor P22077, catalytic dead mutant, or shRNA resulted in decreased stability of 53BP1, suggesting that the deubiquitinating activity of USP7 increased the stability of 53BP1 (5). In addition, the treatment with Cdk1 inhibitor roscovitine reduced the interaction between 53BP1 and USP7 as compared with the control. Thus, it can be concluded that USP7 binds to 53BP1 phosphorylated at S380 and that the phosphorylation is important for the stabilization of 53BP1 (Figure 4B).

5. THE LOCALIZATION OF 53BP1

5.1. The localization of 53BP1 in DNA damage

The localization of 53BP1 to the damaged chromatin is crucial for DNA damage response signaling (59). In the absence of DSB, 53BP1 is abundant in the nucleoplasm; DSBs result in rapid localization of 53BP1 to the damaged chromatin. 53BP1 bears important structural domains such as BRCT repeats, tandem Tudor domains, an oligomerization domain, and UDR motif that mediate the interaction with other DNA damage response proteins and the localization to the site of DNA damage (Figures 2 and 3) (59). As mentioned above, tandem Tudor domains interact with dimethylated histone H4K20 at the site of DNA damage, thereby facilitating the localization of 53BP1 to the damaged chromatin. However, human H4K20 is always methylated in the chromatin and may not be regulated by DNA damage. Therefore, 53BP1 may require an additional marker to recognize the damaged chromatin.

At least three different markers exist at the site of DNA damage. First, gamma-H2A.X and MDC1 are phosphorylated by ATM and localized to the damaged chromatin in the early stage of damage response (9, 11–14). Although gamma-H2A.X has no role in the initial localization of 53BP1 to the damage site, it assists, together with MDC1, RNF8 and RNF168 in the modification of adjacent chromatin. Second, ubiquitinated histone H2AK13/K15 residues are recognized by the 53BP1 UDR motif, which is required for stable localization of 53BP1 to the damaged chromatin (22). Third, acetylation status of H4K16 is important for the binding of tandem Tudor domains of 53BP1 to dimethylated H4K20 (58, 59). Acetylation of H4K16 by histone acetyltransferase Tip60 interferes the interaction between 53BP1 Tudor domain and H4K20, while deacetylation by histone deacetylase (HDAC) 1 and HDAC2 permits the interaction (58, 59). HDAC1 and HDAC2 are shown to deacetylate H4K16 in response to DNA damage and promote the NHEJ repair instead of HR repair, which corresponds to the function of 53BP1 at the damage site (62–64). In addition, the localization of 53BP1 to the damaged chromatin requires selective degradation of 53BP1 in the nucleoplasm during the DNA damage response (Figure 4A). Thus, it is plausible that both the stability and localization of 53BP1 are regulated simultaneously by ATM-mediated phosphorylation, RNF8- and RNF168-mediated ubiquitination, and USP28-mediated deubiquitination based on the proximity of 53BP1 from the site of DSB.

5.2. The localization of 53BP1 in mitosis

Although 53BP1 localizes to the DNA lesion in response to DNA damage as discussed

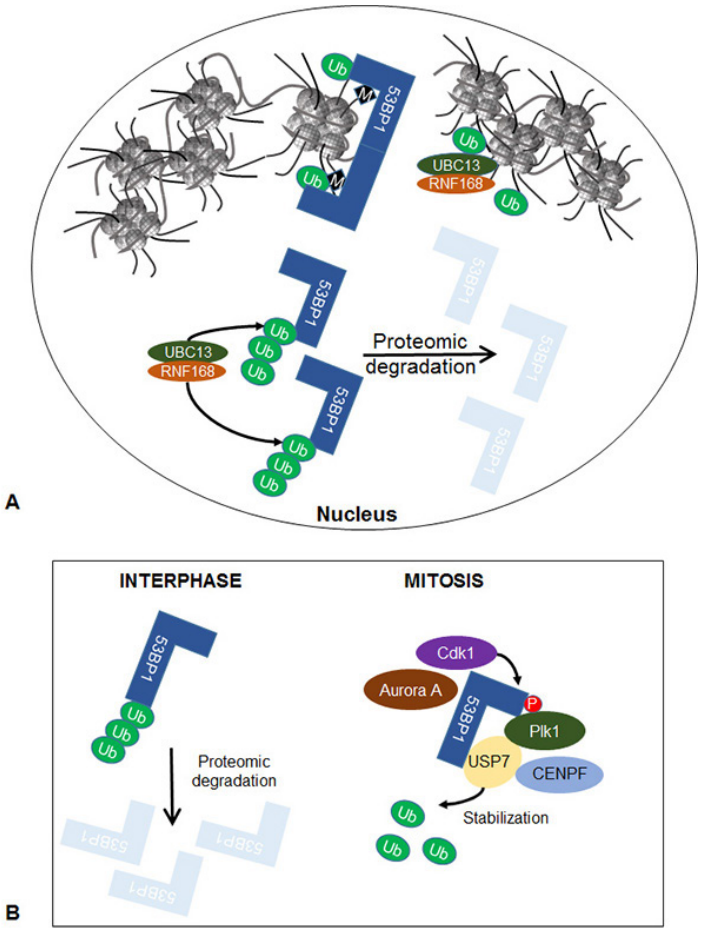

Figure 4. Regulation of 53BP1 stability during DSB repair or mitosis. In the absence of DSB, 53BP1 is in abundance in the nucleus. **(A)** Upon DSB formation, RNF168 mediates polyubiquitination of 53BP1 in the nucleoplasm and promotes its proteomic degradation. UBC13 or other E2 ubiquitin-conjugating enzyme may assist in this process. However, RNF168 ubiquitinates only the histones at the damaged chromatin and allows 53BP1 localization at the damaged chromatin. **(B)** In mitosis, 53BP1 turnover is regulated by its phosphorylation and deubiquitination. The priming phosphorylation of 53BP1 by Cdk1 at S380, a PBD-binding site of Plk1, recruits Plk1 and USP7 complex. The deubiquitinating activity of USP7 increased the stability of 53BP1 in mitotic cells. In addition, the complex of 53BP1-USP7-CENPF increases the stability of CENPF, which maintains the centrosome integrity. Thus phosphorylation of 53BP1 at S380 accelerates the formation of 53BP1-Plk1-USP7-CENPF complex, which is important for the stabilization of 53BP1 and the centrosome integrity.

above, mitotic 53BP1 shows different locations. A previous report revealed the localization of 53BP1 in the kinetochore of chromosomes, which may be in association with its function in checkpoint signaling during mitosis (36). A recent study found that 53BP1 localizes to the centrosome and spindle pole during mitosis, consistent with the localization of Plk1 in the absence of DNA damage (5). Endogenous 53BP1 was found to be localized with gamma-tubulin in the centrosome and spindle pole in mitosis, while exogenous enhanced green fluorescent protein (EGFP)-53BP1 or hemagglutinin (HA)-tagged 53BP1 localized at the centrosome and spindle pole (5). Depletion of 53BP1 using shRNA or CRISPR/Cas9 disrupted the structure of the spindle pole and induced chromosomal misalignment, segregation defects, and chromosome lagging (5). In addition, 53BP1 silencing or expression of the unstable 53BP1 mutant induced supernumerary centrosomes (5). The prolonged mitosis induced by depletion of 53BP1 was dependent on the spindle checkpoint kinase BubR1 that showed increased expression, suggestive of an important role of 53BP1 in mitotic progression.

6. REGULATION OF CENTROSOMAL INTEGRITY BY 53BP1 IN MITOSIS

Aberrations in the number and positioning of centrosomes generate multipolar spindle intermediates, consequently leading to centrosomal missegregation with lagging chromosomes, as observed in many different cancers (3). A recent study reported 53BP1 is a mitotic centrosomal protein (5). Downregulation of 53BP1 expression induces aberrations of positioning of centrosome and mislocalization of the spindle pole, leading to defects in chromosomal alignment and segregation (5). Cytokinetic failure results in polyploid cells with centrosome amplification (1, 3). Loss of

tumor suppressors such as p53, BRCA1, and BubR1 induces extra centrosomes (65–67). Loss of 53BP1 resulted in similar effects, although the phenotypes in 53BP1-knockout cells were less severe than those observed in p53-knockout cells. p53-deficient mouse embryo fibroblasts (MEFs) showed abnormal amplification of centrosome, suggestive of the regulatory role of p53 in centrosome duplication (67, 68). Loss of 53BP1 resulted in the disruption of motor-driven separation force, thereby leading to cytokinetic failure, multinucleation, and extra chromosomes. In a study using cancer cells such as U2OS, NCI-H460, and HeLa, approximately 20% of 53BP1-depleted cells showed extra centrin puncta versus less than 5% for control cells (5). Thus, 53BP1 may display an important role in the suppression of supernumerary centrosome formation.

Previous reports showed that 53BP1 defects induce extra centrosomes in malignant tumor, but not primary epithelial fibroblast cells (69, 70). Thus, the induction of extra centrosomes by loss of 53BP1 is thought to be dependent on cell types (65). Deficiency of p53, but not 53BP1, induces centrosome abnormalities in normal MEFs (69). However, loss of 53BP1 in thymomas, tumors originating from epithelial cells of the thymus, results in moderate centrosome amplification. In addition, 53BP1-deficient tumor cells exhibited a moderate centrosome amplification (24%, n=100) (70), indicating that 53BP1 defects induce centrosome amplification in thymomas. Approximately 20% of cancer cells such as HeLa, U2OS, and NCI-H460 displayed centrosome amplification upon CRISPR/Cas9- or shRNA-induced loss of 53BP1 (5). The cell susceptibility to 53BP1 defects may vary with genomic backgrounds and instabilities of cancer cells.

7. PERSPECTIVES: POSSIBLE REGULATORY MECHANISM FOR CENTROSOMAL INTEGRITY BY 53BP1

Although the molecular mechanisms involved in regulation of centrosome integrity by 53BP1 are unclear, two possible hypotheses are suggested (5). CENPF that interacts with 53BP1 plays a critical role in centrosome positioning by maintaining the tension between microtubules and kinetochores (71, 72). Cancer cells lacking a functional 53BP1 exhibited low level of CENPF protein (5), which may disturb the regulation of centrosome integrity. This is supported by the fact that cells with depleted CENPF or 53BP1 displayed similar phenotypes. Loss of CENPF induced aberrant chromosomal alignment and segregation, owing to the failure of kinetochore assembly. Moreover, it resulted in mitotic delay, with reduced kinetochore tension in metaphase (71–75). Phenotypes such as mitotic delay, chromosome misalignment, and aberrant centrosomes positioning may reflect a common function of CENFP in kinetochore assembly (71). 53BP1 depletion in cells was associated with the downregulation of CENPF; thus, the loss of 53BP1 may affect the stability of CENPF, which is degraded by APC^{Cdc20} at the end of anaphase (76). 53BP1 may prevent its degradation by formation of 53BP1-USP7-CENPF complex in mitosis to ensure proper kinetochore assembly as well as chromosome alignment and segregation. Future studies may throw light on the exact function of these factors affecting centrosome integrity.

The tumor suppressor p53 is another possible regulatory factor in 53BP1-depleted cells; the reduction in p53 level may be associated with phenotypic changes (extra centrosomes) in 53BP1-depleted cells (67, 68). 53BP1 stimulates the transcriptional activation of p53 (77); therefore, the reduction in p53 level in 53BP1-depleted cells may be attributed to the loss of 53BP1, leading to the formation of extra centrosomes (68). In addition, the non-functional p53 encouraged extra centrosomes formation. An increase in extra centrosomes of around 1.5.-fold was observed in $H460^{p53-}$ 53BP1-depleted cells as compared with $H460^{p53+}$ 53BP1-depleted cells (5). Therefore, the supernumerary centrosomes induced by 53BP1 depletion may be a consequence of an increase in genomic instability caused by p53 downregulation. The regulatory mechanisms of 53BP1 in the kinetochore machinery for centrosome integrity including positioning and number await further investigation.

8. ACKNOWLEDGEMENTS

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