Repair versus Checkpoint Functions of BRCA1 Are Differentially Regulated by Site of Chromatin Binding

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Abstract

The product of the Brca1 tumor-suppressor gene is involved in multiple aspects of the cellular DNA damage response (DDR), including activation of cell-cycle arrests and DNA double-stranded break (DSB) repair by homologous recombination. Prior reports demonstrated that BRCA1 recruitment to areas of DNA breakage depended on RAP80 and the RNF8/RNF168 E3 ubiquitin ligases. Here, we extend these findings by showing that RAP80 is only required for the binding of BRCA1 to regions flanking the DSB, whereas BRCA1 binding directly to DNA breaks requires Nijmegen breakage syndrome 1 (NBS1). These differential recruitment mechanisms differentially affect BRCA1 functions: (i) RAP80-dependent recruitment of BRCA1 to chromatin flanking DNA breaks is required for BRCA1 phosphorylation at serine 1387 and 1423 by ATM and, consequently, for the activation of S and G2 checkpoints; and (ii) BRCA1 interaction with NBS1 upon DSB induction results in an NBS1-dependent recruitment of BRCA1 directly to the DNA break and is required for nonhomologous end-joining repair. Together, these findings illustrate that spatially distinct fractions of BRCA1 exist at the DSB site, which are recruited by different mechanisms and execute different functions in the DDR. Cancer Res; 75(13); 2699–707. ©2015 AACR.

Introduction

Both external exposures and natural metabolic processes provide constant challenges to the integrity of our cellular DNA. An intricate network of DNA damage response (DDR) pathways has evolved to enable cells to cope with various types of DNA lesions. One of the most significant components of this machinery is the product of the human cancer suppressor gene, Brca1, which plays a critical role in maintaining genomic integrity. BRCA1 has multiple functions in the human DDR, including repair of DNA double-strand breaks (DSB) and activation of cell cycle checkpoints upon DSB induction (1). Mutations in the Brca1 gene result in genomic instability, predisposing individuals to breast and ovarian cancer (1). On the other hand, loss of functional BRCA1 sensitizes cancer cells to radiation and particular types of chemotherapy, highlighting the critical role of BRCA1 in the DDR (2).

There are two major pathways that repair DSBs in mammalian cells, the error-prone nonhomologous end joining (NHEJ) and the error-free homologous recombination. NHEJ, which is the dominant DSB repair pathway in mammalian cells (3), can function throughout the cell cycle, whereas homologous recombination is limited to the S and G2 phases as it requires a homologous sequence on the sister chromatid. Among its multiple functions in the DDR, BRCA1 plays a well-established role in repair of DSBs by homologous recombination. BRCA1 interacts with the CtBP-interacting protein (CtIP) at the break site (4), promoting DSB processing (5), an event that is required for initiation of homologous recombination. In line with these findings, BRCA1 is required for the generation of the single-stranded DNA regions that are formed as a consequence of the DSB processing (6). Consequently, BRCA1 promotes accumulation of RAD51 at the DSB (7), which facilitates sister chromatid invasion during homologous recombination. However, in addition to the "classic" role of BRCA1 in homologous recombination, some lines of evidence suggest that it may also be involved in DSB repair by NHEJ. Two studies have shown that the religation of a linearized plasmid by NHEJ is impaired in the absence of BRCA1 (8, 9). However, while suggesting a potentially new and intriguing role of BRCA1 in DNA repair, evidence gained from these studies is limited because of the assessment of the NHEJ activity using artificially introduced plasmid DNA, which differs significantly from chromatin. Therefore, the question remains whether BRCA1 is involved in repair of genomic DSBs by NHEJ in mammalian cells.

Another important function of BRCA1 is the activation of cell-cycle checkpoints in response to DNA damage. BRCA1 is a target of the ataxia telangiectasia mutated (ATM) kinase, which is rapidly activated following DSB induction (10) and recruited to the DSB site in an Mre11–Rad50–NBS1 (MRN)-dependent manner, resulting in amplification of ATM signaling (11). ATM phosphorylates BRCA1 at serine residues 1387 and 1423 in response to DSB induction, and these phosphorylation events...
are required for induction of the S and G2–M checkpoints, respectively (12, 13).

In light of the multiple functions of BRCA1 in the DDR, identification of mechanisms involved in recruiting BRCA1 to sites of DNA breakage has been of great interest. The E3 ubiquitin ligase, RNF8, is recruited to γH2AX domains at the DSB site, with MDC1 serving as a mediator between RNF8 and γH2AX (14, 15). RNF8 initiates the formation of ubiquitin conjugates at histone proteins that are sustained by the E3 ubiquitin ligase RNF168 (16, 17). Thereafter, a multiprotein complex containing RAP80 links BRCA1 to the ubiquitin conjugates (18–20), thus recruiting it to the chromatin. However, a previously published study suggested that the RNF8/RNF168–RAP80 pathway might recruit only a portion of the BRCA1 pool to the DSB (7). Using laser microirradiation to induce DSBs in BrdUrd-treated cells, Bekker-Jensen and colleagues investigated the recruitment of various DDR factors to the break site and observed that a downregulation of MDC1, a DDR factor that was later shown to recruit RNF8 to the DSB (15), only partially reduced the presence of BRCA1 in the laser track (7). In contrast, 53BP1 recruitment, which also depends on the RNF8 activity (15), was fully inhibited by the MDC1 knockdown (7). Together, these data suggested that an additional mechanism of BRCA1 recruitment that is independent of the RNF8/RNF168–RAP80 pathway might exist.

Furthermore, BRCA1 has been shown to reside in distinct multiprotein complexes (1, 21), with different functions of BRCA1 in the DDR being attributed to specific protein macrocomplexes (1). Notably, several BRCA1 macrocomplexes do not contain RAP80 (21), suggesting an existence of a RAP80-independent recruitment mechanism of BRCA1 to the break site. Here, we demonstrate that different molecular mechanisms recruit BRCA1 to distinct chromatin regions near the DSB. Although the RAP80-dependent pathway recruits BRCA1 to chromatin regions flanking the DSB, BRCA1 is recruited directly to the DNA break through an interaction with Nijmegen breakage syndrome 1 (NBS1). Further, we show that BRCA1 is involved in recombination of genomic DSBs by NHEJ and that the BRCA1 fraction residing in the vicinity of the DSB, but not in the flanking chromatin, is required for DSB religation. On the other hand, BRCA1 that is recruited to the flanking regions appears to control DSB processing and induction of S-phase and G2–M cell-cycle arrests.

Materials and Methods

Cell culture, chemicals, siRNA
MCF7 and HeLa cells were grown in DMEM supplemented with 10% FBS. 4-OHT (Sigma) was added to a final concentration of 1 μmol/L. Shield-1 (Cheminpharma) was added to a final concentration of 1 μmol/L for 3 hour to stabilize the ddI-Ppo1 fusion protein. ON-TARGETplus SMARTpool siRNA toward BRCA1, NBS1, RAP80, ATM (Dharmacon), or ON-TARGETplus nontargeting siRNA (Dharmacon) was transfected into cells using Dharmafect-1 transfection reagent (Dharmacon) according to the manufacturer's protocols to a final concentration of 100 nmol/L. Olaparib (AZD2281; Selleckchem) and CP466722 (22) were added to the tissue culture medium at a final concentration of 5 μmol/L 1 hour prior to I-Ppo1 induction and remained in the medium for the duration of the experiment.

Antibodies

The following antibodies were used for immunoblotting: β-actin (C4; Chemicon), ATM (MAT3; gift from Y. Shiloh, Sackler School of Medicine, Tel Aviv University, Tel-Aviv, Israel), pKAP1 S824 (Bethyl Laboratories), pCHK2 T68 (Cell Signaling Technology), PATM S1981 (Epitomics), BRCA1 (OP92 and OP93; Calbiochem), NBS1 (NB100-143; Novus), RAP80 (70822; Abcam), pBRCA1 S1387 (A300-007A; Bethyl), pBRCA1 S1423 (2838; Abcam).

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) was performed as previously described (23). The following antibodies were used for the ChIP assay: normal rabbit IgG (Cell Signaling Technology), BRCA1 (OP92 and OP93; Calbiochem), NBS1 (NB100-143; Novus), γH2AX (05-636; Millipore), RAP80 (ab70822; Abcam), MDC1 (Abcam), RNF8 (ab4183; Abcam). Appropriate negative controls using normal rabbit IgG were performed for all ChIP experiments.

DNA repair assays

Assessments of DNA DSB repair by PCR after I-Ppo1 induction were performed as previously described (23). Comet assays were performed using the Trevigen CometAssay Kit (Trevigen) according to the manufacturer's protocol. Cell images were assessed by the TriTek CometScore 1.6.1.21 software. Olive tail moment was determined as a product of DNA in the tail and the mean distance of migration in the tail.

Cell survival analysis

Ten days after cytotoxic treatment, cells were incubated with MIT. The product formazan was eluted with isopropanol containing 0.04 mol/L HCl and quantified using a spectrophotometer. The percentage of survival was calculated in relation to untreated control for each sample.

Immunoblot and protein immunoprecipitation

Immunoblots were performed as previously described (24). For immunoprecipitations, cells were washed with PBS and lysed on ice in TGN buffer [50 mmol/L Tris (pH 7.5), 200 mmol/L NaCl, 50 mmol/L NaF, protease inhibitors] containing 1%, v/v Tween 20 and 0.2%, v/v NP-40 for 30 minutes. Samples were precleared with Protein A/Protein G agarose beads overnight, washed with PBS and lysed on ice in TGN buffer [50 mmol/L Tris (pH 7.5), 200 mmol/L NaCl, 50 mmol/L NaF, protease inhibitors] containing 1%, v/v Tween 20 and 0.2%, v/v NP-40 for 30 minutes. Samples were precleared with Protein A/Protein G agarose beads (Calbiochem) and incubated with indicated antibodies or normal rabbit IgG and Protein A/Protein G agarose beads overnight, washed with TGN buffer, and analyzed by immunoblot. Antibodies used were NBS1 (NB100-143; Novus) and BRCA1 (OP92 and OP93; Calbiochem).

Cell-cycle checkpoint assays

Propidium iodide staining. Cells were fixed in 70% ethanol and stored at –20°C. Cells were washed with PBS, and incubated with 10 μg/ml propidium iodide (PI) and 250 μg/ml RNase A and analyzed by FACSCalibur (BD). Data were assessed by the WinMDI 2.9 software.

BrdUrd/PI staining. Cells were either irradiated with 10 Gy IR or left untreated. Forty-five minutes after irradiation, 20 μmol/L of BrdUrd (bromodeoxyuridine) was added to the culture medium for 15 minutes. Thereafter, cells were fixed in 70% ethanol and
stored at −20°C. Cells were washed with PBS, treated with 2N HCl, and subsequently incubated with anti-BrdUrd (M0744; DaKo) and antimouse IgG (F0257; Sigma) in PBS + 0.5% BSA + 0.1% Tween 20, washed and incubated with 10 μg/mL PI and 250 μg/mL RNase A and analyzed by FACScalibur (BD). Data were assessed by the WinMDI 2.9 software.

**pH3/PI staining.** Cells were either irradiated with 10 Gy IR or left untreated. Thereafter, cells were fixed in 70% ethanol and stored at −20°C. Cells were washed with PBS, incubated in PBS/0.25% Triton X-100 on ice for 15 minutes followed by a subsequent incubation with anti-phospho-H3 (Ser 10) (06-570; Millipore) antibody and goat anti-rabbit IgG FITC conjugated antibody (111-096-144; Jackson ImmunoResearch Labs) in PBS + 1% BSA. Cells were washed and incubated with 10 μg/mL PI and 250 μg/mL RNAse A and analyzed by FACScalibur (BD). Data were assessed by the WinMDI 2.9 software.

**Results**

**Spatiotemporal recruitment kinetics of BRCA1 and members of the RNF8/RAP80 pathway to the DSB site**

Although immunofluorescent focus formation and “laser microirradiation” assays have been quite informative in studying protein dynamics and modifications associated with DNA damage and repair, they are limited in their ability to provide detailed spatial resolution for protein binding to chromatin. We recently reported a system in which DNA breaks could be introduced by the homing endonuclease, I-PpoI, at defined sites in the human genome in a highly regulated manner and in which assessments of DSB repair and protein movements could be done with significantly improved resolution (23). Using this approach, we found that BRCA1 is recruited both directly to the DSB site and to the regions surrounding the DSB, with moderately higher levels being detected in the immediate vicinity of the DSB (Fig. 1A). Consistent with our previous findings (23, 24), NBS1 is detectably recruited only in

![Image](Differential BRCA1 Recruitment to the DNA Double-Strand Break)
the vicinity of the break, whereas γH2AX is reversibly present in the flanking chromatin, but not directly at the DSB (Fig. 1B and C). Notably, γH2AX serves as a platform for recruitment of MDC1 (25, 26). Consistent with this, MDC1 and the downstream factors RNF8 and RAP80 showed a similar recruitment pattern accumulating in the DSB flanking regions, but not directly at the break (Fig. 1D–F). In terms of kinetics, NBS1 recruitment preceded the other proteins, plateauing at 30 minutes after I-PpoI induction, compared with peak binding at 60 minutes for BRCA1 and the members of the γH2AX–MDC1–RNF8/RNF168–RAP80 pathway (Fig. 1). Furthermore, levels of NBS1 and γH2AX remained at the plateau level for the duration of the experiment in contrast to decreasing chromatin binding of BRCA1, MDC1, RNF8, and RAP80 by 120 minutes (Fig. 1). Thus, BRCA1 is recruited directly both to the break and to the DSB surrounding regions, whereas NBS1 is present only in the vicinity of the break and the γH2AX–MDC1–RNF8/RNF168–RAP80 pathway members accumulate only in the DSB flanking chromatin.

Differential roles for the RNF8/RAP80 pathway and NBS1 in BRCA1 recruitment to chromatin

RNF8 and RNF168 activities affect multiple DDR processes, including the recruitment of 53BP1 (15, 17, 27). Thus, to investigate mechanisms involved in BRCA1 recruitment to the DSB and flanking region, we knocked down RAP80 instead of RNF8 in order to minimize the effect on DDR proteins other than BRCA1. RAP80 knockdown abrogated the recruitment of BRCA1 to the DSB flanking regions without affecting the levels of BRCA1 in the vicinity of the break (Fig. 2A), thus indicating that the RNF8/RNF168–RAP80 pathway is responsible for recruiting BRCA1 to the flanking chromatin, but not directly to the DSB. Importantly, RAP80 depletion did not disrupt NBS1 recruitment (Fig. 2B), demonstrating a localized and specific effect. Because a recent study demonstrated that the interaction between BRCA1 and RAP80 is dependent on BRCA1 PARYlation by PARP1 (28), we examined the effect of PARP1 inhibition on BRCA1 recruitment to the DSB. Indeed, PARP1 inhibition reduced BRCA1 recruitment to the DSB surrounding regions without affecting BRCA1 in the vicinity of the DSB (Supplementary Fig. S1A), thus having a similar effect on the BRCA1 recruitment pattern as RAP80 depletion. In addition, we observed that ATM inhibition affected BRCA1 recruitment to the DSB surrounding regions (Supplementary Fig. S1B), consistent with its role in facilitating the formation of γH2AX domains surrounding the DSB that serve as platform for MDC1 recruitment (25, 26).

These results raised the question of the mechanisms responsible for recruitment of BRCA1 directly to the DSB. Because the MRN complex accumulates mainly in the vicinity of the DSB, but not the surrounding regions (e.g., Fig. 1B), it was a logical candidate for recruiting BRCA1 directly to the break. In support of this hypothesis, BRCA1 was reported to be present in a multiprotein complex with MRN (21). Therefore, we asked whether DSB induction by IR affects the interaction between BRCA1 and NBS1, and whether BRCA1 recruitment to the DSB depended on NBS1. Reciprocal commounprecipitation demonstrated an increased interaction between BRCA1 and NBS1 after 10 Gy IR (Fig. 3A) and NBS1 knockdown abolished BRCA1 accumulation both at the DSB and around the break site (Fig. 3B). Because the MRN complex is essential for the initiation of DDR signaling, including ATM recruitment to the DSB, which is required for γH2AX domain formation and MDC1 recruitment (25), we suspected that the abrogation of BRCA1 recruitment to the surrounding chromatin after NBS1 knockdown was likely due to the disruption of the RNF8/RNF168–RAP80 pathway. Consistent with this, we found that NBS1 knockdown abolishes RAP80 accumulation in the DSB surrounding regions (Fig. 3C). These results indicate that two spatially distinct BRCA1 fractions exist at the...
Differential BRCA1 Recruitment to the DNA Double-Strand Break

Figure 3.
NBS1 is required for BRCA1 recruitment to the DSB site. A, reciprocal commounprecipitation of BRCA1 and NBS1 in either untreated MCF7 cells or in MCF7 cells 30 minutes following 10 Gy IR. Normal rabbit IgG was used as control. ChIP of BRCA1 (B) and RAP80 (C) in MCF7 cells expressing ddI-PpoI as described in Fig. 1. Cells were transfected with either nontargeting control siRNA or NBS1 targeting siRNA.

BRCA1 at the DSB is involved in DNA repair

Differential mechanisms involved in the recruitment of spatially distinct BRCA1 fractions raised the possibility of different functions for these two sites of BRCA1 binding in the DDR. Although several studies reported that BRCA1 promotes religation of linearized plasmids by NHEJ (8, 9), plasmid DNA lacks many structural and functional properties of the DNA packaged in chromatin. Thus, the role of BRCA1 in NHEJ repair of genomic DNA has been somewhat unclear. By arresting cells in the G1-phase of the cell cycle, the I-PpoI system can specifically assess religation of DNA by NHEJ repair (23). Downregulation of BRCA1 significantly inhibited NHEJ repair of DSBs induced either by I-PpoI (Fig. 4A; Supplementary Figs. S2 and S3) or by ionizing irradiation (comet assay; Fig. 4B; Supplementary Figs. S2 and S3). This raised the question of which populations of chromatin-bound BRCA1 were affecting DNA repair. Although NBS1 knockdown, which affects BRCA1 recruitment to both direct and flanking sites, had the expected negative impact on NHEJ repair (Fig. 4A and B), RAP80 knockdown, which abrogates the binding of BRCA1 only to the flanking sites, did not affect NHEJ repair (Fig. 4A and B). Thus, the BRCA1 fraction bound to chromatin flanking the DSB is dispensable for NHEJ repair. For completeness, we also tested the effects of BRCA1, NBS1, and RAP80 knockdowns on DSB repair in cycling cells, in which both NHEJ and homologous recombination would contribute to repair of DSBs in cells containing S and G2 populations. Similar to the observations in G1-arrested cells, depletion of RAP80 did not affect DSB repair in cycling cells, whereas depletion of either BRCA1 or NBS1 significantly reduced DSB repair (Supplementary Fig. S4).

Chromatin-bound BRCA1 in the DSB flanking regions is required for cell-cycle checkpoints and regulates DSB processing

Because BRCA1 residing in the DSB flanking regions was dispensable for DSB repair, we sought to identify the role of this BRCA1 fraction in the DDR. A previously published report suggested that the BRCA1–RAP80 protein complex suppresses excess DNA end resection at the DSB (29, 30). Consistent with this finding, we observed that a knockdown of RAP80 in proliferating cells resulted in elevated levels of RPA32 (replication

Figure 4.
DSB religation by NHEJ depends on BRCA1 and NBS1 but not RAP80. A, DNA repair measured by quantitative real-time PCR spanning the unique I-PpoI cleavage site at chromosome 1 in MCF7 cells expressing ddI-PpoI that were cultivated in medium containing 0.1% FBS for 24 hours before DSB induction. Time indicated is hours following the addition of 4-OHT. Data of three independent experiments are shown as mean ± SEM. B, the comet assay was performed in MCF7 cells that were untreated (time point 0 h) or treated with 10 Gy IR and harvested at indicated time points after irradiation. Cells were cultivated in medium containing 0.1% FBS for 24 hours before DSB induction. A and B, cells were transfected with either nontargeting control siRNA, BRCA1, NBS1, or RAP80 targeting siRNA. OTM, olive tail moment.
protein A, 32 kDa subunit) at the break site (Supplementary Fig. S5), which reflects an increase in the amount of DNA resection. Importantly, an increase in RPA32 levels due to RAP80 depletion was only observed in the DSB surrounding regions (Supplementary Fig. S5), where BRCA1 recruitment is mediated by RAP80, but not directly at the break.

DSB flanking chromatin is also a region where ATM-dependent signaling is amplified in a γH2AX-dependent manner (25). Following DSB induction, activated ATM phosphorylates BRCA1 at serine residues 1387 and 1423, modifications required for activation of the S and G2–M checkpoints, respectively (12, 13). Therefore, we asked whether BRCA1 recruitment to the DSB surrounding regions by RAP80 facilitates the phosphorylation of BRCA1 by ATM, thus triggering the DNA damage-induced cell-cycle checkpoints. RAP80 depletion in two different cell lines significantly reduced the phosphorylation of BRCA1 at serine residues 1387 and 1423 without affecting the phosphorylation of other ATM targets, such as Kap1 and CHK2 (Fig. 5A and B). A knockdown of NBS1, which would abrogate BRCA1 recruitment to both the DSB and the flanking regions, resulted in a similar degree of reduction of the S and G2–M checkpoints (25, 26). Here, we identified in a manner that depends on the formation of the DSB-anchoring region, ATM-mediated phosphorylation of Brca1 at serine 1387 and 1423 and the activation of the ATM pathway in MCF7 (A) and HeLa cells (B) 30 minutes after treatment with 10 Gy IR. Cells were transfected with either nontargeting control siRNA, RAP80, NBS1, or ATM targeting siRNA. —-, ATM band; *, nonspecific band.

Discussion

By taking advantage of the spatial resolution afforded by introduction of ε-PPol–induced DSBs at defined sites in the human genome, we found that BRCA1 recruitment to DSBs actually represents two separate processes (Fig. 7). In response to DNA breakage, BRCA1 binds to NBS1 protein and is recruited directly to the sites of DNA breaks, in which its recruitment is required for efficient DNA repair. The results definitively implicated BRCA1 residing in the direct vicinity of the DSB in NHEJ repair of endogenous DNA. Given that the initiation of DSB processing that is required for homologous recombination is dependent on the MRN complex (25), although the RAP80–BRCA1 complex represses DNA resection as discussed below, we speculate that BRCA1 bound directly to the break may also facilitate repair by homologous recombination. In addition, BRCA1 is recruited to chromatin flanking sites of DNA breakage in a manner dependent on RAP80. In this DSB flanking region, ATM-mediated phosphorylation of Brca1 occurs and is required for both S-phase and G2–M checkpoints. Notably, our model is consistent with a previous report showing that a simultaneous knockdown of NBS1 and H2AX is required to achieve a complete abrogation of BRCA1 foci formation following irradiation (31).

Consistent with previous reports implicating the RAP80–BRCA1 complex in suppression of excessive DSB processing (28–30), we observed an increase in DNA resection in the DSB flanking regions in the absence of RAP80. These findings suggest an additional role for the BRCA1 fraction that is recruited by RAP80 to the DSB flanking chromatin in tuning homologous recombination by preventing excessive DSB processing. We suspect that a much larger fraction of BRCA1 protein is bound to the DSB flanking regions than directly to the DSB site, thus making it difficult to assess the relative amount and importance of the directly bound fraction of BRCA1 by other techniques. The approaches described here were sufficient to clarify that this fraction is both recruited by a different mechanism and functionally distinct. However, some questions remain with regard to the MRN-dependent BRCA1 recruitment in the vicinity of the break, including whether the BRCA1–NBS1 interaction is direct or whether it requires other proteins as mediators. In addition, it remains to be clarified which domains of NBS1 and BRCA1 are responsible for the interaction and whether it is regulated by the posttranslational modifications of BRCA1 and NBS1 that occur following DNA damage induction.

It is important to note that the DSB-flanking chromatin is considered to be a region where ATM signaling is amplified in a manner that depends on the formation of the γH2AX domains that spread around the DSB (25, 26). Here, we identified an additional role of the DSB-flanking regions in promoting ATM-dependent signaling by showing that these
regions serve as a platform for BRCA1 phosphorylation by ATM, which is required for an efficient checkpoint activation. Interestingly, although activation of the BRCA1-dependent cell-cycle checkpoints relies on RAP80, we found that a knockdown of RAP80 only moderately affects cell survival after exposure to IR. In contrast, a depletion of NBS1 and BRCA1 strongly reduces the surviving fraction. These observations are consistent with RAP80 lacking a role in DSB religation. Further, our findings are in line with previously published reports showing different phenotypes of mice lacking NBS1 or BRCA1 versus RAP80-deficient mice. Although a knockout of Brca1 or

Figure 6.
BRCA1 recruitment to the DSB surrounding regions promotes induction of the cell-cycle arrest in S and G2. A, S-phase checkpoint analysis in MCF7 cells by quantification of BrdUrd incorporation into DNA following 10 Gy IR. BrdUrd incorporation is shown normalized to nonirradiated control. B, G2–M checkpoint analysis in MCF7 cells by phospho-H3 staining following 10 Gy IR. C, MTT assay in MCF7 cells showing cell survival following IR. A–C, MCF7 cells were transfected with either nontargeting control siRNA, NBS1, BRCA1, or RAP80 targeting siRNA. Data of three independent experiments are shown as mean ± SEM. C, the SEM is too small to be shown for several time points.

Figure 7.
Model of spatially differential BRCA1 recruitment to the DSB. The data presented herein support the following steps involved in Brca1 recruitment to chromatin and function after DNA breakage. (1) Following DSB induction, BRCA1 interacts with NBS1, resulting in the recruitment of a small fraction of BRCA1 directly to the DNA break site, where it facilitates DSB repair. (2) ATM phosphorylates histone protein H2AX, resulting in the formation of γH2AX domains in the DSB flanking regions that serve as a platform for MDC1 and RNF8 recruitment. (3) RNF8 and RNF186 E3 ubiquitin ligases facilitate the conjugation of ubiquitin chains to the histone H2A. (4) RAP80 links Brca1 to these polyubiquitin chains, thus recruiting a larger Brca1 fraction to the DSB flanking chromatin. (5) BRCA1 recruitment to the flanking chromatins increases the amount of BRCA1 molecules interacting with active ATM, resulting in an efficient phosphorylation of BRCA1 at serine residues 1387 and 1423, which is required for the activation of S-phase and G2–M checkpoints, respectively. (6) The RAP80-BRCA1 complex residing in the DSB flanking regions also represses an excessive DSB processing, thus tuning homologous recombination.
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Nbs1 in mice is lethal (32, 33). Rap80Δ/C mice are viable (34). Consistent with our results, MEFs derived from Rap80Δ/C mice are only moderately hypersensitive to IR compared with wild-type mice (34). Thus, efficient DNA repair appears to be the major determinant of cell survival after exposure to agents that induce DSBs, whereas activation of cell-cycle arrests plays a minor role in cell survival, consistent with numerous studies in the literature. Taken together, these results provide new insights into the regulation of the functional diversity of BRCA1 in the DDR and demonstrate the functional importance and role of the spatial arrangement of BRCA1 at the DSB site.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: M. Goldstein, M.B. Kastan

Development of methodology: M. Goldstein, M.B. Kastan

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Goldstein

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Goldstein

Writing, review, and/or revision of the manuscript: M. Goldstein, M.B. Kastan

Study supervision: M. B. Kastan

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