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BRCA PROTEINS AND DNA DAMAGE CHECKPOINTS

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1. ABSTRACT

BRCA1 and BRCA2 are frequently mutated in breast cancer patients, responsible for the majority cases of familial breast cancer. Through genetic and biochemical analysis, the function of BRCA1 and BRCA2 is emerging. This review examines recent progresses in understanding the roles of BRCA proteins in DNA damage checkpoint control and how they are regulated in response to DNA damage.

2. INTRODUCTION

BRCA1 and BRCA2 are tumor suppressors that are frequently mutated in breast cancer patients (1-3). How BRCA proteins mediate tumor suppression is of significant interest to scientists and clinicians. Recent progresses in BRCA1 and BRCA2 research reveal potential mechanisms of their tumor suppression function. Increasing evidence suggest that BRCA1 and BRCA2 play important roles in preserving genomic stability through their involvement in DNA damage repair and cell cycle checkpoint control. There are some excellent reviews that discuss DNA damage repair function of BRCA proteins (4,5). This review focuses on cell cycle checkpoint control mediated by BRCA proteins.

Cell cycle checkpoints are series of molecular events that are initiated to arrest cells at certain stages of cell cycle in response to DNA damage. They are cellular surveillance mechanisms that help preserve genomic stability by allowing time for cells to repair damaged DNA. Dysfunction of proteins involved in cell cycle checkpoint control, such as p53 and ATM, often results in genomic instability and tumorigenesis (6). Generally, DNA damage checkpoints are divided into G1/S, S, and G2/M controls. Recent studies have implicated BRCA1 and BRCA2 in S and G2/M cell cycle checkpoint control. Loss of their cell cycle checkpoint function could potentially contribute to the development of breast cancer.

3. INVOLVEMENT OF BRCA PROTEINS IN DNA DAMAGE CHECKPOINT

3.1. S-PHASE CHECKPOINT

S-phase checkpoint prevents cell from undergoing replication immediately after DNA damage. Defection in S-phase checkpoint results in radioresistant DNA synthesis (RDS). The exact molecular mechanism of S-phase checkpoint is still not clear, although it has been suggested that S-phase checkpoint mainly targets late replication origin firing (7). Profound RDS phenotype occurs in ATM-deficient cells following DNA damage, suggesting that ATM play an important role in S-phase checkpoint (8). Downstream of ATM, Chk1, Chk2 and NBS1 have been shown to be involved in S-phase checkpoint (9-11). Mechanistically, Chk1 and Chk2 are suggested to target Cdc25A, which is critical for S phase progression (9,12).

In addition to Chk1, Chk2 and NBS1, BRCA1 emerges as a potential regulator of S-phase checkpoint downstream of ATM. Using BRCA1-deficient HCC1937 cells, Xu *et al*. show that HCC1937 cells have defective S-phase checkpoint (RDS phenotype) in response to DNA damage (13). Complementation of HCC1937 cells with full-length BRCA1 restores S-phase checkpoint (13), suggesting the important role of BRCA1 in S-phase checkpoint.

How does BRCA1 regulate S-phase checkpoint? It has been shown that BRCA1 interacts with

Mre11/Rad50/NBS1 (MRN) complexes (14-16). Therefore, BRCA1 might regulate S-phase checkpoint through MRN complexes. Using HCC1937 cells, Zhong *et al.* have shown that BRCA1 is required for MRN foci formation in response to DNA damage (14). However, later studies suggest that the foci formation of MRN complexes is independent of BRCA1 (15,16). It is possible that BRCA1 regulates S-phase checkpoint through Chk1-Cdc25A pathway (12), whose activation requires BRCA1 (discussed later).

A recent report also suggests that BRCA2 is involved in S-phase checkpoint. Using Chinese hamster cell V-C8, van der Zwet *et al.* show that the RDS phenotype of V-C8 cells following radiation can be rescued by transfection of human chromosome 13, which contains BRCA2 gene, or mouse BRCA2 cDNA (17). These data for the first time suggested a role of BRCA2 in S-phase checkpoint. However, the molecular mechanism for BRCA2's S-phase checkpoint function is not yet understood. Given that BRCA2 exists in the same multi-subunit complex with BRCA1 (18), it is possible that BRCA2 works together with BRCA1 in S-phase checkpoint.

One caveat for the studies using the somatic "deficient" cell lines mentioned above is that the genetic background of these cells are complex. Cell derived from knockout mice should be an useful model to address this concern. It remains to be determined whether or not the S-phase checkpoint is intact in $BRCA1^{-/-}$ murine cells. Paradoxically, studies using cells derived from BRCA2 knockout mice did not reveal a defect in S-phase checkpoint (19). One possible reason is the species difference. The other possible reason is that there are other gene mutations in V-C8 cells that partially responsible for the S-phase checkpoint deficiency observed in these cells. Therefore, further studies are needed to establish the role and molecular mechanisms of BRCA proteins in S-phase checkpoint.

3. 2. G2/M CHECKPOINT

G2/M checkpoint allows for repair of DNA prior to mitosis, thus prevents damaged DNA being passed to daughter cells. The mechanism of G2/M checkpoint regulation is well established. Studies up to date suggest that Cdc2 is a major target of G2/M checkpoint (20,21). Cdc2 is inhibited by Wee1, which phosphorylates Cdc2 at Tyr 15. Cdc2 is also activated by Cdc25c, which dephosphorylates Cdc2 at Tyr 15. A model has been proposed that upon DNA damage, checkpoint kinases Chk1 and Chk2 are first activated by ATM/ATR. Chk1 and Chk2 then phosphorylate Wee1 and Cdc25c, resulting in the activation of Wee1 and nuclear exclusion of Cdc25c. The combined effect of Wee1 activation and Cdc25c nuclear exclusion leads to the inhibition of Cdc2 and the blockade of cells from G2 to M transition (22).

Using mouse embryonic fibroblast (MEF) derived from mice carrying a targeted deletion of exon 11 of the BRCA1 gene, Xu *et al.* show that G2/M checkpoint is defective in $BRCA1^{-/-}$ cells (23). The defective G2/M checkpoint is accompanied with extensive chromosomal abnormalities (23). Later studies in BRCA1-deficient HCC1937 cells also show a defective G2/M checkpoint, which is restored by the reconstitution of wild-type BRCA1 (13). Thus, it is likely that BRCA1 plays a role in G2/M checkpoint control.

Information about downstream effectors of BRCA1 in G2/M checkpoint has also been illustrated recently. Yarden *et al.* show that Chk1 activation and G2/M checkpoint in response to ionizing radiation is defective in HCC1937 cells (24). Reconstitution of BRCA1 in HCC1937 cells restored Chk1 activation and G2/M checkpoint. These data suggest that BRCA1 regulates G2/M checkpoint through Chk1. Since BRCA1 has been shown to interact with both ATM and Chk1, it is possible that BRCA1 may bridge Chk1 and ATM. Given that ATM also phosphorylates BRCA1, it will be interesting to determine whether ATM-dependent phosphorylation of BRCA1 is required for Chk1 activation and G2/M checkpoint control.

There is no evidence supporting a role of BRCA2 in damage-induced G2/M checkpoint control. In fact, cells derived from $BRCA2^{-/-}$ mice have intact G2/M checkpoint in response to DNA damage (19). However, several reports suggest that BRCA2 may contribute to mitotic checkpoint regulation. One study shows that BRCA2 interacts with hBUBR1 (25), which is the homologue of S. cerevisiae mitotic checkpoint protein BUB1. Furthermore, BRCA2 is phosphorylated by hBUBR1 *in vitro*. However, the functional significance of the BRCA2-BUBR1 interaction in mitotic checkpoint remains to be determined. Another study shows that BRCA2 interacts with a novel protein BRAF35, which preferentially binds to cruciform DNA (26). Injection of antibodies against either proteins delays metaphase progression, implying a role of BRCA2 in mitosis.

4. REGULATORS OF BRCA PROTEINS IN DNA DAMAGE CHECKPOINT

ATM and ATR are proximal kinases that regulate a number of proteins in DNA damage pathway (27,28). While BRCA1 has been shown to be a downstream substrate of ATM and ATR (29-31), it is not yet known whether BRCA2 is also a direct substrate of ATM/ATR. ATM phosphorylates BRCA1 at several residues including Ser1387, Ser1423 and Ser1457 (29,30). To investigate the regulation of BRCA1 by ATM in S-phase checkpoint, Xu *et al.* monitored S-phase checkpoint using HCC1937 cells transfected with wild type BRCA1 or BRCA1 with individual ATM phosphorylation site mutated. Interestingly, one specific ATM phosphorylation site of BRCA1, Ser1387, is required for S-phase checkpoint function of BRCA1 (32). On the other hand, another ATM phosphorylation site of BRCA1- Ser1423- is specifically required for BRCA1's G2/M checkpoint function in HCC1937 cells (13). Therefore, distinctive ATM phosphorylation sites of BRCA1 have different roles in regulating checkpoint functions.

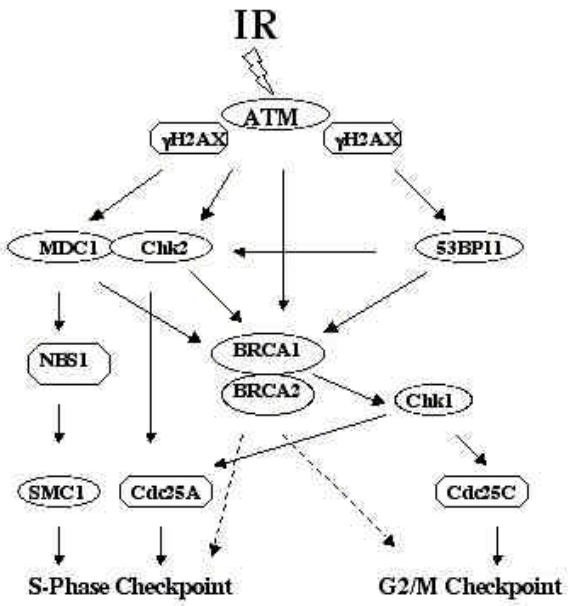

Figure 1. Schematic representation of BRCA1/2-dependent checkpoint pathways.

Several recent studies shed some new light into the regulation of BRCA1 following DNA damage. BRCA1 foci formation following IR is defective in murine H2AX $^{-/-}$ cells (33). Furthermore, 53BP1, whose foci formation also requires H2AX, appears to regulate BRCA1 foci (34). Using siRNA to downregulate 53BP1, Wang *et al*. show that BRCA1 foci formation is defective in 53BP1 siRNA transfected cells. Therefore, a linear pathway of H2AX-53BP1-BRCA1 has been proposed (Figure 1) (34). Consistent with this notion, H2AX and 53BP1 are also suggested to be involved in the regulation of G2/M checkpoint (34-36). However, some inconsistencies exist that need to be addressed in the future. For example, BRCA1 phosphorylation in response to IR seems normal in murine H2AX $^{-/-}$ cells (A. Nussenzweig, personal communication). In addition, the G2/M checkpoint defects in H2AX $^{-/-}$ or 5BP1$^{-/-}$ cells are modest compared to that observed in BRCA1$^{-/-}$ cells. Thus, it is likely that, besides H2AX/53BP1, additional pathways exist in regulating BRCA1 following DNA damage.

Recently, Mediator of DNA Damage Checkpoint protein 1, MDC1, previously known as Kiaa0170/NFBD1, emerges as another important regulator of BRCA1. Results from several groups show that MDC1 is phosphorylated in an ATM-dependent manner (37,38), and regulates S-phase checkpoint, probably through influencing NBS1 and Chk2 pathways (39-42). Furthermore, we found that downregulation of MDC1 resulted in defective BRCA1 foci formation and decreased phosphorylation of BRCA1 and Chk1 following DNA damage (43). These defects are accompanied by a defective G2/M checkpoint in response to ionizing radiation (42,43). Similar to 53BP1, MDC1 foci formation also depends on H2AX (unpublished results). Therefore, current data suggest that downstream of ATM-γH2AX, both 53BP1 and MDC1 regulates BRCA1 in DNA damage checkpoint (Figure 1).

5. CONCLUSION

Many studies up to date suggest the role of BRCA protein in DNA damage checkpoint. However, there are some discrepancies that exist between the murine knockout model and somatic cells deficient of BRCA proteins. Future studies are required to resolve these issues. Moreover, the molecular mechanisms underlying BRCA protein's checkpoint function are emerging (Figure 1), but far from clear. Insights into the mechanisms of BRCA in checkpoint regulation will help us understand how and in what extent their checkpoint control functions will contribute to tumor suppression *in vivo.*

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