Fanconi Anemia Complementation Group D2 (FANCD2) Functions Independently of BRCA2- and RAD51-associated Homologous Recombination in Response to DNA Damage*

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The BRCA2 breast cancer tumor suppressor is involved in the repair of double strand breaks and broken replication forks by homologous recombination through its interaction with DNA repair protein Rad51. Cells defective in BRCA2-FANCD1 are extremely sensitive to mitomycin C (MMC) similarly to cells deficient in any of the Fanconi anemia (FA) complementation group proteins (FANC). These observations suggest that the FA pathway and the BRCA2 and Rad51 repair pathway may be linked, although a functional connection between these pathways in DNA damage signaling remains to be determined. Here, we systematically investigated the interaction between these pathways. We show that in response to DNA damage, BRCA2-dependent Rad51 nuclear focus formation was normal in the absence of FANCD2 and that FANCD2 nuclear focus formation and mono-ubiquitination appeared normal in BRCA2-deficient cells. We report that the absence of BRCA2 substantially reduced homologous recombination repair of DNA breaks, whereas the absence of FANCD2 had little effect. Furthermore, we established that depletion of BRCA2 or Rad51 had a greater effect on cell survival in response to MMC than depletion of FANCD2 and that depletion of BRCA2 in FANCD2 mutant cells further sensitized these cells to MMC. Our results suggest that FANCD2 mediates double strand DNA break repair independently of Rad51-associated homologous recombination.

Breast cancer is one of the most common cancers affecting women. About half of all familial cases of breast cancer are caused by mutation in the breast cancer susceptibility genes BRCA1 and BRCA2. The BRCA2 gene encodes a 3,418-amino acid protein (1) containing eight conserved BRC motifs (2, 3) through which BRCA2 binds to Rad51, a protein with a crucial role in DNA recombination and repair (4–6). Because BRCA2 forms a complex with Rad51, a protein directly involved in DNA repair, BRCA2 may link DNA damage signaling pathways with the DNA damage repair machinery.

Recent studies indicate that Brca2-defective V-C8 lung fibroblasts are extremely sensitive to DNA cross-linking agents (7). This phenotype is similar to that of Fanconi anemia (FA)1 cells (8, 9), suggesting that the BRCA2 and FA proteins may function together (10). Fanconi anemia is an autosomal recessive chromosomal disorder (8, 9). There are 11 complementation groups (A, B, C, D1, D2, E, F, G, and L) (11–13), and 9 of the FA genes have been cloned (A, B, C, D1/BRCA2, D2, E, F, G, and L/PHF9) (8, 9, 13–16). Seven of the proteins (A, B, C, E, F, G, and L) form a nuclear complex and play a role in the mono-ubiquitination of FANCD2 protein in response to DNA damage (16–21). This modification is required for the repair of DNA cross-links and the accumulation of FANCD2 at the sites of DNA damage (19, 22). Recent studies have suggested genetic interactions between FA genes and the breast cancer susceptibility genes BRCA1 and BRCA2. It has been reported that BRCA1 protein is required for mono-ubiquitination (19) and FANCD2 nuclear focus formation (19, 23). Furthermore, BRCA1 directly interacts with ubiquitinated FANCD2 and FANCA protein (19, 24). These reports strongly suggest that the BRCA1 and FA pathways are linked. In addition, it was recently reported that FA-D1 cells contain biallelic BRCA2 inactivating mutations and that expression of full-length BRCA2 in FA-D1 cells can partially reverse the MMC sensitivity of these cells (14). These findings indicate that BRCA2 is the gene responsible for defects observed in FA-D1 cells. Supporting the involvement of BRCA2 and its associated protein Rad51 in the classical FA pathway, Digweed et al. (25) have reported that Rad51 focus formation in response to x-rays depends on the integrity of the FA pathway. In addition it has been suggested that mono-ubiquitination of FANCD2 promotes IR-induced BRCA2 focus formation (26). However, Godthelp et al. (27) reported normal Rad51 focus formation after MMC or IR treatment in all FA cells other than FA-D1 cells. Thus, the role of BRCA2 in the classical FA pathway in response to DNA damage remains to be resolved.

In this study, we further investigated the functional interaction between BRCA2 and FANCD2 following DNA damage. We used BRCA2- and FANCD2-deficient cells and cells treated with siRNAs specifically targeting BRCA2, Rad51, and FANCD2 and demonstrated that FANCD2 does not have a

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1 The abbreviations used are: FA, Fanconi anemia; FANC, FA protein; IR, ionizing radiation; MMC, mitomycin C; siRNA, small interfering RNA; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; HR, homologous recombination; GFP, green fluorescent protein; Gy, gray.
direct role in BRCA2- and Rad51-associated homologous recombination repair following DNA damage.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Culture Conditions—**V-C8 (Brcar-defective lung fibroblast cells) and BRCA2-reconstituted V-C8 cell lines were cultivated in Ham’s F-10 (Biowhittaker Inc.) supplemented with 10% fetal bovine serum (7). The reconstituted V-C8 cell lines cell lines were established by transfecting with a full-length wild-type BRCA2 mammalian expression plasmid and selected for G418-resistant clones (5). BRCA2 expression in reconstituted clones was determined by Western blotting following immunoprecipitation using rabbit anti-BRCA2 antibody (5). Drs. Alan D. D’Andrea and Toshiyasu Taniguchi kindly provided FA-D1 cells (UV423/MMPA), FA-D1 cells stably expressing BRCA2 (A913 423/2-33). FA-D2 cells (PD20), and FA-D2-derivative cells stably expressing FANC2 (PD20 + FANC2). PD20 cells were cultured in RPMI 1640 medium supplemented with 15% fetal bovine serum. PD20 + FANC2 cells also received 1 μg/ml puromycin. UV423/MMPA cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 15% fetal bovine serum containing 1 μg/ml puromycin. A913 423/2-33 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 15% fetal bovine serum containing hypoxanthine/aminopterin/thymidine and 200 μg/ml hygromycin.

**Clonogenic Survival Assays—**Cells were trypsinized, and 500–1,000 cells (2,500–5,000 cells for high doses) were seeded in a 100-mm dishes and left to attach for 4 h. For MMC treatment, cells were continuously exposed to 0, 0.01, 0.1, 1, or 10 ng/ml MMC for 8 days. For IR treatment, cells were exposed to γ radiation at the dose of 0, 4, 8, or 12 Gy. Eight days after those treatments, cells were stained and counted. Each survival curve represents the mean of at least two independent experiments. Error bars represent the standard deviations of the means.

**Immunoprecipitation, Immunoblotting, and Immunostaining—**Immunoprecipitation was carried out as described previously (5). Briefly, immunoprecipitation of BRCA2 was carried out using 1 μg of anti-BRCA2 polyclonal antibody (BRCA2C) (5). Proteins bound to protein G agarose were eluted by boiling in SDS sample buffer and separated by SDS-PAGE. Immunoblotting was performed using anti-BRCA2 antibodies (BRCA2C) (5), anti-Rad51 antibodies (5), and anti-FANC2 antibodies (FI17, Santa Cruz Biotechnology, Inc.) at a concentration of 5 μg/ml. Immunooblotted proteins were visualized by chemiluminescence. Immunofluorescence was performed (5) using affinity-purified anti-Rad51 antibodies and anti-FANC2 antibodies at a concentration of 1–2 μg/ml. Treatment with MMC or IR was carried out as described previously (14, 19, 22, 25). Images were captured with a 100× oil lens on an Axioplan fluorescence microscope. At least 150 cells in 5–10 different fields were counted for each experiment.

**Homologous Recombination (HR) Repair Assay—**An HR repair assay was carried out as described previously (28). Briefly, the efficiency of HR was assessed using an I-Scel expression plasmid (pCBAsCe) and an I-Scel-positive plasmid (DR-GFP) composed of two differentially mutated GFP genes, one of which contained a unique I-Scel restriction site. The assay works through gene conversion repair of a double strand break caused by I-Scel digestion. DR-GFP plasmids repaired by homologous recombination express GFP (29). The relevant cells were transfected with either 2 μg of DR-GFP plus 8 μg of pCBAsCe or 2 μg of DR-GFP plus 8 μg of control plasmids. 48 h after transfection, the cells were harvested, and the numbers of GFP-expressing cells were assessed by flow cytometry.

**RNA Interference—**RNA interference was performed using the Smart Pool siRNA (Dharmacon) for BRCA2, Rad51, and FANC2. A pSSHUP vector with BRCA2 short hairpin RNA was used for PD20 cells or PD20 + FANC2 cells. A pSSHUP vector (courtesy of Daniel Billadeau, Mayo Clinic) that produced a short hairpin RNA against BRCA2 was generated with the following oligonucleotides: forward, 5′-gateccGTTTTagAAACGCAAGTACcTaCcaagaGTAAGTCTGCTGTTCAATAAATTggigaaa-3′; reverse, 5′-ggcaACTTCTTCTGTTGGAATgaGttctctCATCGAACG-GAAAGATTTGGAaacaacttt-3′. Transfection of siRNA oligonucleotides was carried out with LipofectAMINE 2000 (Invitrogen) in 6-well plates according to the manufacturer’s specifications. Cells were incubated for 48 h prior to transfection prior to MMC treatment.

**MTT Assay—**The sensitivity of siRNA-transfected cells to MMC was evaluated using an MTT assay as described previously (30). 48 h after transfection, 5,000 cells were seeded into 96-well plates with 100 μl of culture medium, incubated for 16 h, and exposed to various concentrations of MMC for 72 h. MTT solution (CellTiter 96 Aqueous One Solution Reagent, Promega) (20 μl) was added to each well and incubated for 1.5 h. The absorbance of each well was measured in a microplate reader at 490 nm, and the absorbance in treated cells relative to untreated cells was displayed.

**RESULTS**

**BRCA2 Is Required for Cell Survival and Rad51 Focus Formation following MMC and Ionizing Radiation—**Recent studies indicate that BRCA2-defective cells are extremely sensitive to cross-linking agents (7). To confirm that this hypersensitivity in V-C8 cells deficient in Brca2 is due to the loss of Brca2, we generated V-C8 derivative cell lines stably expressing wild-type human BRCA2 (V-C8 + BRCA2). Expression of human BRCA2 in these V-C8 cells was confirmed by immunoblotting (Fig. 1A). We noted that the exogenously expressed human BRCA2 associated with endogenous Rad51 in these cells (Fig. 1A, middle panel) and that the expression level of Rad51 remained the same as that in V-C8 cells (Fig. 1A, bottom panel). The reconstitution of a BRCA2-Rad51 DNA repair complex in the V-C8 + BRCA2 cells using exogenous human BRCA2 suggested that BRCA2-dependent DNA repair was active in these cells. This was confirmed by demonstrating that the sensitivity of V-C8 cells to double strand breaks and cross-links induced by IR and MMC was largely rescued by the introduction of human BRCA2 into these cells (Fig. 1, B and C). In addition, although V-C8 cells showed defective Rad51 focus formation following MMC or IR treatment, we found that BRCA2-reconstituted...
V-C8 cells displayed a 64.3% increase in the number of cells containing ≥5 foci following MMC treatment and a 78.0% increase after IR treatment (Fig. 2, A and B). Taken together, these data confirm that BRCA2 is required for Rad51 focus formation following DNA damage and suggest that the absence of BRCA2 confers hypersensitivity to DNA cross-linking agents through an inability to recruit Rad51 homologous recombination complexes to sites of DNA damage.

**FANCD2 Functions Independently of Rad51 and BRCA2 in Response to DNA Damage.** A, V-C8 reconstituted with BRCA2 form Rad51 foci. V-C8 and V-C8 + BRCA2 cells were treated with 10 ng/ml MMC or 8 Gy of IR and immunostained with anti-Rad51 antibodies. B, quantification of Rad51 focus formation in V-C8 and reconstituted cells. Nuclei were grouped by the number of Rad51 foci per nucleus. Data are presented as the mean ± S.E. (n = 3). C, disruption of FANCD2 does not alter Rad51 focus formation. PD20 cells and PD20 FANCD2-reconstituted cells were treated with 40 ng/ml MMC or 12 Gy of IR and immunostained with anti-Rad51 antibodies. D, quantification of Rad51 focus formation in FA-D2 and reconstituted cells. Nuclei were grouped as in B. E, BRCA2 is not required for FANCD2 focus formation after DNA damage. FA-D1 and FA-D1 + BRCA2 cells were treated with 25 ng/ml MMC or 12 Gy of IR and immunostained with anti-FANCD2 antibodies. F, quantification of FANCD2 focus formation in FA-D1 and reconstituted cells. Nuclei were grouped by number of foci. Data are presented as the mean ± S.E. (n = 3). G, BRCA2 does not influence FANCD2 mono-ubiquitination. FA-D1 and FA-D1 + BRCA2-reconstituted cells were treated with 40 ng/ml MMC or 12 Gy of IR and immunoblotted with anti-FANCD2 antibody. The letters u and l indicate the mono-ubiquitinated (FANCD2-L) and nonubiquitinated (FANCD2-S) forms of FANCD2, respectively. Band intensity of FANCD2 was measured by densitometry. The lower panel shows band intensity of FANCD2-L relative to FANCD2-S.

**FANCD2 Is Not Required for Damage-induced Rad51 Focus Formation**—Fanconi anemia cells, like V-C8 cells, are extremely sensitive to MMC. To explore the connection between BRCA2 and FANCD2, we examined whether Rad51 damage-induced nuclear focus formation depends on FANCD2. For this study, we used FA-D2 cells (PD20) and an FA-D2-derivative cell line stably expressing human FANCD2 (PD20 + FANCD2). As shown in Fig. 2C, Rad51 localized to nuclear foci following MMC or IR treatment in both PD20 cells and PD20 + FANCD2 cells. The percentage of Rad51-foci positive cells in PD20 cells following MMC treatment or IR treatment was very similar to that in PD20 cells reconstituted with wild-type FANCD2 (Fig. 2D). In contrast, FANCD2 foci were detected only in FA-D2 + FANCD2 cells and not in FA-D2 cells (data not shown). Thus, it appears that FANCD2 is not required for the localization of Rad51 to nuclear DNA damage foci.

**BRCA2 Is Not Required for FANCD2 Mono-ubiquitination or FANCD2 Focus Formation Following DNA Damage**—Although FANCD2 is not required for Rad51 localization to nuclear DNA damage foci, it is possible that the BRCA2-Rad51 complex may
be involved in the regulation of FANCD2. To investigate whether BRCA2 affects FANCD2 regulation following DNA damage, we evaluated FANCD2 DNA damage focus formation in the absence of functional BRCA2 using BRCA2 mutant FA-D1 cells (VU423/PMMP) cells. Following MMC or IR treatment, the percentage of FA-D1 cells with FANCD2 foci was similar to that in FA-D1/H11001BRCA2 cells (Fig. 2, E and F). Similarly to V-C8 cells, these FA-D1 cells showed defective Rad51 focus formation after MMC or IR treatment, which could be restored upon expression of exogenous human BRCA2 (data not shown). This suggests that BRCA2 is not required for the formation of FANCD2 foci following DNA damage.

Previous studies have shown that FANCD2 is mono-ubiquitinated following DNA damage and that this modified form of FANCD2 can be detected as an upward mobility shift on SDS-PAGE (19, 22). Therefore, we further evaluated the influence of BRCA2 on FANCD2 function by examining the effect of the absence of functional BRCA2 on FANCD2 ubiquitination in BRCA2 mutant FA-D1 cells. As shown in Fig. 2G, the mono-ubiquitinated form of FANCD2 was prominent in both FA-D1 cells and FA-D1 cells reconstituted with human BRCA2 (A913423/2-33; FA-D1 + BRCA2) following MMC or IR treatment. Importantly, the expression levels of FANCD2 in these cells remained the same (data not shown). Taken together these data strongly suggest that BRCA2 is not required for FANCD2 activity in response to DNA damage.

**FANCD2 Is Not Required for Homologous Recombination Repair of DNA Breaks**—To confirm the requirement for BRCA2 in HR repair and to investigate whether FANCD2 is involved in the same HR repair pathway as BRCA2, we used an I-SceI-dependent GFP reporter assay to measure homologous recombination activity in BRCA2- or FANCD2-deficient cells (29). This assay depends on reconstitution of the GFP signal as a consequence of repair of an I-SceI-induced double strand break, as described under “Experimental Procedures.” We transfected V-C8, V-C8 + BRCA2, PD20, and PD20 + FANCD2 cells with the DR-GFP reporter plasmid and an I-SceI expression plasmid (pcBA5e). 48 h after transfection the proportion of cells expressing GFP following homologous recombination repair of the I-SceI-induced double strand break was measured by flow cytometry. As shown in the example in Fig. 3A, GFP positive cells were rare in both V-C8 + BRCA2 and V-C8 cells. The -fold increase in GFP positive cells relative to vector control in V-C8 + BRCA2 cells is shown. C, quantification of HR repair in PD20 + FANCD2 and PD20 cells. The -fold increase in GFP positive cells relative to vector control in PD20 + FANCD2 cells is shown.
with wild-type FANCD2 (13.7-fold increase), suggesting that FANCD2 does not make a substantial contribution to HR repair, whereas BRCA2 is required for this process.

**FANCD2 Mediates the Response to MMC Treatment in a BRCA2- and Rad51-independent Manner**—To assess whether BRCA2 and Rad51 contribute independently to cell survival in response to MMC, we utilized RNA interference approaches. 293T cells or HeLa cells were transfected with siRNAs targeting BRCA2, Rad51, and FANCD2. Each of these siRNAs significantly reduced the expression of targeted proteins (Fig. 4A). In addition, treatment of cells with combinations of these siRNAs resulted in similar reductions in protein levels (data not shown). As expected, depletion of each of these proteins substantially reduced cell survival following MMC treatment. Specifically, depletion of BRCA2 or Rad51 resulted in hypersensitivity to MMC (Fig. 4B). As sensitivity to MMC was not increased when both proteins were depleted in unison, it appears that BRCA2 and Rad51 respond to MMC treatment through the same pathway. In contrast, we noted that depletion of FANCD2 had a small but nonetheless significant effect on cell survival following MMC treatment (Fig. 4C), suggesting that FANCD2 might not influence MMC sensitivity in the same manner as BRCA2 and Rad51. Importantly, when FANCD2 was depleted along with Rad51, we observed enhanced MMC sensitivity compared with cells with either Rad51 or FANCD2 depletion alone (Fig. 4C). This increase in MMC sensitivity following co-depletion of FANCD2 was also observed in the cells depleted of BRCA2 (data not shown). The additive effect of FANCD2 deficiency and either Rad51 or BRCA2 deficiency on MMC sensitivity suggests that FANCD2 functions through a BRCA2- and Rad51-independent repair pathway. However, it is formally possible that combinations of partial defects in the same functional pathway, as might be caused by combined siRNA treatments, could lead to a greater effect than for a single defect. To address this possibility, we evaluated the influence of reduced BRCA2 levels on MMC sensitivity in FANCD2-deficient PD20 cells and in FANCD2-reconstituted PD20 cells. As shown in Fig. 4D, depletion of BRCA2 in both...
PD20 and PD20-reconstituted cells enhanced MMC sensitivity. Given that the FANCD2 pathway is inactive in these cells, the added MMC sensitivity following BRCA2 depletion provides strong evidence against cooperation of partial defects in the same pathway. Overall, these results suggest that FANCD2 and BRCA2-Rad51 function independently in cell survival following MMC treatment.

**DISCUSSION**

In this study, we investigated the relationship between BRCA2-Rad51 and FANCD2 in DNA damage-signaling pathway and DNA repair. We have shown that Rad51 focus formation following DNA damage depends on BRCA2 but not FANCD2. In addition, the regulation of FANCD2 following DNA damage is independent of BRCA2, suggesting that FANCD2 may function independently of BRCA2-Rad51. In agreement with this hypothesis, we have shown that FANCD2 is dispensable for a BRCA2-Rad51-dependent homology-directed recombination. Moreover, cells with deficiency in both FANCD2 and BRCA2-Rad51 pathways are more sensitive to MMC, supporting the observation that FANCD2 and BRCA2-Rad51 function in different DNA repair pathways.

BRCA2 mutant and deficient cells display extreme sensitivity to DNA damage. In this study, we confirm that the DNA damage hypersensitivity in V-C8 cells (Brc2-deficient) is due to the lack of full-length Brc2. In addition, we show that Rad51 focus formation in response to DNA damage depends on BRCA2 (31), as previously reported for CAPAN-1 cells and Brc2-deficient mouse embryonic fibroblasts (32, 33). Taken together, these results suggest that BRCA2 is directly involved in DNA repair by recruiting Rad51 to the sites of DNA damage. Our findings support recent studies showing that BRCA2 and Rad51 are recruited together to sites of double strand DNA breaks (34) to facilitate homologous recombination repair (29) as opposed to single strand annealing (35).

BRCA2 and Rad51 mutant and deficient cells display great sensitivity to DNA cross-linking agents such as MMC, a phenomenon similar to that observed in Fanconi anemia cells (10). As biallelic mutations of BRCA2 have been identified in FA-D1 cells (14), it is now accepted that BRCA2 mutations are responsible for the phenotype observed in FA-D1 cells. These observations led to the hypothesis that the FA pathway is linked with the BRCA2 and Rad51 homologous recombination DNA repair pathway. Data in support of this came from the observations by Digweed et al. (25) that Rad51 foci are attenuated in FA-A, FA-C and FA-G cells after IR exposure. In addition, it appears that FANCJ interacts with BRCA2 and co-localizes with BRCA2 after DNA damage (36). It has also been reported that FANCJ interacts with BRCA2 (37) and is required for BRCA2 focus formation (26). In addition, mono-ubiquitination of FANCJ is needed for the assembly of IR-induced FANCJ and BRCA2 foci (26). However, Godthelp et al. (27) reported that normal Rad51 focus formation is observed in all FA cells except FA-D1 cells in response to DNA damage induced by MMC or X-rays, suggesting that the BRCA2-associated Rad51 monoubiquitinated recombination repair pathway and the FA repair pathway may function independently in the response to these types of DNA damage.

In this study, we attempted to clarify these apparently inconsistent findings by systematically examining the contribution of FANCJ to the BRCA2- and Rad51-dependent DNA damage response. Our data show the normal appearance of nuclear Rad51 foci in FANCJ-deficient cells following MMC or IR treatment. We also noted that Rad51 focus formation appeared normal in FA-F cells following MMC or IR treatment (data not shown), whereas Rad51 foci failed to form in BRCA2-deficient cells. Our results are in keeping with those of Godthelp et al. (27) and strongly suggest that FANCJ is not required for Rad51 focus formation following DNA damage. The results also appear to suggest that FANCJ functions independently of BRCA2 and Rad51 in DNA repair.

To further address this possibility, we evaluated the influence of BRCA2 deficiency on FANCJ function. We found that FANCJ foci formed normally in BRCA2-deficient cells following MMC exposure to DNA damaging agents. We also failed to detect any difference in damage-induced mono-ubiquitination of FANCJ2 in BRCA2-defective or reconstituted cells, suggesting that BRCA2 is not involved in FANCJ mono-ubiquitination after DNA damage. In support of our studies, Bruun et al. (38) have recently shown that BRCA2 depletion does not affect FANCJ ubiquitination after DNA damage. Taken together, these findings establish that BRCA2 is not upstream of FANCJ in the DNA damage-signaling pathway.

We next assessed the role of FANCJ in homologous recombination repair of DNA breaks. It has been clearly established using a homology-directed repair reporter assay (29) that BRCA2 and Rad51 are required for HR repair of DNA double strand breaks. We used the same assay to confirm the requirement for BRCA2 in HR repair in V-C8 cells and to demonstrate that FANCJ does not have a significant involvement in this BRCA2- and Rad51-dependent HR repair using FANCJ-deficient PD20 cells. Similarly, Nakanishi et al. (39) have reported that a deficiency in FANCJ has a very mild effect on HR repair as measured by this assay (39). In addition, we used RNA interference to show that depletion of FANCJ resulted in mild MMC sensitivity compared with the effects of BRCA2 or Rad51 depletion. Moreover, we observed that FANCJ depletions or inactivation in combination with depletion of BRCA2 or Rad51 enhanced Rad51- and BRCA2-dependent MMC sensitivity, whereas double depletion of BRCA2 and Rad51 did not enhance MMC sensitivity.

On the basis of these data we propose a model in which FANCJ mediates repair of IR-induced and other DNA damage through a pathway that is independent of the Rad51 homologous recombination repair pathway. Similarly, Nakanishi et al. (39) have proposed a role for FANCJ in a second BRCA2-independent double strand break pathway involving homology. Likewise, based on the recent finding that FANCJ2-disrupted chicken DT40 cells exhibit normal Rad51 repair foci and do not display decreased spontaneous sister chromatid exchange, Yamamoto et al. (40) have proposed that FANCJ2 functions in a sub-DNA repair pathway that is independent of DNA cross-over and sister chromatid exchange. Importantly these FANCJ2-defective chicken cells were deficient in HR-mediated DNA repair. In contrast, studies in human cells have shown that deficiency in FANCJ does not have a substantial effect on HR repair in human cells (39). The difference in these observations may be explained by the use of different HR assays to detect homology-directed repair. Certainly, a number of different recombination assays exist that appear to measure different HR and double strand break repair processes, some of which are yet to be fully understood. Alternatively, the contrasting results may derive from differences in double strand break repair between chicken DT40 cells and human cells. Nevertheless, it is clear that FANCJ2 functions in a DNA repair pathway that is different from that of BRCA2 and Rad51. Future studies are needed to elucidate the molecular mechanisms of the FA- or FANCJ2-dependent DNA repair pathway.

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