BRCA2 Regulates Homologous Recombination in Response to DNA Damage: Implications for Genome Stability and Carcinogenesis

Christine Abaji, Isabelle Cousineau, and Abdellah Belmaaza

Abstract

BRCA2 has been implicated in the maintenance of genome stability and RAD51-mediated homologous recombination repair of chromosomal double-strand breaks (DSBs), but its role in these processes is unclear. To gain more insight into its role in homologous recombination, we expressed wild-type BRCA2 in the well-characterized BRCA2-deficient human cell line CAPAN-1 containing, as homologous recombination substrates, either direct or inverted repeats of two inactive marker genes. Whereas direct repeats monitor a mixture of RAD51-dependent and RAD51-independent homologous recombination events, inverted repeats distinguish between these events by reporting RAD51-dependent homologous recombination, gene conversion, and crossover events only. At either repeats, BRCA2 decreases the rate and frequency of spontaneous homologous recombination, but following chromosomal DSBs, BRCA2 increases the frequency of homologous recombination. At direct repeats, BRCA2 suppresses both spontaneous gene conversion and deletions, which can arise either from crossover or RAD51-independent sister chromatid replication slippage (SCRS), but following chromosomal DSBs, BRCA2 highly promotes gene conversion with little effect on deletions. At inverted repeats, spontaneous or DSB-induced crossover events were scarce and BRCA2 does not suppress their formation. From these results, we conclude that (i) BRCA2 regulates RAD51 recombination in response to the type of DNA damage and (ii) BRCA2 suppresses SCRS, suggesting a role for BRCA2 in sister chromatids cohesion and/or alignment. Loss of such control in response to estrogen-induced DNA damage after BRCA2 inactivation may be a key initial event triggering genome instability and carcinogenesis. (Cancer Res 2005; 65(10): 4117-25)

Introduction

Germ line mutations in BRCA2 predispose humans to breast, ovarian, and other cancers (1). In addition, hypomorphic mutations in BRCA2 have been found in cells from patients in the FANC-B and FANC-D1 subgroups of Fanconi’s anemia, an autosomal-recessive cancer susceptibility syndrome (2). Tumor cells derived from predisposed individuals show loss of heterozygosity (LOH) for the wild-type allele with retention of the mutated inherited allele, indicating that BRCA2 acts as a tumor suppressor gene. BRCA2 is ubiquitously expressed and encodes a large nuclear protein localized to the nucleus of S-phase cells. BRCA2 has been implicated in processes fundamental to all cells, including chromatin remodelling, regulation of transcription, and RAD51-mediated recombination repair, although its role in these processes remains unclear and the mechanisms underlying tissue specificity of its tumor suppressive property are unknown (3–5).

Mouse and human BRCA2-deficient cells accumulate spontaneous chromosome aberrations during cell division in culture, implicating BRCA2 in the maintenance of genome stability (4). The abnormalities include gross chromosomal rearrangements, chromosome and chromatid breaks as well as triradial and quadriradial chromosomes, markers of defective mitotic recombination that are typical of the human diseases Bloom’s syndrome, ataxia telangiectasia, and Fanconi’s anemia, mutated in BLM, ATM, and FANC genes, respectively (6). BRCA2-deficient cells are hypersensitive to genotoxic agents that have the potential to cause DNA double-strand breaks (DSBs), implicating BRCA2 in cell cycle signaling and/or DSB repair. However, because cell cycle checkpoints are largely preserved in BRCA2-deficient cells in the presence of DNA damage, a role for BRCA2 in DSB repair was acknowledged (3, 4).

Mammalian mitotic cells can repair DNA DSBs by two major recombination mechanisms, nonhomologous end joining (NHEJ) and homologous recombination (7). In NHEJ, DNA ends are joined with little or no base pairing at the joining site and the end-joining product can suffer insertion or deletion mutations (8). In contrast, DSB repair by homologous recombination requires the presence of an intact DNA duplex with extensive homology to the region flanking the break to serve as a repair template. The preferred template for homologous recombination repair is the sister chromatid (9). A key step in DSB repair by homologous recombination is the invasion of a 3’ single-strand DNA (ssDNA) end into the intact template. RAD51 protein carries out this reaction. RAD51 is functional as a polymer, made up of hundreds of monomers that coat ssDNA and form a nucleoprotein filament that catalyzes the strand invasion reaction, which is followed by new DNA synthesis (10). The resulting intermediate can either disassemble (i.e., the newly synthesized strand can be displaced and anneal with the noninvading 3’ ssDNA end to elicit noncrossover gene conversion only) or be processed to a Holliday junction intermediate to yield gene conversion with or without crossover (11, 12). Homologous recombination is considered to be error free when it involves sister chromatids (9), but it can also be deleterious when it takes place between repetitive sequences, and in excess, it can promote genome instability and cause diseases (6, 13, 14).

The first evidence linking BRCA2 to homologous recombination was its direct interaction with RAD51. The interaction is mediated by six of eight internal BRC repeats (BRC1–BRC4, BRC7, and BRC8) that are encoded by BRCA2 exon 11 and highly conserved between mammals (10). BRCA2 and RAD51 colocalize to subnuclear foci following DNA damage and during the S and G2 phases of the
cell cycle (3). Structural, cell biological, and biochemical evidence indicates that BRCA2 peptides can inhibit and promote RAD51 function, suggesting that full-length BRCA2 might prevent or assist RAD51 assembly at sites of DNA damage (15).

Direct evidence of a role for BRCA2 in assisting RAD51-mediated chromosomal repair was provided by the demonstration that with a chromosomal DSB in direct repeats, gene conversion was decreased by >100-fold in the BRCA2-deficient human cancer cell line CAPAN-1 and 4- to 6-fold in Brca2-deficient mouse cells compared with wild-type cells (16, 17). The 4-fold decrease in gene conversion was accompanied by a 2- to 3-fold increase in deletion events, suggesting that DSB repair by error-prone mechanisms predominates in BRCA2-deficient cells (17), raising a scenario wherein chromosomal instability provoked by BRCA2 deficiency is the result of incorrect routing of DSB processing down error-prone pathways because error-free processing by homologous recombination is unavailable (4, 17).

However, it remains uncertain whether the shift from gene conversion to deletion at direct repeats is due to error-free or error-prone homologous recombination pathways (17). DSB-induced deletion between direct repeats can result either from RAD51-dependent crossover or RAD51-independent, single-strand annealing (SSA), an error-prone homologous recombination pathway that anneals complementary ssDNA ends, culminating in deletion of one repeat and the intervening sequences (11). The distinction between SSA and crossover becomes important for models of BRCA2 function in homologous recombination and the maintenance of genome stability. Mechanistically, a shift from gene conversion to deletion by SSA would indicate a defect in initiation of RAD51-mediated homologous recombination (18) and thus a role for BRCA2 at this step, whereas a shift from gene conversion to deletion by crossover would indicate a defect in crossover suppression (19) and thus a role for BRCA2 in the processing of Holliday junction intermediates. A parallel can be drawn with RAD51C and XRCC3, thus a role for BRCA2 in the processing of Holliday junction intermediates would indicate a defect in crossover suppression (19) and deletion by SSA would indicate a defect in initiation of RAD51-mediated homologous recombination (19).

Cell lines, culture, and transfections. The cell lines CAPAN-1 and MCF-7 used in these experiments originated from the American Type Culture Collection (Manassas, VA).

To generate the parental CAPAN-1 cell lines D1-10, D2, and D26, cells were electroporated at 350 V/960 μF with 1 to 20 μg of pCAttW (inverted repeat) or pCAstr (direct repeat) constructs linearized with AklI restriction enzyme. The electroporated cells were subjected to Hg selection (85 μg/mL) 48 hours later, and several Hg-resistant (HgR) cell clones were picked and amplified individually for PCR and Southern blot analyses to identify intact, single-copy integration events.

The recombination reporter cell lines were transfected with pcDNANeo-based expression vector containing HA-tagged BRCA2 (pCAdr) or HA-BRCA2 (pCAinv) linearized with PvuI and using the Effectene Transfection Reagent kit (Qiagen, Valencia, CA). Forty-eight hours later, the transfected cells were cultured in medium containing G418 (225 μg/mL). G418R cell clones were picked and amplified individually for BRCA2 expression analysis by reverse transcription-PCR (RT-PCR) and Western blotting.

Reverse transcription-PCR. mRNA from individual cell clones was extracted with Trizol reagent and transcribed to cDNA using reverse transcriptase and primers (hexamers) of the SuperScript First-Strand Synthesis System RT-PCR kit (Invitrogen, Carlsbad, CA). The primer pairs employed for PCR, brca2Fwt (5’-aagaggttgttagacagcaggt-3’) and brca2B6983 (5’-gaagggtgtagcatagtttgg-3’), amplifying an 829-bp fragment of BRCA2 cDNA only, whereas 1885F (5’-taaggacctagattaaagg-3’) and 1885R (5’-ctcgtagctgtagtga-3’) amplify a 642-bp fragment of 18s cDNA of rRNA. PCR amplifications were carried out with the Expand High Fidelity PCR System kit (Boehringer Mannheim, Indianapolis, IN) for 30 cycles (25).

Protein manipulations. Protein extracts were prepared by sonicating cell pellets from individual cell clones with lysis buffer [62.5 mmol/L Tris-HCl (pH 6.8), 6 mol/L urea, 10% glycerol, and 2% SDS] containing protease inhibitors (1 mmol/L phenylmethylsulfonyl fluoride, 1.5 μg/mL apratatin, 1 μg/mL leupeptin, and 1 μg/mL pepstatin). The Bicinchoninic Acid Protein Assay kit (Pierce, Rockford, IL) served to measure protein content; 50 μg of protein extract were separated on 5% SDS-PAGE, transferred to a nitrocellulose membrane (Amersham, Piscataway, NJ), and probed with anti-BRCA2 antibody (Ab2; Oncogene, Boston, MA), which recognizes a COOH-terminal epitope of wild-type BRCA2, or a monoclonal anti-HA antibody for HA-tagged BRCA2.

Homologous recombination frequency and rate. For spontaneous homologous recombination, 1 to 5 × 105 cells were subjected to Puro (0.5 μg/mL) selection 48 hours after plating. The frequency of homologous recombination was assessed by dividing the number of PuroR colonies by the number of cells plated for selection. Because homologous recombination at direct repeats can delete the Hgy gene, cells were maintained for 2 weeks without Hgy before Puro selection. The homologous recombination rate was quantified from 15 sublines (1-100 cells each) of each cell line. When the sublines reached confluence in 100-mm Petri dishes, the cells were trypsinized, counted, and a portion was taken for plating efficiency estimation. The remaining cells were plated under Puro selection, and the resulting PuroR colonies were used to calculate the frequency of recombination. The recombination rate was calculated from these frequencies by a fluctuation test (26).

For I-SceI experiments, the p’sactineSce1 and pFRED25 vectors that express the meganuclease I-Sce1 (7) and green fluorescent protein (GFP),
respectively, were cotransfected into 1 to 4 × 10^5 cells with the Fugene 6 reagent kit (Boehringer Mannheim). In parallel experiments, the same number of cells were transfected with pFRED25 and pmC1neo, the latter to correct for DNA content, as a control for I-Sce1 efficiency (spontaneous homologous recombination). Puro selection was done 10 days after transfection. The frequency of I-Sce1–induced homologous recombination was assessed by dividing the number of I-Sce1 Puro^R colonies by the number of GFP-expressing cells.

**PCR analysis of Puro^R colonies.** Genomic DNA from individual Puro^R cell clones was extracted, and 400 ng subjected to PCR, as described previously (25). The following primer pairs were employed in PCR, Hyg4419 (5′-gcttggtgaagactgctgc-3′), Hyg2829 (5′-aaccttctgcaacagtcggctg-3′), pUC469 (5′-tgacagttattcgaagcag-3′), and pUC315 (5′-aagggggtgtgtctgcaagcag-3′).

**Results**

**Stable expression of wild-type BRCA2 in CAPAN-1 recombination reporter cell lines.** The pancreatic adenocarcinoma cell line CAPAN-1 is the only well-characterized human cell line known to be defective in BRCA2 (see references in ref. 5). CAPAN-1 has lost a wild-type BRCA2 allele and carries a 6174delT mutation on the remaining allele. This frame-shift mutation, which is frequent in families with hereditary breast and ovarian cancer (1), leads to a truncation after amino acid 1981 within BRCT. Because the truncation removes the nucleic localization signal, the truncated BRCA2 protein localizes to the cytoplasm but still retains the RAD51 binding motifs and interacts with RAD51. CAPAN-1 has been reported to be defective in chromosomal DSB repair by homologous recombination compared with other human cell lines (16). Complementation of CAPAN-1 cells with wild-type BRCA2 increases homology-directed chromosomal integration of plasmid DNA and enhances radiation resistance (27).

To determine the effect of BRCA2 on chromosomal homologous recombination, we expressed wild-type BRCA2 in CAPAN-1 recombination reporter cell lines. These were transfected with the pcDNA-Neo based expression vector containing HA-tagged wild-type human BRCA2 (pcDNA3/HA/BRCA2; ref. 24) or the empty vector. G418-resistant derivative cell clones were amplified and analyzed for BRCA2 expression at the mRNA and protein levels (Fig. 1A and B). The different BRCA2-expressing derivative lines, listed in Fig. 1 and Table 1, show the highest expression level but lower than in MCF-7 cells that contain two wild-type BRCA2 alleles. However, they show no apparent changes in growth rate or plating efficiency compared with their parents (data not presented; ref. 27).

**BRCA2 suppresses spontaneous homologous recombination in CAPAN-1 cells.** The recombination reporter CAPAN-1 cell lines carry in their genome either direct or inverted repeats of two inactive Puro resistance genes, separated by the Hyg resistance gene (Fig. 2A). Whereas the promoterless wild-type Puro gene contains a gene inactivating 5′ deletion, deleting EagI and BseHII restriction sites and inserting one I-Sce1 cleavage site have inactivated the full-length Puro gene. An homologous recombination event between the two Puro cassettes would reconstitute a functional Puro gene through loss of I-Sce1 and gain of wild-type EagI/BseHII sites, restoring resistance to the drug Puro in a colony assay.

We determined whether spontaneous homologous recombination (i.e., without treatment of CAPAN-1 cells with exogenous DNA damaging agents) is affected by BRCA2. The direct repeat D2 and D26 lines measure a mixture of RAD51-dependent and RAD51-independent homologous recombination events, whereas inverted repeat I-10 cell lines report RAD51-dependent homologous recombination only (refs. 12, 28; Fig. 2A).

Expression of wild-type BRCA2 in direct repeat lines decreases the frequency of homologous recombination by 3- to 5-fold, whereas a similar expression level in inverted repeat lines does so by 22- to 36-fold (Table 1). BRCA2 also diminishes the rate of homologous recombination (events/cell/generation) by up to 3-fold in direct repeat lines, but by 4- to 10-fold in inverted repeat lines (Table 1). These results reveal that CAPAN-1 exhibits a spontaneous hyper-recombination phenotype.

**Loss of BRCA2 expression in CAPAN-1 cells restores hyper-recombination.** To ascertain that suppression of spontaneous RAD51 recombination is specific to BRCA2 rather than resulting from clonal selection, we repeated the experiments with the inverted repeat line I-10/B49R that no longer expresses BRCA2. This reverting cell line derives from culturing the inverted repeat line 1-I-10/B49 for several passages in G418-free medium to interrupt selection for BRCA2 expression. The frequency of spontaneous homologous recombination in the reverting line 1-I-10/B49R is similar to the parental line I-10, which was grown in parallel for the same number of passages (Table 1).

**BRCA2 inhibits both spontaneous RAD51-dependent and RAD51-independent homologous recombination without affecting the processing of recombination intermediates.** In mitotic cells, RAD51-dependent homologous recombination occurs mainly by gene conversion, a nonreciprocal exchange of genetic information, which is rarely associated with crossover, a reciprocal exchange (9). Gene conversion keeps intact the structure of the locus, whereas crossover inverts the intervening Hyg gene between

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**Figure 1.** BRCA2 expression in the CAPAN-1 cell lines D2/D26 and I-10. A, RT-PCR was carried out with a primer pair that is specific for wild-type BRCA2 mRNA to amplify a 829-bp fragment in the expressing cell lines, whereas a primer pair specific for ribosomal RNA chosen as a control for RT-PCR amplifies a 624-bp fragment in both BRCA2-expressing and nonexpressing cell lines. B, Western blot analysis of protein extract using an antibody directed against either the COOH terminus of wild-type HA-BRCA2 or the HA tag.
Inverted repeat and deletes it between direct repeats. However, spontaneous deletions between direct repeats can also result from RAD51-independent, sister chromatid replication slippage (SCRS; Fig. 2A; ref. 28).

To determine which homologous recombination pathway is more affected by BRCA2, we analyzed the structure of recombinants (PuroR colonies) by PCR (Fig. 2B). In direct repeat parental lines, 26 of 29 (90%) recombination events represent deletions, and the remaining 10% (3 of 29), gene conversion (Table 2). This proportion remained the same in BRCA2-expressing derivative lines (Table 2).

To determine whether spontaneous deletions result from RAD51-dependent crossover or SCRS, we analyzed inverted repeat recombination events. In the inverted repeat parental line I-10, 13 of 14 (93%) recombination events represent gene conversion and the remaining 7% (1 of 14) represents inversion (Table 2). BRCA2-expressing derivative lines yielded similar results in that 93% (14 of 15) seemed to be gene conversion and 7% (1 of 15), inversion (Table 2). In addition, all homologous recombination events in parental lines or BRCA2-expressing derivative lines seemed flanked on both sides by homologous junctions with no apparent rearrangements. These results indicate that BRCA2 does not affect the processing of recombination intermediates towards gene conversion or against crossover, implying that spontaneous deletions between direct repeats result mainly from SCRS (see Discussion).

**BRCA2 promotes gene conversion at chromosomal double-strand breaks.** Expression of I-Sce1 can increase homologous recombination in wild-type cells by up to 10,000-fold (7). When compared with wild-type human cell lines, CAPAN-1 showed more than a 100-fold decrease in gene conversion following I-Sce1 expression (16). To study the effect of BRCA2 on chromosomal DSB repair, parental lines and BRCA2-expressing derivatives were transiently cotransfected in parallel with the p3′actin-α6Sce vector, which expresses I-Sce1 (7), and a GFP-expressing vector employed to determine transfection efficiency, which varied from 0.5% to 1.5%. When the I-Sce1 site in the full-length Puro gene is cleaved, homologous recombination will repair the DSB to generate PuroR colonies.

In direct repeat parental lines, I-Sce1 expression increases the frequency of homologous recombination by 200-fold compared with spontaneous homologous recombination (Table 3). PCR analysis of DSB repair products revealed deletion events exclusively (10 of 10; Table 2). Deletion events could not be detected in previous studies with CAPAN-1, as the assay system employed was designed to report gene conversion events only (16). In BRCA2-expressing derivative lines, I-Sce1 expression heightens homologous recombination frequency by up to 600-fold (Table 3), but in contrast to their parent, the increase is equally split between gene conversion and deletion events (Table 2). Because DSB repair involving direct repeats occurs mainly by gene conversion in wild-type cells (9), the partial shift to gene conversion events could reflect partial complementation of CAPAN-1 cells (Fig. 1B).

I-Sce1–induced deletion between direct repeats can result either from crossover or SSA events. At inverted repeats, crossover inverts the intervening sequences, whereas SSA cannot take place because the exposed ssDNA flanking a DSB would be identical and unable to anneal. In the inverted repeat parental line I-10, the frequency of I-Sce1–induced homologous recombination is similar to that of spontaneous homologous recombination, whereas in BRCA2-expressing derivative lines the frequency of I-Sce1–induced homologous recombination increases by 246-fold (Table 3). PCR analysis of DSB repair products indicates that the proportions of I-Sce1–induced error-free gene conversion (93%) and crossover (inversion; 7%) events are similar to those of spontaneous homologous recombination events (Table 2). These results suggest that BRCA2 also does not affect the processing of recombination intermediates at chromosomal DSBs. Thus, deletions between direct repeats must have occurred almost exclusively by SSA. These results indicate that in BRCA2-deficient cells, the error-prone SSA pathway predominates and BRCA2 promotes error-free repair of chromosomal DSBs by gene conversion, providing good confirmation of previous reports (16, 17).

**Discussion**

We compared homologous recombination before and after transfection of wild-type BRCA2 in the BRCA2-deficient human CAPAN-1 cell line. The results show that CAPAN-1 cells exhibit not only a defect in homology-directed error-free repair of chromosomal DSBs but also a spontaneous hyper-recombination phenotype. The ability of BRCA2 to both suppress spontaneous homologous recombination and promote error-free repair of chromosomal DSBs provides evidence of a dual role for this protein in regulating homologous recombination in response to the type of DNA damage.

CAPAN-1 cells express a truncated form of BRCA2 that retains significant RAD51-binding capacity but is primarily localized to the cytoplasm (29); its exclusion from the nucleus may explain the spontaneous hyper-recombination phenotype. Although in CAPAN-1 cells RAD51 was found in the cytoplasm, presumably sequestered by the truncated BRCA2, an appreciable level of
Figure 2. Chromosomal homologous recombination between direct and inverted repeats. A, spontaneous or DSB-induced homologous recombination events reconstitute a functional Puro gene through loss of the I-SceI site and gain of the EagI/BssHII sites, restoring Puro<sup>R</sup>. Because I-SceI insertion mutation in ScePuro entails deletion of the EagI and BssHII sites, only homologous recombination events between the two Puro cassettes restore a functional ScePuro gene. Gene conversion restores one functional Puro gene without affecting the overall structure of the locus, whereas crossover between inverted repeats inverts the intervening sequences. Deletion can result from different RAD51-dependent intrachromatid or mispaired sister chromatid crossovers, or RAD51-independent SCRS or SSA.

B, PCR analysis of Puro<sup>R</sup> recombinants. a, inverted and direct repeat gene conversion products: the primer pair Hyg4419/pUC469 amplifies an I-SceI–resistant 2,324-bp fragment (lane 2) that can be cut with EagI into 1,283 and 1,041 bp (lane 3). b, inverted repeat crossover (inversion): the primer pair Hyg2829 and pUC469 amplifies an inverted, I-SceI–resistant 2,491-bp fragment (lane 2) that can be cut with EagI into three fragments: 1,041, 850, and 600 bp (lane 3). c, direct repeat deletion (crossover/SSA/SCRS) products: the primer pair pUC315 and pUC469 amplifies an I-SceI–resistant 1,836-bp fragment (lane 2) that can be cut with EagI into two fragments: 822 and 1,041 bp. Lane 1, a 1-kbp ladder.
nuclear RAD51 that can carry out spontaneous homologous recombination was found associated with S-phase chromatin and capable of forming nuclear foci during the S phase, presumably at replication fork arrest sites (30).

BRCA2 may regulate RAD51 recombination according to the type of DNA damage response (Fig. 3), a complex cascade of phosphorylation events that ultimately serve to influence or effect DNA repair, cell cycle delay or apoptosis with the overall objective of maintaining genome stability. In mammalian cells, two related protein kinases, ATM and ATR, orchestrate such a response (31). Whereas ATM responds exclusively to DSB-inducing agents. Both SSBs and DSBs may occur spontaneously during the growth of CAPAN-1 cells due to failure to reactivate stalled DNA replication forks (32). The assembly of RAD51 foci following DNA damage depends on RAD51 phosphorylation by C-ABL, which in response to DSBs, is activated by ATM (31). However, RAD51 phosphorylation by C-ABL inhibits its strand exchange activity in vitro (33), presumably by disrupting RAD51 nucleoprotein filament formation (34), but activates it in vivo in response to DNA damage (35). As BRCA2 promotes RAD51-dependent homologous recombination at DSBs, the disparate effect of C-ABL on RAD51 could be reconciled if BRCA2 activates a phosphorylated form of RAD51 or prevents its phosphorylation after such damage (Fig. 3). In this hypothetical model, BRCA2 acts to coordinate DNA damage signaling with repair. However, whether BRCA2 suppresses spontaneous RAD51 recombination via ATR is not known. Whereas evidence of a direct connection between ATR and BRCA2 is still lacking, BRCA2 interacts with BRCA1, which interacts with C-ABL, RAD51, and ATR (3, 36). ATR phosphorylates BRCA1 upon exposure to hydroxyurea, an agent that induces SSBs at replication forks (3), and BRCA1 regulates RAD51 recombination as BRCA2.4 Thus, BRCA2 may regulate RAD51 recombination, at least in part, in conjunction with BRCA1.

Table 2. Effect of BRCA2 on homologous recombination pathways

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Spontaneous HR GC (%)</th>
<th>CO/SCRS (%)</th>
<th>I-SceI HR GC (%)</th>
<th>CO/SSA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct repeat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2/D26 (BRCA2)</td>
<td>3/29 (10)</td>
<td>26/29 (90)</td>
<td>0/10 (0)</td>
<td>10/10 (100)</td>
</tr>
<tr>
<td>D2/D26 (BRCA2)−</td>
<td>2/20 (10)</td>
<td>18/20 (90)</td>
<td>6/12 (50)</td>
<td>6/12 (50)</td>
</tr>
<tr>
<td>Inverted repeat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-10 (BRCA2)</td>
<td>13/14 (93)</td>
<td>1/14 (7)</td>
<td>13/14 (93)</td>
<td>1/14 (7)</td>
</tr>
<tr>
<td>I-10 (BRCA2)−</td>
<td>14/15 (93)</td>
<td>1/15 (7)</td>
<td>14/15 (93)</td>
<td>1/15 (7)</td>
</tr>
</tbody>
</table>

Abbreviations: GC, gene conversion; CO, crossover; HR, homologous recombination.

Table 3. Effect of BRCA2 on spontaneous and I-SceI–induced HR

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Frequency*</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spontaneous</td>
<td>I-SceI</td>
</tr>
<tr>
<td>Direct repeat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>5.5 ± 0.6 × 10−6</td>
<td>9.7 ± 2.8 × 10−4</td>
</tr>
<tr>
<td>D2/B74</td>
<td>1.1 ± 0.8 × 10−6</td>
<td>6.4 ± 3.4 × 10−4</td>
</tr>
<tr>
<td>D26</td>
<td>3.2 ± 0.7 × 10−6</td>
<td>3.8 ± 3.2 × 10−4</td>
</tr>
<tr>
<td>D26/B12</td>
<td>8.5 ± 5.9 × 10−7</td>
<td>5.0 ± 5.4 × 10−4</td>
</tr>
<tr>
<td>Inverted repeat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-10†</td>
<td>5.7 ± 1.6 × 10−5</td>
<td>4.1 ± 1.1 × 10−5</td>
</tr>
<tr>
<td>I-10/B29</td>
<td>2.6 ± 0.1 × 10−6</td>
<td>6.4 ± 1.2 × 10−4</td>
</tr>
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</table>

*Frequency represents the mean of four experiments. I-Sce1 frequency was calculated from the number of PuroR colonies relative to the number of cells expressing GFP. Puro selection was applied 10 d after transfection of cells with I-Sce1 expressing vector or a control vector for spontaneous HR.
†The I-Sce1 frequency of this cell line was calculated from the number of PuroR colonies relative to the number of plated cells.

4 I. Cousineau et al., unpublished data.
RAD51C, MSH2, or XRCC3, our results indicate that BRCA2 does not affect the processing of recombination intermediates towards gene conversion or against crossover, nor is it required for accurate termination of gene conversion. All gene conversion products in CAPAN-1 cells were flanked on both sides by homologous junctions; inaccurate termination of gene conversion is characterized by rearrangements at one homologous junction (25, 39). In addition, unlike BLM-deficient cells, BRCA1/2-, ATM-, and FANC-deficient cells exhibit normal or decreased SCEs but increased frequency of spontaneous deletion and gene conversion events (refs. 6, 15 and this study).4 Thus, the spontaneous hyper-recombination phenotype of BRCA1/2-, ATM-, and FANC-deficient cells seems more consistent with a loss in homologous recombination regulation rather than a defect in the processing of recombination intermediates.

Spontaneous hyper-recombination also has the potential to trigger chromosomal instability in the presence of cell cycle checkpoints. Gene conversion leads to LOH when acting between two heteroalleles, or to gene inactivation when acting between a gene and a pseudogene (13). Gene conversion has been shown to lead to LOH when acting between two heteroalleles, or to gene inactivation when acting between a gene and a pseudogene (13). Gene conversion leads to LOH when acting between two heteroalleles, or to gene inactivation when acting between a gene and a pseudogene (13). Gene conversion leads to LOH when acting between two heteroalleles, or to gene inactivation when acting between a gene and a pseudogene (13). Gene conversion leads to LOH when acting between two heteroalleles, or to gene inactivation when acting between a gene and a pseudogene (13). Gene conversion leads to LOH when acting between two heteroalleles, or to gene inactivation when acting between a gene and a pseudogene (13). Gene conversion leads to LOH when acting between two heteroalleles, or to gene inactivation when acting between a gene and a pseudogene (13).

Thus, the spontaneous hyper-recombination phenotype of BRCA1/2-, ATM-, and FANC-deficient cells seems more consistent with a loss in homologous recombination regulation rather than a defect in the processing of recombination intermediates.

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Figure 3. A model of BRCA2 in genome stability. In normal cells, BRCA2 sequesters RAD51 to prevent spontaneous homologous recombination at a SSB, thereby promoting SSBR and preventing conversion of a SSB to a DSB. After a DSB, ATM inactivates free RAD51 through C-ABL but activates the BRCA2/RAD51 complex. In BRCA2-deficient cells, RAD51 is active and initiates spontaneous HR at a SSB, thereby decreasing the efficiency of SSBR. Upon a DSB, RAD51 becomes inactive by phosphorylation, leaving the damage unrepaired or shunting it to error-prone repair pathways (see text for further explanation).
SSBR components. The interaction of BRCA1/2 with one or more of these components may also be important. A SSBR model postulates that continued DNA synthesis at the 3' end of a SSB would displace the 5' terminus as a single-strand flap (44), an efficient substrate for RAD51 (Fig. 3; ref. 45). The flap can be removed by FEN and XRCC1, which in turn may be stimulated by proliferating cell nuclear antigen (PCNA; ref. 44). An association between BRCA1/2 and SSBR proteins is also suggested by the observation that BRCA1/2 foci partially localize with RAD51 foci during the S phase and relocalize to PCNA-containing structures and in response to SSBR-inducing agents, such as UV light and hydroxyurea (23). Recognition and/or removal of the flap by FEN, PCNA, and XRCC1 (44) may also implicate BRCA1/2. BRCA2 has the ability to bind different forms of DNA (46), and BRCA1 binds DNA flaps in vitro (47). In addition, BRCA1 interacts with MSH2 and BLM that have been implicated in DNA flap removal from recombination intermediates (12, 25, 38).

It is currently thought that BRCA1/2 protect breast and ovarian tissues from estrogen-induced DNA damage, but a molecular basis has been lacking. As estrogen metabolites adduct DNA, cause SSBRs, and induce genome instability (48), BRCA1/2 may protect against estrogen-induced spontaneous hyper-recombination. Given that cells from BRCA1/2 mutation carriers seem more radiosensitive (haplosufficient) than cells from wild-type individuals (49), estrogen-induced hyper-recombination may rearrange delete the remaining wild-type BRCA1/2 allele in heterozygotes for BRCA1/2 mutations or in wild-type individuals with decreased BRCA1/2 expression (50). Estrogen-induced hyper-recombination may, therefore, underlie tissue specificity of the tumor suppressive properties of BRCA1/2, and the increased risk of breast and ovarian cancers.

In summary, our results provide the first genetic evidence that BRCA2 regulates RAD51 recombination according to the type of DNA damage. As BRCA2 is required for RAD51-dependent homologous recombination activation following chromosomal DSBs, selected RAD51 peptides or compounds that interfere with BRCA2 function may thus present new reagents to sensitize BRCA2-expressing tumor cells to the lethal effect of therapeutic DSBR-inducing agents.

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