Commentary
Caretaker Brca1: keeping the genome in the straight and narrow

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Abstract

Inheritance of germline BRCA1 mutations is associated with a high risk of breast and ovarian cancers. A multitude of cellular functions has been ascribed to BRCA1, including transcription activation and various aspects of DNA repair. So far, indirect evidence has indicated a role for BRCA1 in the repair of double-strand breaks. Recently, an elegant gene targeting design was used to provide definitive evidence that BRCA1 promotes homologous recombination and limits nonhomologous mutagenic repair processes. This reaffirms the role of BRCA1 as caretaker in preserving genomic integrity.

Keywords: Brca1, Brca2, homologous recombination, nonhomologous end-joining, double-strand break, Rad51, RecA, breast cancer

Introduction

Ever since the cloning of the two breast cancer susceptibility genes *BRCA1* and *BRCA2*, intense scientific effort has been invested into elucidating their biologic functions. Both genes encode large proteins (1863 and 3418 amino acids, respectively) that are coordinately expressed during cellular proliferation and differentiation [1]. The BRCA1 and BRCA2 proteins are expressed in most tissues and most abundantly during the S phase of the cell cycle. A plethora of functions have been ascribed to BRCA1 and BRCA2, including transcription activation [2–4], chromatin remodelling [5,6], centrosome duplication [7,8], apoptosis [9], and transcription-coupled [10] and homology directed [11–13] DNA repair.

Murine models for *BRCA1* and *BRCA2* mutations establish that both genes are essential for development [14–17], because early embryonic lethality is a common phenotype. This early lethal phenotype can be partly rescued by introducing a null mutation in the damage checkpoint gene *p53* or *p21* [18]. This suggests that loss of BRCA1 or BRCA2 may trigger the activation of a cell-cycle checkpoint through the accumulation of DNA damage. In agreement with this, both Brca1$^{-/-}$ and Brca2$^{-/-}$ homozygous cells have been found to be hypersensitive to γ-irradiation [17,18] and spontaneously exhibit chromosomal abnormalities [11,13].

The first indication that BRCA1 and BRCA2 function in homologous recombination came from coimmunoprecipitation and yeast two-hybrid studies [17,19,20], which demonstrated that both BRCA1 and BRCA2 interacted with Rad51, a mammalian homologue of the *Escherichia coli* RecA protein that is involved in DNA damage repair. (The association of BRCA1 with Rad51, however, is likely to be indirect and possibly mediated by BRCA2 [21]).

DSB = double-strand break; NHEJ = nonhomologous end-joining.

Immunostaining further suggests that BRCA1, BRCA2 and Rad51 work together in a protein complex as they coalesce to form discrete nuclear dots during S phase and after DNA damage [21,22]. Moreover, in meiotic cells extensive colocalization of BRCA1, BRCA2 and Rad51 have been demonstrated on synaptonemal complexes [21].
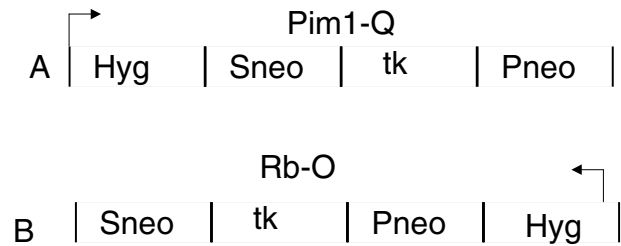
As already mentioned, Rad51 is part of homologous recombination. In *E coli*, and the budding yeast *Saccharomyces cerevisiae*, homologous recombination is a major pathway for repairing double-strand breaks (although no homologues of BRCA1 or BRCA2 have yet been identified in these organisms). In mammalian cells, it has been estimated that around 30–50% of DNA breaks are repaired by homologous recombination [23], the remainder being dealt with mainly by a process known as nonhomologous end-joining (NHEJ). Unlike homologous recombination, which repairs through genetic exchange with a homologous chromosome, NHEJ simply involves direct joining of the broken ends. This frequently results in small deletions or insertions at the site of breakage. Whereas NHEJ is a relatively well-understood pathway in mammalian cells [24], little is known about the mechanisms involved in mammalian homologous recombination. Despite all speculations that BRCA1 and BRCA2 participate in Rad51-mediated homologous recombination, direct evidence is lacking. Furthermore, to delineate the players involved in homologous recombination in mammalian cells, it is necessary to have a robust reporter assay system. It is to this challenge that Jasin [25] provided a valuable tool.

BRCA1 controls homologous recombination

A Brca1-deficient murine embryonic stem cell line developed by Gowen *et al* [14] was employed. The mutant *Brca1* gene here was created by replacing the terminal portion of intron 10 and the beginning of exon 11 with a selectable marker gene, resulting in the loss of the full-length *Brca1* transcript. Exon 11 is the largest coding exon of *Brca1* and encodes approximately 60% of the protein, including the putative Rad51-interacting domain [20]. Murine embryos that harbour the *Brca1* mutation described by Gowen *et al* [14] typically survive to embryonic day 9 or 10.

Moynahan *et al* [12] devised two assays to test the integrity of homologous recombination in the Brca1$^{-/-}$ embryonic stem cells. First, the rate of targeted integration of transfected DNA was evaluated (Fig. 1). Moynahan *et al* found that gene-targeting efficiency was 23-fold lower in Brca1$^{-/-}$ cells when the promotorless pim1-Q targeting vector was transfected. This was not due to the lack of a promotor, because when an Rb-O targeting vector that contained a pgk promotor was used, Brca1$^{-/-}$ cells had a 13-fold decrease in gene targeting and a fourfold increase in random integration compared with Brca1$^{+/-}$ control cells.

Figure 1

Schematic representations of the (A) pim1-Q and (B) Rb-O targeting vectors. (A) Gene targeting strategy at the pim1 locus. Expression of the promotorless hygromycin resistance relies on the endogenous pim1 promotor once it is integrated by homologous recombination. Transfected cells are selected in hygromycin. (B) Gene targeting strategy at the Rb locus. The Rb-O contains a hygromycin gene expressed from a pgk promotor. Therefore, hygromycin resistance is exhibited regardless of the site of integration
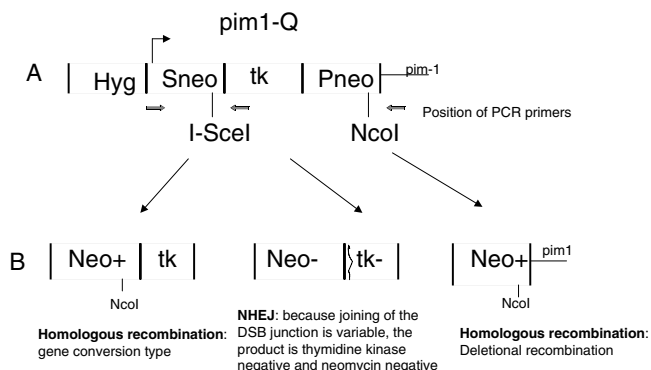
Next, Moynahan and colleagues tested the ability of Brca1-deficient cells to repair chromosomal double-strand breaks. They made use of a rare-cutting endonuclease, I-SceI, which has a unique 18-base-pair nonpalindromic recognition sequence that is not found in the mammalian genome [26]. A direct repeat recombination substrate containing this sequence was integrated at the *pim1* and *Rb* loci. Introduction of the enzyme I-SceI would, therefore, create a double-strand break (DSB) in the direct repeat that consisted of two differentially mutated neomycin phosphotransferase (*neo*) genes separated by a thymidine kinase (*tk*) gene (Fig. 2). It has already been demonstrated that the presence of a single DSB was a potent stimulus for homologous recombination [25]. By selecting for the loss of the *tk* gene, it would be possible to estimate the frequency of deletional homologous recombination (Fig. 2). In agreement with the previous assay, the Brca1$^{-/-}$ cell line exhibited a fivefold lower frequency of spontaneous and DSB-induced deletion compared with the heterozygous control. This was further supported by detailed examination of the substrates by polymerase chain reaction and Southern blotting.

Using primers flanking the DSB site and the NcoI site in the substrate (Fig. 2), Moynahan *et al* [12] were able to distinguish end-products from both homologous recombination and NHEJ. They concluded that Brca1$^{-/-}$ cells had a consistently fivefold to sixfold lower level of homologous recombination by both noncrossover gene conversion and deletion. On the contrary, NHEJ was intact and its frequency was even increased by 1.5-fold to 1.6-fold in Brca1$^{-/-}$ cells.

Conclusion

The work of Moynahan *et al* [12] is significant, because it is the first direct demonstration of the role of Brca1 in controlling homologous recombination. Cells deficient in

Figure 2

Outcomes of the repair of a double-strand break (DSB) introduced by I-SceI. (A) The integrated pim1-Q vector. Open arrows indicate the positions of PCR primers that are used to distinguish between the various repair products. (B) Possible repair products. NHEJ, nonhomologous end-joining.

Brca1 were found to have decreased gene targeting as well as impaired homologous recombination of an experimentally introduced DSB. Interestingly, the rate of homologous recombination in Brca1$^{-/-}$ embryonic stem cells was reduced by around fivefold, but was not absent. The authors proposed that this was due to the particular Brca1$^{-/-}$ cell line chosen. The mutation in the *Brca1* gene apparently does not produce a complete null allele, because an in-frame fusion product is formed from expression of an exon 10 12 splice variant [12]. In this Brca1$^{-/-}$ cell line, Rad51 focus formation following DNA damage is partly, but not totally defective. This may explain the relative, but not complete loss of homologous recombination in these cells. Alternatively, the partial Brca1 product expressed might not have any role in homologous recombination, but other as yet unidentified Brca1-independent pathways that govern homologous recombination could be responsible for the residual recombination observed.

This work also raises other interesting questions. For example, an increase in random integration of transfected DNA was observed. As Moynahan *et al* [12] suggested, this could be a direct result of increased NHEJ, or simply due to the elevated level of spontaneous DSBs in the genome into which transfected DNA could insert. Alternatively, other undefined mutagenic mechanisms may be at work, because evidence is now gathering that multiple DNA repair pathways can potentially compete for the same repair substrate, in this case the DSB [27,28].

It has been suggested that Brca1 plays a crucial role in maintaining genetic stability. Like Brca2-deficient cells [13], Brca1-deficient murine [11] and human [29] cells have been reported to exhibit spontaneous chromosomal aberrations. This is akin to the gross chromosomal aberra-

tions that are typical of chromosome instability disorders such as Bloom syndrome, Werner syndrome, Nijmegen breakage syndrome and ataxia–telangiectasia [30]. The mechanism for the maintenance of genetic stability is not known, but it has been suggested that genes that are mutated in the chromosome instability syndromes function as 'caretakers' [31] to prevent the occurrence of gross chromosomal rearrangements. Most of these genes participate in some aspect of DNA damage recognition and/or repair, and Brca1 has been shown to interact with at least two of them. For example, Brca1 associates with the hRad50-hMre11-p95/nibrin complex (nibrin is the gene mutated in Nigmegen breakage syndrome) in response to irradiation [32]. These nuclear foci are distinct from the Rad51 foci described earlier, suggesting Brca1 may participate in more than one damage response pathway. Recently, Cortez *et al* [33] provided evidence that the DNA damage sensor ATM (the gene mutated in ataxia–telengiectasia) was required for the phosphorylation of Brca1 in response to DSBs. A model is now emerging in which phosphorylation of Brca1 by ATM recruits or regulates multiple repair pathways that may involve the hRad50-hMre11-p95/nibrin complex, transcription coupled repair, or Rad51-mediated homologous recombination [34].

Homologous recombination may be an important mechanism for maintaining genetic stability. Evidence already exists that cells that harbour mutations in other genes in the Rad51-family, *XRCC2* and *XRCC3*, are defective in homologous recombination [35,36] and exhibit spontaneous chromosome instability [37,38]. It is not yet known what pathways are responsible for generating the abnormal chromosomes in the absence of normal homologous recombination. Cells that are defective in NHEJ *per se* do not exhibit abnormal chromosomes [39]. In Brca1-deficient cells, Moynahan *et al* [12] believe that nonhomologous processes may promote aberrant types of repair. Interestingly, Brca2-deficient cells that exhibit gross chromosomal aberrations also have intact NHEJ [13]. Although this idea is very plausible, further studies are required to clarify this issue.

Finally, the correlation between chromosomal instability and cancer has long been known, but we are just beginning to unravel the mechanisms responsible for maintaining genetic stability. As the number of potential candidates for homologous recombination increases, the strategy devised by Moynahan *et al* [12] remains a useful screening tool. Although our understanding of homologous recombination in mammalian cells is still in its infancy, the enormous research interest in the field means that our understanding will move at a considerable speed. At this juncture, one can only say 'watch this space' – knowledge of the caretakers of the genome may one day change the manner in which we manage cancers.

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