Commentary

Role of *BRCA* gene dysfunction in breast and ovarian cancer predisposition

Ralph Scully

Dana Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts, USA

Received: 12 April 2000
Revisions requested: 22 May 2000
Revisions received: 31 May 2000
Accepted: 14 June 2000
Published: 13 July 2000

Breast Cancer Res 2000, **2**:324–330

Abstract

Tumor suppressor genes that perform apparently generic cellular functions nonetheless cause tissue-specific syndromes in the human population when they are mutated in the germline. The two major hereditary breast/ovarian cancer predisposition genes, *BRCA1* and *BRCA2*, appear to participate in a common pathway that is involved in the control of homologous recombination and in the maintenance of genomic integrity. How might such functions translate into the specific suppression of cancers of the breast and ovarian epithelia? Recent advances in the study of *BRCA1* and *BRCA2*, discussed herein, have provided new opportunities to address this question.

Keywords: breast cancer, DNA repair, homologous recombination, ovarian cancer, tumor suppressor genes

Introduction

Familial breast or ovarian cancer predisposition syndromes have long been recognized. Their genetic bases have become clear with the cloning of two major disease susceptibility genes, *BRCA1* and *BRCA2*, termed herein '*BRCA*' genes [1–4]. Each has characteristics of a tumor suppressor gene: inheritance within affected families follows an autosomal-dominant pattern of inheritance; and loss of heterozygosity (LOH) at the relevant gene locus is seen in familial tumors, with retention of the disease-predisposing allele [5–8]. The spectrum of disease-associated mutations includes frequent truncating mutations and less frequent missense mutations. Although LOH is frequently detected at the *BRCA1* or *BRCA2* locus in sporadic breast cancer, the retained allele is almost always wild-type [9,10]. Thus, in contrast to the causal role of *BRCA* gene mutation in the hereditary syndrome, *BRCA* gene mutation in sporadic breast or ovarian cancer seldom conforms to Knudson's model for tumor suppressor genes [11]. Cancer risk in *BRCA* gene mutation carriers may be increased modestly in other organs. However, highly penetrant, early-onset, site-specific cancer is restricted to the breast and ovary.

BRCA1 and *BRCA2* homologs have been detected in several mammalian species, including the mouse. *BRCA1* and *BRCA2* are expressed ubiquitously [12–14]. Gene targeting experiments in the mouse [15–22] have revealed functional differences between true null and partial loss-of-function (hypomorphic) mutant alleles of *BRCA* genes. If these distinctions can be made in murine development, the same might be true in human disease. At its simplest,

DSBR = double-strand break repair; LOH = loss of heterozygosity; MEF = mouse embryonic fibroblast; MMR = mismatch repair.

a hypomorphic *BRCA* allele might exhibit lower penetrance for cancer than a true null allele. Because of the relative scarcity of individual *BRCA* mutant alleles and the existence of unidentified modifier genes, it has been difficult to estimate the penetrance of different disease-predisposing *BRCA* alleles. However, the position of a mutation in the *BRCA1* or *BRCA2* gene can affect the relative incidences of breast and ovarian cancer within a kindred [23,24]. These observations suggest that the breast and ovarian epithelia differ in their requirements for *BRCA* gene function. Whether this is a qualitative or quantitative difference is unknown.

BRCA1 germline mutations differ from those that affect *BRCA2* with regard to the relative risk of developing other solid tumors. *BRCA2* germline mutation carries an increased risk for cancers of the prostate, pancreas, gallbladder/bile duct, and stomach, as well as for malignant melanoma [25]. *BRCA2* mutation also appears to confer higher risks for male breast cancer [26]. *BRCA1* mutation confers a higher incidence of ovarian cancer than does *BRCA2* mutation [26]. There appear to be histologic differences between *BRCA1*-linked and *BRCA2*-linked breast tumors [27,28]. Taken together, these point to subtle differences between *BRCA1* and *BRCA2* germline mutations in their impact on tumorigenesis. At present, the mechanistic basis of these differences is unknown.

Role of *BRCA1* and *BRCA2* in maintenance of genome integrity

The *BRCA1* and *BRCA2* gene products (BRCA1 and BRCA2, respectively) function in the maintenance of genomic integrity, at least in part by cooperating with recombinational repair proteins. Both BRCA1 and BRCA2 form a complex with Rad51, a protein that has an established role in homologous recombination [17,29,30]. The BRCA1, BRCA2, and Rad51 proteins are coexpressed in the developing mouse embryo, and gene targeting of each revealed similar lethal phenotypes of nullizygous mouse embryos [14,16,17,31,32]. BRCA1, BRCA2, and Rad51 colocalized in S-phase nuclear foci in somatic cells, and upon the axial element of the developing synaptonemal complex of cells in meiotic prophase I [29,33]. The BRCA1 and BRCA2 proteins were also found to be complexed with one another in cell extracts [33], suggesting that these proteins collaborate on a common pathway of tumor suppression. A specific role for this complex in the S phase was implied by the rapid relocalization of BRCA1, BRCA2, and associated proteins to sites of DNA synthesis after exposure of cells to certain DNA-damaging agents in S phase [34,35]. The BRCA1 protein also interacts with the Rad50/MRE11/NBS1 complex, implicated in the response to double-stranded DNA breaks [36].

Functional data from the study of *BRCA1*- or *BRCA2*-mutated mice has confirmed a role for these genes in the maintenance of genomic integrity. Both *BRCA2* and *BRCA1* homozygous mutant cells exhibit ionizing radiation sensitivity, a frequent indicator of a DNA repair defect [17,20]. *BRCA2* or *BRCA1* homozygous mutant mouse embryonic fibroblasts (MEFs) from embryos reveal spontaneous chromosomal anomalies and chromosome breakage, which is consistent with a recombination defect [20,37]. A unique *BRCA1* homozygous mutant embryonic stem cell clone revealed reduced efficiency of gene conversion in response to a site-specific double-stranded DNA break [38]. A defect in gene targeting (a process that is dependent on homologous recombination) in the same embryonic stem cell clone was improved by re-expression of wild-type *BRCA1* [39]. *BRCA1* or *BRCA2* mutated cancer cell lines reveal abnormally delayed kinetics of double-strand break repair (DSBR) [18,40]. Definitive evidence linking *BRCA1* function in DSBR with its tumor suppressor role came from the finding that wild-type, but not clinically defined mutant *BRCA1* alleles, can restore efficient DSBR to a *BRCA1*-mutated breast cancer cell line [41]. The biochemical mechanisms of action of *BRCA1* and *BRCA2* in DSBR have yet to be determined. Where examined, *BRCA* mutant cells revealed defects in homologous recombination, but not in nonhomologous end-joining [22,27,38].

Although recombination would seem to be a unifying theme in these processes, other repair functions, such as the transcription-coupled repair of oxidative DNA damage, are defective in some *BRCA1*-mutated cells [22,42]. *BRCA1* also plays a role in the G2/M checkpoint response to ionizing radiation, although this has not been observed in all *BRCA1*-mutated cells [21,41]. *BRCA1* or *BRCA2* homozygous mutation leads to severe aneuploidy, accompanied by centrosome amplification [21,43].

Genomic instability is characteristic of cancer cells, and it is not difficult to imagine that mutation in *BRCA1* or *BRCA2* might accelerate tumorigenesis, for example through promoting aneuploidy, chromosomal translocation or LOH events. If *BRCA* gene functional inactivation destabilizes the genome, tumor development might be compressed into a shorter time frame. In diseases such as breast or ovarian cancer, the incidence of which increases with advancing age, the 'mere' acceleration of disease progression could contribute to the early onset of disease that is characteristically seen in carriers of *BRCA* mutations. However, this does not provide an obvious explanation of the specifically increased risk of breast/ovarian cancer in *BRCA* gene mutation carriers. The following sections explore hypotheses that could account for this specificity.

Collaboration between repair and checkpoint functions in tumorigenesis

BRCA1 and *BRCA2* nullizygous embryos die early in development, with a severe growth deficit accompanied

by elevated expression of the p53-responsive cell cycle inhibitor, p21 [16,44,45]. This suggests that not only failed DNA repair, but also the cell's response to that failure might be relevant to *BRCA* gene biology. If *BRCA1* and *BRCA2* are DNA repair genes, then the p53/p21-mediated growth arrest seen in *BRCA* mutant tissue might represent a 'checkpoint' response to spontaneous DNA damage arising as a result of the failure of DNA repair processes. The above-noted chromosome breakage syndrome, described in *BRCA2* or *BRCA1* homozygous mutant MEFs, supports this idea [20,37].

These observations suggest a way to understand the role of *BRCA* gene mutation in tumorigenesis. Perhaps loss of *BRCA* gene function in an otherwise intact epithelial cell might lead to its death or arrest, because of activation of checkpoint functions. However, if *BRCA* gene mutation were to occur within a cell that had already suffered inactivation of critical DNA damage-responsive checkpoints, then the abnormalities in DNA structure resulting from *BRCA* gene loss might be tolerated, and might then manifest their potential as accelerators of tumor progression. This hypothesis would predict that checkpoint loss is a necessary precursor of *BRCA* gene inactivation in tumorigenesis.

Several recent developments in mouse models of *BRCA*-linked disease support this hypothesis. *BRCA1* or *BRCA2* mutant MEFs undergo growth arrest at early passage [20,37]. Both p53-dependent and p53-independent checkpoints appear to play a part in this growth arrest [20,22,46]. Recently, by use of the Cre-lox system, *BRCA1* gene inactivation was achieved specifically in the breast of the adult mouse [47]. Such mice developed late-onset breast cancer, with frequent *p53* mutation seen in tumors. When these mice were re-examined on a $p53^{+/-}$ genetic background, breast cancer was detected at higher frequency and with earlier onset [47]. This model indicates a permissive role for *p53* mutation in *BRCA*-linked disease. Hemizygosity in *p53* was also found to play a permissive role for breast tumor development in $BRCA1^{+/-}$ mice that had been exposed to ionizing radiation [48]. Mutation in *p53* is common (occurring in approximately 30%) in sproadic breast/ovarian cancer, but is considerably more common (occurring in approximately 60%) in *BRCA*-linked disease [49]. *BRCA*-linked disease has also been found to be associated with rare *p53* mutant alleles, suggesting that novel p53 functions may be lost in *BRCA*-linked tumorigenesis [46,50]. The full spectrum of checkpoint(s) that are responsible for restraining cells mutated for *BRCA1* or *BRCA2* from continued proliferation remains to be defined. One recently identified candidate is the spindle checkpoint [46].

If *BRCA*-linked disease requires inactivation of checkpoint(s) followed by *BRCA* gene loss, then the tissue specificity of *BRCA*-linked disease might arise from a specific predisposition of the breast and ovarian epithelium to lose the function of such checkpoints. If so, the question shifts sideways – what determines the timing of inactivation of DNA damage checkpoints in breast/ovarian cancers?

Recombination and breast development

The breast epithelium undergoes distinct developmental programs during puberty and pregnancy. During puberty, in particular, rapid proliferation of breast tissue occurs, and the progeny of this proliferative burst are retained within the breast lobule. This is demonstrated by the finding that lobules of the breast are clonal [51–53]. In this way, breast epithelial cells have the potential to retain 'memory' of genetic alterations that occurred earlier in breast development. In contrast, some other epithelia that are characterized by rapid proliferation, such as the intestinal epithelium, shed cells continuously.

Radiation exposure in young women – in atomic bomb survivors or from iatrogenic causes – carries with it a specifically increased risk of subsequent breast cancer [54,55]. Women who were exposed to A bomb radiation at less than 20 years of age developed breast cancer with normal latency, suggesting that that risk was increased but that disease progression was unaffected. More recently, it became clear that A bomb exposure below the age of 10 years, before the onset of puberty, increased the risk of subsequent adult-onset breast cancer [56]. Perhaps this early impact of radiation exposure on breast cancer risk reflects the 'memory' of breast epithelial stem cells for genotoxic damage. Interestingly, a cohort of women who survived A bomb exposure developed early-onset breast cancer (<35 years of age), suggesting the existence of a susceptible genetic subgroup [57]. It has been suggested that this cohort may represent women with pre-existing *BRCA* gene germline mutations [58].

Do such observations tie in with *BRCA* gene biology? If a $BRCA1^{+/-}$ or $BRCA2^{+/-}$ mammary cell were to develop checkpoint defects and undergo LOH at the relevant *BRCA* locus early in breast development, then a number of daughter cells exhibiting this LOH event could be produced and retained within the affected lobule. Cancer risk could be multiplied in the breast epithelium by such an event, in proportion to the number of daughter cells retained after the LOH event (which might, at a maximum, constitute an entire lobule, comprising millions of at-risk epithelial cells). By contrast, other rapidly proliferating epilthelia in which daughter cells are rapidly shed (such as the colonic epithelium) would not encounter this risk amplification mechanism. In this way, the clonality of the breast epithelium may, in part, account for the enhanced sensitivity of the breast to genotoxic damage as a mechanism of carcinogenesis.

Furthermore, if the $BRCA^{+/-}$ genotype exhibits haploinsufficiency, then the risk of such early LOH events might be

increased. This model predicts that the years surrounding puberty are particularly important for *BRCA*-linked disease, and that the breast epithelium may develop detectable genetic lesions in checkpoint and *BRCA* genes before the end of puberty. Although there is relatively little information that is directly pertinent to such mechanisms, one study [59] suggested that LOH events connected with *BRCA*-linked disease might occur early in breast development.

Mutagenesis models

At first glance, generic cellular functions such as DNA repair/recombination would seem unlikely candidates for having tissue-specific functions. However, the precise genetic consequences of *BRCA* gene inactivation have yet to be fully defined. Defects in DNA repair are frequently accompanied by an increase in the mutation rate. An interesting example of potentially tissue-specific effects of mutagenesis came from a study of the impact of mismatch repair (MMR) defects on colon cancer. MMR dysfunction gives rise to characteristic frame-shift mutations across certain nucleotide repeat sequences. In MMR-defective colon cancers, frame-shift mutations were detected repeatedly within a purine-rich sequence in the type II transforming growth factor-β receptor gene sequence, resulting in its inactivation [60]. This suggested that MMR defects might promote colon cancer specifically by virtue of their characteristic mutagenic 'signature'. Could an analagous effect connect *BRCA* gene dysfunction to breast/ovarian cancer? There is as yet no indication that *BRCA* gene inactivation gives rise to a mutagenic event that is capable of delivering such specificity. However, 'forward mutagenesis' studies, which can provide unbiased information regarding mutagenesis, have not yet been reported for the *BRCA* genes. The full spectrum of mutagenesis attributable to *BRCA* gene inactivation is therefore unknown.

One similarity between the breast and the ovary is their regulation by estrogenic hormones. A positive correlation has been observed between estrogen exposure and breast cancer risk [61]. This effect may in large part reflect proliferative effects of estrogen upon its target tissues. In addition, however, some estrogen metabolites, which might be expected to accumulate in estrogen target tissues, have been shown to chemically modify DNA *in vitro*, and can promote carcinogenesis in some rodent models [62,63]. An estrogen target tissue might therefore suffer increased DNA damage directly from estrogen metabolites, giving rise to a 'remote carcinogenesis' mechanism (i.e. although potentially carcinogenic in other tissues, the pharmacokinetics of the carcinogen dictates a restricted site of action *in vivo*).

A defect in recombination could amplify the carcinogenic potential of this tissue-specific DNA damage. For example, work in prokaryotes has revealed a key role for homologous recombination in maintaining genomic integrity after DNA polymerase stalling/replication arrest (for review [64]). Bulky DNA adducts, such as those formed by estrogen metabolites, might be expected to induce DNA polymerase stalling when encountered by the replication machinery, placing particular stress on efficient recombination to prevent genomic instability. Error-prone recombination can give rise to chromosome translocation, LOH events, and other large-scale genome rearrangements that are characteristic of tumor cells (for review [65]). One can imagine how tissue-specific DNA damage, *BRCA* gene dysfunction, and the clonal expansion of breast epithelial cells within the lobule (as discussed above) might collaborate to promote breast cancer above other cancers in *BRCA*-linked disease.

Clearly, the interplay between genotoxic damage and carcinogenesis is not limited to the breast. The gastrointestinal tract, for example, must handle heavy loads of genotoxic agents. However, this epithelium may be protected by the rapid shedding of epithelial cells, which would ensure that only stem cells could potentially form tumors. Such a difference in the physiology of these epithelia may make the gastrointestinal tract less prone to a recombination defect than the breast. At the same time, experimental evidence for this concept is lacking.

The accumulation of a carcinogen at the target site would seem to be a prerequisite for local carcinogen action. This process might therefore also involve other genotoxic agents that accumulate in the breast epithelium or surrounding fat. Hints of this are seen in the property of human mammary lipid extracts to promote single-stranded DNA breaks in cultured primary human mammary epithelial cells [66].

Transcriptional functions of *BRCA1* and *BRCA2*

BRCA1 and BRCA2 have each been proposed to function as transcriptional regulators [67–70]. Indeed, BRCA1 and BRCA2 can form complexes with various transcription factors and chromatin remodeling proteins [71–75]. If the *BRCA* genes regulate the expression of a specific set of target genes, then the identification of these targets might reveal tissue-specific functions of BRCA1 or BRCA2 that are relevant to breast and ovarian cancer. Several candidate target genes of BRCA1 have been identified. Notably, some of these are DNA damage/stress responsive genes and, in some cases, are *p53* dependent [76–78]. Both BRCA1 and BRCA2 can interact with p53 [79–81]. Overexpressed *BRCA1* is toxic to cells and can stabilize *p53* [82,83]. In view of the repair functions of the *BRCA* genes and the genetic interactions between *p53* mutation and *BRCA* gene mutation, discussed above, the relationship between *BRCA* gene products and *p53* may be complex. Evaluation of *BRCA–p53* interactions may reveal novel functions of *p53* [46,50].

Transient overexpression of *BRCA1* can modify estrogen receptor-dependent promoter functions [84]. However, estrogen receptor mutation is a frequent event in *BRCA*-linked breast cancer, suggesting that tumorigenesis caused by *BRCA* gene mutation affects pathways other than those controlled by the estrogen receptor. A broader analysis of the physiologic effects of *BRCA* gene products on transcription functions may clarify which genes are directly transcriptionally regulated by *BRCA* gene products, and which of these are relevant to tumor suppression.

Conclusion

It is not yet clear which properties of the *BRCA* genes account for their tissue-specific actions. Genome stability and transcription functions may each be relevant to *BRCA* gene-mediated tumor suppression. How such functions are applied to the breast and ovary may become clear from a more detailed understanding of the biology of the *BRCA* genes and of these epithelia.

Acknowledgements

I should like to thank many colleagues for helpful discussions, especially Drs David Livingston, Jean Feunteun, Carl Barrett, Joe Gray and Terry Rabbitts, and colleagues in the Livingston laboratory.

References

1. Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King MC: **Linkage of early-onset familial breast cancer to chromosome 17q21.** *Science* 1990, **250**:1684–1689.
2. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, *et al*: **A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1.** *Science* 1994, **266**:66–71.
3. Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, Nguyen K, Seal S, Tran T, Averill D, *et al*: **Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13.** *Science* 1994, **265**:2088–2090.
4. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G: **Identification of the breast cancer susceptibility gene BRCA2.** *Nature* 1995, **378**:789–792.
5. Smith SA, Easton DF, Evans DG, Ponder BA: **Allele losses in the region 17q12-21 in familial breast and ovarian cancer involve the wild-type chromosome.** *Nature Genet* 1992, **2**:128–131.
6. Neuhausen SL, Marshall CJ: **Loss of heterozygosity in familial tumors from three BRCA1-linked kindreds.** *Cancer Res* 1994, **54**: 6069–6072.
7. Collins N, McManus R, Wooster R, Mangion J, Seal S, Lakhani SR, Ormiston W, Daly PA, Ford D, Easton DF, *et al*: **Consistent loss of the wild type allele in breast cancers from a family linked to the BRCA2 gene on chromosome 13q12-13.** *Oncogene* 1995, **10**:1673–1675.
8. Gudmundsson J, Johannesdottir G, Bergthorsson JT, Arason A, Ingvarsson S, Egilsson V, Barkardottir RB: **Different tumor types from BRCA2 carriers show wild-type chromosome deletions on 13q12-q13.** *Cancer Res* 1995, **55**:4830–4832.
9. Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM, Haugen-Strano A, Swensen J, Miki Y, *et al*: **BRCA1 mutations in primary breast and ovarian carcinomas.** *Science* 1994, **266**:120–122.
10. Lancaster JM, Wooster R, Mangion J, Phelan CM, Cochran C, Gumbs C, Seal S, Barfoot R, Collins N, Bignell G, Patel S, Hamoudi R, Larsson C, Wiseman RW, Berchuck A, Iglehart JD, Marks JR, Ashworth A, Stratton MR, Futreal PA: **BRCA2 mutations in primary breast and ovarian cancers.** *Nature Genet* 1996, **13**:238–240.
11. Knudson AG Jr: **Mutation and cancer: statistical study of retinoblastoma.** *Proc Natl Acad Sci USA* 1971, **68**:820–823.
12. Marquis ST, Rajan JV, Wynshaw-Boris A, Xu J, Yin GY, Abel KJ, Weber BL, Chodosh LA: **The developmental pattern of Brca1 expression implies a role in differentiation of the breast and other tissues.** *Nature Genet* 1995, **11**:17–26.
13. Lane TF, Deng C, Elson A, Lyu MS, Kozak CA, Leder P: **Expression of Brca1 is associated with terminal differentiation of ectodermally and mesodermally derived tissues in mice.** *Genes Dev* 1995, **9**:2712–2722.
14. Rajan JV, Marquis ST, Gardner HP, Chodosh LA: **Developmental expression of Brca2 colocalizes with Brca1 and is associated with proliferation and differentiation in multiple tissues.** *Dev Biol* 1997, **184**:385–401.
15. Gowen LC, Johnson BL, Latour AM, Sulik KK, Koller BH: **Brca1 deficiency results in early embryonic lethality characterized by neuroepithelial abnormalities.** *Nature Genet* 1996, **12**:191–194.
16. Hakem R, de la Pompa JL, Sirard C, Mo R, Woo M, Hakem A, Wakeham A, Potter J, Reitmair A, Billia F, Firpo E, Hui CC, Roberts J, Rossant J, Mak TW: **The tumor suppressor gene Brca1 is required for embryonic cellular proliferation in the mouse.** *Cell* 1996, **85**: 1009–1023.
17. Sharan SK, Morimatsu M, Albrecht U, Lim DS, Regel E, Dinh C, Sands A, Eichele G, Hasty P, Bradley A: **Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2.** *Nature* 1997, **386**:804–810.
18. Connor F, Bertwistle D, Mee PJ, Ross GM, Swift S, Grigorieva E, Tybulewicz VL, Ashworth A: **Tumorigenesis and a DNA repair defect in mice with a truncating Brca2 mutation.** *Nature Genet* 1997, **17**: 423–430.
19. Friedman LS, Thistlethwaite FC, Patel KJ, Yu VP, Lee H, Venkitaraman AR, Abel KJ, Carlton MB, Hunter SM, Colledge WH, Evans MJ, Ponder BA: **Thymic lymphomas in mice with a truncating mutation in Brca2.** *Cancer Res* 1998, **58**:1338–1343.
20. Shen SX, Weaver Z, Xu X, Li C, Weinstein M, Chen L, Guan XY, Ried T, Deng CX: **A targeted disruption of the murine Brca1 gene causes gamma-irradiation hypersensitivity and genetic instability.** *Oncogene* 1998, **17**:3115–3124.
21. Xu X, Weaver Z, Linke SP, Li C, Gotay J, Wang XW, Harris CC, Ried T, Deng CX: **Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells.** *Mol Cell* 1999, **3**:389–395.
22. Cressman VL, Backlund DC, Avrutskaya AV, Leadon SA, Godfrey V, Koller BH: **Growth retardation, DNA repair defects, and lack of spermatogenesis in BRCA1-deficient mice.** *Mol Cell Biol* 1999, **19**: 7061–7075.
23. Gayther SA, Warren W, Mazoyer S, Russell PA, Harrington PA, Chiano M, Seal S, Hamoudi R, van Rensburg EJ, Dunning AM, *et al*: **Germline mutations of the BRCA1 gene in breast and ovarian cancer families provide evidence for a genotype-phenotype correlation.** *Nature Genet* 1995, **11**:428–433.
24. Gayther SA, Mangion J, Russell P, Seal S, Barfoot R, Ponder BA, Stratton MR, Easton D: **Variation of risks of breast and ovarian cancer associated with different germline mutations of the BRCA2 gene.** *Nature Genet* 1997, **15**:103–105.
25. The Breast Cancer Linkage Consortium: **Cancer risks in BRCA2 mutation carriers.** *J Natl Cancer Inst* 1999, **91**:1310–1316.
26. Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, Weber B, Lenoir G, Chang-Claude J, Sobol H, Teare MD, Struewing J, Arason A, Scherneck S, Peto J, Rebbeck TR, Tonin P, Neuhausen S, Barkardottir R, Eyfjord J, Lynch H, Ponder BA, Gayther SA, Zelada-Hedman M, *et al*: **Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium.** *Am J Hum Genet* 1998, **62**:676–689.
27. Marcus JN, Watson P, Page DL, Narod SA, Lenoir GM, Tonin P, Linder-Stephenson L, Salerno G, Conway TA, Lynch HT: **Hereditary breast cancer: pathobiology, prognosis, and BRCA1 and BRCA2 gene linkage.** *Cancer* 1996, **77**:697–709.
28. Lakhani SR, Jacquemier J, Sloane JP, Gusterson BA, Anderson TJ, van de Vijver MJ, Farid LM, Venter D, Antoniou A, Storfer-Isser A, Smyth E, Steel CM, Haites N, Scott RJ, Goldgar D, Neuhausen S, Daly PA, Ormiston W, McManus R, Scherneck S, Ponder BA, Ford D, Peto J, Stoppa-Lyonnet D, Easton DF, *et al*: **Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations.** *J Natl Cancer Inst* 1998, **90**:1138–1145.
29. Scully R, Chen J, Plug A, Xiao Y, Weaver D, Feunteun J, Ashley T, Livingston DM: **Association of BRCA1 with Rad51 in mitotic and meiotic cells.** *Cell* 1997, **88**:265–275.
30. Mizuta R, LaSalle JM, Cheng HL, Shinohara A, Ogawa H, Copeland N, Jenkins NA, Lalande M, Alt FW: **RAB22 and RAB163/mouse BRCA2: proteins that specifically interact with the RAD51 protein.** *Proc Natl Acad Sci USA* 1997, **94**:6927–6932.

31. Lim DS, Hasty P: **A mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53.** *Mol Cell Biol* 1996, **16**:7133–7143.
32. Tsuzuki T, Fujii Y, Sakumi K, Tominaga Y, Nakao K, Sekiguchi M, Matsushiro A, Yoshimura Y, MoritaT: **Targeted disruption of the Rad51 gene leads to lethality in embryonic mice.** *Proc Natl Acad Sci USA* 1996, **93**:6236–6240.
33. Chen J, Silver DP, Walpita D, Cantor SB, Gazdar AF, Tomlinson G, Couch FJ, Weber BL, Ashley T, Livingston DM, Scully R: **Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells.** *Mol Cell* 1998, **2**: 317–328.
34. Scully R, Chen J, Ochs RL, Keegan K, Hoekstra M, Feunteun J, Livingston DM: **Dynamic changes of BRCA1 subnuclear location and phosphorylation state are initiated by DNA damage.** *Cell* 1997, **90**: 425–435.
35. Chen JJ, Silver D, Cantor S, Livingston DM, Scully R: **BRCA1, BRCA2, and Rad51 operate in a common DNA damage response pathway.** *Cancer Res* 1999, **59 (7 suppl)**:1752s–1756s.
36. Zhong Q, Chen CF, Li S, Chen Y, Wang CC, Xiao J, Chen PL, Sharp ZD, Lee WH: **Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response.** *Science* 1999, **285**: 747–750.
37. Patel KJ, Vu VP, Lee H, Corcoran A, Thistlethwaite FC, Evans MJ, Colledge WH, Friedman LS, Ponder BA, Venkitaraman AR: **Involvement of Brca2 in DNA repair.** *Mol Cell* 1998, **1**:347–357.
38. Moynahan ME, Chiu JW, Koller BH, Jasin M: **Brca1 controls homology-directed DNA repair.** *Mol Cell* 1999, **4**:511–518.
39. Snouwaert JN, Gowen LC, Latour AM, Mohn AR, Xiao A, DiBiase L, Koller BH: **BRCA1 deficient embryonic stem cells display a decreased homologous recombination frequency and an increased frequency of non-homologous recombination that is corrected by expression of a brca1 transgene.** *Oncogene* 1999, **18**:7900–7907.
40. Foray N, Randrianarison V, Marot D, Perricaudet M, Lenoir G, Feunteun J: **Gamma-rays-induced death of human cells carrying mutations of BRCA1 or BRCA2.** *Oncogene* 1999, **18**:7334–7342.
41. Scully R, Ganesan S, Vlasakova K, Chen J, Socolovsky M, Livingston DM: **Genetic analysis of BRCA1 function in a defined tumor cell line.** *Mol Cell* 1999, **4**:1093–1099.
42. Gowen LC, Avrutskaya AV, Latour AM, Koller BH, Leadon SA: **BRCA1 required for transcription-coupled repair of oxidative DNA damage.** *Science* 1998, **281**:1009–1012.
43. Tutt A, Gabriel A, Bertwistle D, Connor F, Paterson H, Peacock J, Ross G, Ashworth A: **Absence of Brca2 causes genome instability by chromosome breakage and loss associated with centrosome amplification.** *Curr Biol* 1999, **9**:1107–1110.
44. Suzuki A, de la Pompa JL, Hakem R, Elia A, Yoshida R, Mo R, Nishina H, Chuang T, Wakeham A, Itie A, Koo W, Billia P, Ho A, Fukumoto M, Hui CC, Mak TW: **Brca2 is required for embryonic cellular proliferation in the mouse.** *Genes Dev* 1997, **11**:1242–1252.
45. Hakem R, de la Pompa JI, Elia A, Potter J, Mak TW: **Partial rescue of Brca1 (5-6) early embryonic lethality by p53 or p21 null mutation.** *Nature Genet* 1997, **16**:298–302.
46. Lee H, Trainer AH, Friedman LS, Thistlethwaite FC, Evans MJ, Ponder BA, Venkitaraman AR: **Mitotic checkpoint inactivation fosters transformation in cells lacking the breast cancer susceptibility gene, Brca2.** *Mol Cell* 1999, **4**:1–10.
47. Xu X, Wagner KU, Larson D, Weaver Z, Li C, Ried T, Hennighausen L, Wynshaw-Boris A, Deng CX: **Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation.** *Nature Genet* 1999, **22**:37–43.
48. Cressman VL, Backlund DC, Hicks EM, Gowen LC, Godfrey V, Koller BH: **Mammary tumor formation in p53- and BRCA1-deficient mice.** *Cell Growth Differ* 1999, **10**:1–10.
49. Crook T, Brooks LA, Crossland S, Osin P, Barker KT, Waller J, Philp E, Smith PD, Yulug I, Peto J, Parker G, Allday MJ, Crompton MR, Gusterson BA: **p53 mutation with frequent novel condons but not a mutator phenotype in BRCA1- and BRCA2-associated breast tumours.** *Oncogene* 1998, **17**:1681–1689.
50. Smith PD, Crossland S, Parker G, Osin P, Brooks L, Waller J, Philp E, Crompton MR, Gusterson BA, Allday MJ, Crook T: **Novel p53 mutants selected in BRCA-associated tumours which dissociate transformation suppression from other wild-type p53 functions.** *Oncogene* 1999, **18**:2451–2459.
51. Kordon EC, Smith GH: **An entire functional mammary gland may comprise the progeny from a single cell.** *Development* 1998, **125**: 1921–1930.
52. Chepko G, Smith GH: **Mammary epithelial stem cells: our current understanding.** *J Mammary Gland Biol Neoplasia* 1999, **4**:35–52.
53. Tsai YC, Lu Y, Nichols PW, Zlotnikov G, Jones PA, Smith HS: **Contiguous patches of normal human mammary epithelium derived from a single stem cell: implications for breast carcinogenesis.** *Cancer Res* 1996, **56**:402–404.
54. Wolden SL, Lamborn KR, Cleary SF, Tate DJ, Donaldson SS: **Second cancers following pediatric Hodgkin's disease.** *J Clin Oncol* 1998, **16**:536–544.
55. Tokunaga M, Norman JE Jr, Asano M, Tokuoka S, Ezaki H, Nishimori I, Tsuji Y: **Malignant breast tumors among atomic bomb survivors, Hiroshima and Nagasaki, 1950-74.** *J Natl Cancer Inst* 1979, **62**:1347–1359.
56. Tokunaga M, Land CE, Yamamoto T, Asano M, Tokuoka S, Ezaki H, Nishimori I: **Incidence of female breast cancer among atomic bomb survivors, Hiroshima and Nagasaki, 1950–1980.** *Radiat Res* 1987, **112**:243–272.
57. Tokunaga M, Land CE, Tokuoka S, Nishimori I, Soda M, Akiba S: **Incidence of female breast cancer among atomic bomb survivors, 1950–1985.** *Radiat Res* 1994, **138**:209–223.
58. Land CE: **Studies of cancer and radiation dose among atomic bomb survivors. The example of breast cancer.** *JAMA* 1995, **274**: 402–407.
59. Lakhani SR, Chaggar R, Davies S, Jones C, Collins N, Odel C, Stratton MR, O'Hare MJ: **Genetic alterations in 'normal' luminal and myoepithelial cells of the breast.** *J Pathol* 1999, **189**:496–503.
60. Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B, *et al*: **Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability.** *Science* 1995, **268**:1336–1338.
61. Key TJ: **Serum oestradiol and breast cancer risk.** *Endocr Relat Cancer* 1999, **6**:175–180.
62. Liehr JG, Avitts TA, Randerath E, Randerath K: **Estrogen-induced endogenous DNA adduction: possible mechanism of hormonal cancer.** *Proc Natl Acad Sci USA* 1986, **83**:5301–5305.
63. Fishman J, Osborne MP, Telang NT: **The role of estrogen in mammary carcinogenesis.** *Ann N Y Acad Sci* 1995, **768**:91–100.
64. Cox MM, Goodman MF, Kreuzer KN, Sherratt DJ, Sandler SJ, Marians KJ: **The importance of repairing stalled replication forks.** *Nature* 2000, **404**:37–41.
65. Jasin M: **Chromosome breaks and genomic instability.** *Cancer Invest* 2000, **18**:78–86.
66. Martin FL, Venitt S, Carmichael PL, Crofton-Sleigh C, Stone EM, Cole KJ, Gusterson BA, Grover PL, Phillips DH: **DNA damage in breast epithelial cells: detection by the single-cell gel (comet) assay and induction by human mammary lipid extracts.** *Carcinogenesis* 1997, **18**:2299–2305.
67. Chapman MS, Verma IM: **Transcriptional activation by BRCA1.** *Nature* 1996, **382**:678–679.
68. Monteiro AN, August A, Hanafusa H: **Evidence for a transcriptional activation function of BRCA1 C-terminal region.** *Proc Natl Acad Sci USA* 1996, **93**:13595–13599.
69. Milner J, Ponder B, Hughes-Davies L, Seltmann M, Kouzarides T: **Transcriptional activation functions in BRCA2.** *Nature* 1997, **386**: 772–773.
70. Scully R, Anderson SF, Chao DM, Wei W, Ye L, Young RA, Livingston DM, Parvin JD: **BRCA1 is a component of the RNA polymerase II holoenzyme.** *Proc Natl Acad Sci USA* 1997, **94**:5605–5610.
71. Siddique H, Zou JP, Rao VN, Reddy ES: **The BRCA2 is a histone acetyltransferase.** *Oncogene* 1998, **16**:2283–2285.
72. Fuks F, Milner J, Kouzarides T: **BRCA2 associates with acetyltransferase activity when bound to P/CAF.** *Oncogene* 1998, **17**: 2531–2534.
73. Yu X, Wu LC, Bowcock AM, Aronheim A, Baer R: **The C-terminal (BRCT) domains of BRCA1 interact in vivo with CtIP, a protein implicated in the CtBP pathway of transcriptional repression.** *J Biol Chem* 1998, **273**:25388–25392.
74. Yarden RI, Brody LC: **BRCA1 interacts with components of the histone deacetylase complex.** *Proc Natl Acad Sci USA* 1999, **96**:4983–4988.
75. Pao GM, Janknecht R, Ruffner H, Hunter T, Verma IM: **CBP/p300 interact with and function as transcriptional coactivators of BRCA1.** *Proc Natl Acad Sci USA* 2000, **97**:1020–1025.
76. Somasundaram K, Zhang H, Zeng YX, Houvras Y, Peng Y, Zhang H, Wu GS, Licht JD, Weber BL, El-Deiry WS: **Arrest of the cell cycle by the tumour-suppressor BRCA1 requires the CDK-inhibitor p21WAF1/CiP1.** *Nature* 1997, **389**:187–190.

77. Harkin DP, Bean JM, Miklos D, Song YH, Truong VB, Englert C, Christians FC, Ellisen LW, Maheswaran S, Oliner JD, Haber DA: **Induction of GADD45 and JNK/SAPK-dependent apoptosis following inducible expression of BRCA1.** *Cell* 1999, **97**:575–586.
78. MacLachlan TK, Somasundaram K, Sgagias M, Shifman Y, Muschel RJ, Cowan KH, El-Deiry WS: **BRCA1 effects on the cell cycle and the DNA damage response are linked to altered gene expression.** *J Biol Chem* 2000, **275**:2777–2785.
79. Ouchi T, Monteiro AN, August A, Aaronson SA, Hanafusa H: **BRCA1 regulates p53-dependent gene expression.** *Proc Natl Acad Sci USA* 1998, **95**:2302–2306.
80. Marmorstein LY, Ouchi T, Aaronson SA: **The BRCA2 gene product functionally interacts with p53 and RAD51.** *Proc Natl Acad Sci USA* 1998, **95**:13869–13874.
81. Zhang H, Somasundaram K, Peng Y, Tian H, Zhang H, Bi D, Weber BL, El-Deiry WS: **BRCA1 physically associates with p53 and stimulates its transcriptional activity.** *Oncogene* 1998, **16**:1713–1721.
82. Wilson CA, Payton MN, Elliott GS, Buaas FW, Cajulis EE, Grosshans D, Ramos L, Reese DM, Slamon DJ, Calzone FJ: **Differential subcellular localization, expression and biological toxicity of BRCA1 and the splice variant BRCA1-delta11b.** *Oncogene* 1997, **14**:1–16.
83. Somasundaram K, MacLachlan TK, Burns TF, Sgagias M, Cowan KH, Weber BL, el-Deiry WS: **BRCA1 signals ARF-dependent stabilization and coactivation of p53.** *Oncogene* 1999, **18**:6605–6614.
84. Fan S, Wang J, Yuan R, Ma Y, Meng Q, Erdos MR, Pestell RG, Yuan F, Auborn KJ, Goldberg ID, Rosen EM: **BRCA1 inhibition of estrogen receptor signaling in transfected cells.** *Science* 1999, **284**:1354–1356.

Author's affiliation: Dana Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts, USA

Correspondence: Ralph Scully, Dana Farber Cancer Institute and Harvard Medical School, 44 Binney Street, Boston, MA 02115, USA. Tel: +1 617 632 4375; fax: +1 617 632 4381; e-mail: ralph_scully@dfci.harvard.edu