[Cancer Biology & Therapy 3:6, 528-533, June 2004]; ©2004 Landes Bioscience

Focused Review Series: BRCA1 at 10 Years Since Discovery

BRCA1 and Transcription

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Received 06/18/04; Accepted 06/24/04

Previously published online as a *Cancer Biology & Therapy* E-publication: http://www.landesbioscience.com/journals/cbt/abstract.php?id=843

KEY WORDS

RNA polymerase II (RNAPII), BRCA1-associated transcriptional (BAT) complex, BRCA1-associated surveillance (BASC)

ACKNOWLEDGEMENTS

The author would like to thank Dr. Susan A. Krum and other members of the lab for comments on this review.

T.F.L. is supported by funds from the Cancer Research Coordinating Committee of the University of California, the Stop Cancer Foundation, the Council on Research for the University of California, and a grant from the Jonsson Comprehensive Cancer Center.

ABSTRACT

The BRCA1 tumor suppressor gene is expressed in all mammalian cells. Within these cells, the BRCA1 protein product interacts with several seemingly distinct nuclear complexes. Proteins within these complexes are potential targets for the E3-ubiquitin ligase activity associated with BRCA1:BARD1 complexes. Recent breakthroughs have centered on elucidating critical DNA repair and chromatin-remodeling functions associated with BRCA1 activity. During both DNA replication and DNA repair, BRCA1 appears to serve both adaptor and enzymatic functions. Roles include transient physical recruitment of NBS1, γH2AX, FANCD2 and other proteins in specific repair associated complexes, and enzymatic activity as an E3-ubiquitin ligase against a subset of these proteins. BRCA1 has also been implicated as a regulator of transcription. It is in this second capacity that progress has been much more difficult to assess. In particular, unambiguous adaptor and enzymatic functions have yet to be demonstrated in transcriptional machinery. Addressing the critical gap in our understanding of enzymatic targets of BRCA1 will be required for significant future progress in this field. The following review puts forward a model for BRCA1 interactions with the transcriptional complex in undamaged cells, and a potential mechanism for substrate switching between transcription and DNA-repair complexes following exposure of cells to proliferative or genotoxic stress. This model incorporates recent evidence that BRCA1 interacts predominantly with hyper-phosphorylated, enzymatically active, RNA polymerase II (RNAPII) in undamaged cells. The model proposes that BRCA1 binds processive RNA polymerase as part of a genome surveillance function, upstream of critical roles in DNA repair.

BACKGROUND

BRCA1 is a tumor suppressor protein first identified from germline mutations linked to familial breast and ovarian cancer in humans.[1] Most of the BRCA1 mutations observed in cancer result in truncated or aberrant BRCA1 protein products unable to form stable complexes with verified BRCA1 interacting proteins. Thus, BRCA1 disease results from absence, or severely diminished expression, of functional BRCA1 protein.[2-4] An expanding array of studies link BRCA1-deficiency to radiation hypersensitivity, defective double-strand break repair, defective homology-based repair, and defective S and G_2/M checkpoint control (reviewed in refs. 5–8 and references therein). These studies also demonstrate that loss of BRCA1 is linked to genetic instability and eventual loss of other tumor suppressor genes. Specifically, wild type p53 function appears incompatible with proliferation in BRCA1-defective cells and mutation or elimination of p53 function is a reliable indicator of BRCA1 status in these tumors (see refs. 9,10 and others). This result is supported by animal studies in which removal of p53 can restore proliferative capacity in BRCA1-defective mice (see refs. 11,15 and others).

Alterations in the stability of DNA repair machinery and chromatin structure appear to be the defining defects leading to genomic instability in BCRA1 associated disease. Key to these activities, BRCA1 acts both downstream and upstream of critical regulators of genotoxic stress including ATM, ATR, NBS1 and γH2AX, FANCD2 and others.[8-16] These interactions form elements of a BRCA1-associated repair/surveillance (BASC) complex and generally require phosphorylation of the BRCA1 protein by ATM/ATR family kinases, and/or by chk2.[17-18] Mutations in a number BRCA1 associated proteins lead to DNA-repair phenotypes that partially overlap with phenotypes found in BRCA1 deficient cells: ATM,[19] chk2 ,[20-22] Fanconi anemia,[8] H2AX.[23-25]

In contrast, a large percentage of BRCA1 is unphosphorylated or hypo-phosphorylated in undamaged and G_0/G_1 cells[26] and the majority of this unphosphorylated BRCA1 associates with transcriptional complexes.[27,28] The BRCA1-associated transcriptional (BAT)

complex appears to contain, and potentially require, prior association of several proteins with implicated roles in activated/processive transcription, including: RNA helicase A,[29,30] and transcriptional enhancers NUPIF and the p-TEFb.[31] BRCA1 does not appear to be a target of p-TEFb/cdk9 kinase activity, but the p220 enzymatic subunit of RNAPII appears to be heavily labeled by both cdk9 and cdk7, a component of TFIIH, prior to BRCA1 binding. Interestingly, BAT associated RNAPII is highly processive and may include a large fraction of processive RNAPII in some cell types.[28,32,33] Binding of BRCA1 to the core RNAPII complex appears to involve hRPB2 and hRPB10a[34] and association of RNApolII with p-TEFb.[31] All these features suggest that BRCA1 associates with a major post promoter form of RNAPII. Excellent reviews covering evidence that BRCA1 plays a role in transcriptional activation/coactivation can be found.[33,47,48]

Genetic and biochemical data have supported roles for BRCA1 in homologous recombination and other types of DNA repair,[35-37] chromatin remodeling,[38,39] X-inactivation,[40] and as an E3-ubiquitin ligase.[41-44] Intact ATM and/or ATR signaling appears essential for many aspects of these chromatin associated and repair functions.[45] While there is strong biochemical data for roles of BRCA1 in transcription, ATM/ATR and other modifiers of BRCA1 function have not been shown be to positively regulate these functions and cellular responses to genotoxic stress may actually block interactions of BRCA1 with transcriptional machinery.[46] This chapter will discuss the roles of BRCA1 in transcription and DNA repair, and will attempt to link the two areas together in one model.

BRCA1 AND TRANSCRIPTION

Binding of the transcriptional machinery by the C-terminus of BRCA1 (BRCA1-CTD) was the first biochemical activity ascribed to the BRCA1 protein.[49,50] Since that report, a series of experiments have demonstrated that the C-terminus of human BRCA1 (amino acids 1528–1863) can be used to recruit RNA polymerase II (RNAPII) to synthetic reporters leading to widely held view that BRCA1 plays some role in transcriptional activation (reviewed in refs. 47 and 51). However, direct evidence that intact BRCA1 binds to promoter regions of genes is lacking.

DIRECT EVIDENCE THAT BRCA1 BINDS COMPONENTS OF THE TRANSCRIPTIONAL APPARATUS

Yeast-two hybrid and coprecipitation experiments originally supported the assignment of BRCA1 as a binding partner for RNAPII.[27,49,50] This interaction appears to involve one or more proteins associated with the core polymerase complex.[34] Initial studies provided evidence that the acidic C-terminus of BRCA1 was sufficient to mediate such interactions,[27] and may do so by binding RNA helicase A.[29,34] More recently, it has been shown that other regions of BRCA1 may also contribute in contacts with RNAPII subunits.[52]

Previous models of BRCA1 function(s) in transcription are poorly defined, but favor regulation of various preinitiation steps, including, but not limited to: recruitment of RNAPII to promoters, transcriptional activation[53,54] transcriptional coactivation[33,55] and transcriptional inhibition.[56-59] It is clear that the BRCA1 protein bound to RNAPII is present as a dimmer with BARD1 suggesting that the complex is capable of acting as a fully active E3-ubiquitin ligase,[52] however no published evidence exists to suggest that BRCA1 is capable of transferring ubiquitin to any component of the transcriptional apparatus. Identification of in vivo targets for BRCA1 E3 ligase activity will clearly be an area of intense interest. In vitro assays have demonstrated that purified BRCA1: BARD complex enhances RNA synthesis when mixed with partially purified components of RNA polymerase.[52]

A distinct, but not mutually exclusive, model would suggest that BRCA1 interacts with elements of the RNAPII holoenzyme linked to post-promoter activities of the complex. Evidence for this model include interactions of BRCA1 with the elongation factor NELF-B/COBRA1[39] and the CstF-50 component of the polyadenylation complex.[60,61] Consistent with this new model, we recently showed that BRCA1 proteins from several species interacted with phosphorylated RNAPII and preferentially bound to hyper-phosphorylated RNAPII, typically associated with highly processive active transcriptional units.[28] We provided evidence that unphosphorylated BRCA1 associated with the most processive polymerase complexes within cells and that the catalytic subunit (p220) within these complexes was hyper-phosphorylated. Following exposure of cells to DNA damaging agents, BRCA1 was shown to detach from these elongating RNAPII complexes [60,61] and subsequently associates with sites of DNA damage.[28] These results suggest that BRCA1 is specifically associated with processive RNAPII complexes in preference to promoter bound or prepromoter transcriptional complexes in undamaged cells.

TRANSCRIPTIONAL ACTIVATION ASSAYS USING BRCA1

For several years now, BRCA1 has been commonly described as a transcriptional activator or coactivator. Given the general lack of evidence that BRCA associates with gene promoters, or promoter bound RNAPII, these terms should be reconsidered. The general notion that BRCA1 acts as a transcriptional activator/coactivator evolved following early reports that the acidic C-terminus of the BRCA1 protein (particularly amino acids 1528–1863) provided modest levels of transcriptional activation activity when engineered into a DNA binding complex using the so called Gal4-UAS assays.[49,50] Subsequently, direct binding of BRCA1 to components of RNAPII complex [27] loosely supported this view. Recently, engineered overexpression of BRCA1 in cells has been used in combination with cDNA array expression analysis to identify potential transcriptional targets of BRCA1-mediated transcriptional activation[62-65] and provided additional, yet not specific, support for a role in classic transcriptional activation. Since no "promoter-associated" DNA-binding activity has ever been demonstrated for BRCA1, more recent work on BRCA1 effects on activation of transcription have suggested that BRCA1 acts as a coactivator of transcription. This allowed continued reference to the original hypothesis that BRCA1 could act to recruit RNA polymerase to specific mammalian promoters by binding to specific transcription factors (reviewed in ref. 66). However, to date, there is no direct evidence that BRCA1 interacts, directly or indirectly, with the promoter region of any of the genes proposed to be directly regulated by BRCA1.

Several lines of evidence have now emerged which call into question the ability of BRCA1 to act as an endogenous recruiter of RNAPII to promoters.[28] First and foremost, we found that full-length BRCA1 proteins, or the naturally occurring Δ11 splice variants, do not activate transcription in yeast or mammalian one-hybrid assays.[28,46] Secondly, one-hybrid transcriptional activation activity of BRCA1-CTDs is poorly conserved and correlates, in part, with negative charge. Negative charge is known to give problematic false

positives within the GAL4-UAS assay.[67,69] Third, closely related BRCA1-CTDs from mouse and cow lack significant one-hybrid transcriptional activation activity when tested in multiple cell types and under multiple conditions.[28,46] Fourth, in-frame fusion of the mouse CTD onto portions of the human CTD sequence severely dissipate CTD-associated one-hybrid activity.[28] Fifth, in vivo, BRCA1 proteins from multiple species interact specifically with hyper-phosphorylated (IIO) RNAPII, widely considered to represent post-promoter forms of the pol II complex.[28] Sixth, in vivo, BRCA1 proteins fail to interact with hypo-phosphorylated (IIA) forms of RNAPII that would be expected in preinitiation states of the transcriptional apparatus.[28]

Importantly, in vivo, BRCA1 interacts specifically with a very large percentage of the catalytically active RNAPII while binding to a very small percentage of the total RNAPII found in cells, suggesting that BRCA1 interacts preferentially with post-promoter (late) forms of the transcriptional machinery.[28] We observed that full-length BRCA1 from mouse and bovine species also copurified with a large percentage of hyper-phosphorylated (IIO) RNAPII found in cycling epithelial cells. This distinction, based on fractionation of the endogenous proteins, suggested that a major role of BRCA1 in undamaged cells is directed toward post-initiation functions of RNAPII. These results provide evidence that BRCA1 is not a transcriptional activator *per se*, and suggest that roles in transcriptional regulation would likely involve post-initiation functions, or aspects of genomic surveillance, that are linked to RNAPII transcriptional machinery.

DIFFERENTIAL EXPRESSION OF GENES IN BRCA1 OVEREXPRESSING AND UNDEREXPRESSING CELLS

BRCA1 expression cassettes have been created to study a diverse array of BRCA1 functions and as potential therapeutic vectors. While obviously necessary within the context of a developing field, studies based on this class of reagents must be carefully considered based on knowledge available at present. Foremost, endogenous BRCA1 proteins are expressed at very low levels and are tightly regulated during the cell cycle or in response to genotoxic stress.[70,71] Peak steady-state levels of mRNA and protein occur at the G_1/S transition[72,75] and protein levels are reduced during M-phase. Control of expression requires transcriptional regulation by wild type p53,[76] and rescue of wild type BRCA1 function by gene therapy requires continuous expression of the rescuing allele from the endogenous promoter.[77,78] Because expression of BRCA1 from constitutive promoters results in expression of genes not normally associated with BRCA1 expressing cells in vivo,[71] the utility of constitutive BRCA1 expression cassettes is a necessary but controversial aspect of many studies on BRCA1 function.

So what is to be made of the evidence that BRCA1 expression can induce cell cycle inhibitory factors? Exogenous BRCA1 in mammalian cells is associated with increased transcription of several stress-response genes including $p21^{waf1/cip1}$,[79] p27[80] and GADD45[62] and decreased transcription of other genes, including certain estrogen receptor regulated genes.[56] The GADD45 response to BRCA1 in particular is p53 dependent [81] and the p21 response appears indirect as well. Both BRCA1 overexpressing and underexpressing cells show evidence of cell cycle arrest mediated by p21. In BRCA1-null cells, this phenotype is partially alleviated by loss of p53 expression, suggesting activation of various checkpoint functions.[15] Examination of the expression of BRCA1 mRNA in mammalian embryos shows no overlap with p21 or GADD45 expression, two of the better studied potential transcriptional targets,[71] suggesting that normal tissues with "elevated" BRCA1 expression are not responding with expression of cell cycle arrest markers. On the contrary, normal expression of BRCA1 is always highest in proliferating cells, both in the embryo, and in adult mammary tissue.[74,82,83] Evidence that BRCA1 acts as a transcriptional activator (in the classic sense) is thus limited.

Perhaps the best evidence for a role of BRCA1 as a transcriptional coactivator centers on interactions with p53, a known transcription factor previously shown to interact with BRCA1. BRCA1 interacts with p53 via the C-terminal BRCT domains of BRCA1.[84,86] Evidence for alterations of p53 responsive gene expression have been demonstrated in cells expressing exogenous BRCA1.[15] However, these studies are also complicated by reliance on overexpression of BRCA1 as indicated by stabilization of nuclear p53 to levels indicative of genotoxic response.[85] The complexities associated with identifying transcriptional targets of BRCA1 may be daunting, but there is room to hope. RNA expression profiling of BRCA1-null mice have shown very few differences in p53 response genes. However, 14-3-3σ has been shown to be downregulated in BRCA1-null embryos and restoration of expression requires both wild type BRCA1 and p53[87] Clearly, approaches, which take advantage of engineered mice, and conditionally null cells, will have great utility in sorting through potential targets. Analysis of coexpression in embryonic or adult tissues at times of high BRCA1 expression may also prove valuable.[71] Ultimately, demonstration of direct interaction of BRCA1 with each of these factors while bound to arrested transcriptional complexes will be required for demonstration of specific coactivator function.

A SWITCH MODEL TO EXPLAIN THE TRAFFICKING OF BRCA1 BETWEEN RNAPII COMPLEXES AND SITES OF CHROMATIN REPAIR

Figure 1 presents a general model describing the mechanical basis for the BRCA1 movement between processive RNAPII in undamaged cells, and components of the DNA repair complex following exposure to DNA, or chromatin, damaging agents. The model indicates that that BRCA1 binds to various phosphorylated proteins via its BRCT domains. This region of BRCA1 thus forms a phospho-protein binding module. Substrate selection is modified first by differential phosphorylation of the substrates (NBS1, H2AX, FANCD2 etc), but also requires modulation of binding affinity by phosphorylation of BRCA1 itself. Phosphorylation during S-phase by chk2 and ATR kinases lead to specific target selection, and distinct or additional targets may be recognized following ATM kinase activation. Phosphorylation by ATM/ATR also reduces contacts with phosphorylated RNAPII, allowing dissociation from sites of transcription and diffusion to sites of DNA/chromatin repair. Dissociation from RNAPII may assist but is not required for polymerase stalling, however, the proximity of active transcription complexes to expressed regions of the genome allows for increased repair activity of the transcribed strand, known as transcription coupled repair.

Following genotoxic stress, BRCA1 becomes phosphorylated by ATM/ATR family kinases and dissociates from processive RNAPII complexes.[28] Subsequently, phospho-BRCA1 associates with sites of DNA repair.[26,88] This separation of phosphorylated BRCA1 from active sites of transcription can be visualized directly in pachytene spermatocytes where phosphorylated BRCA1 decorates the transcriptionally silent XY body in an ATM/ATR dependent fashion.[40] In normal testes, the XY bivalent is decorated by ATR and an array of phosphorylated targets, including γH2AX and BRCA1, which are

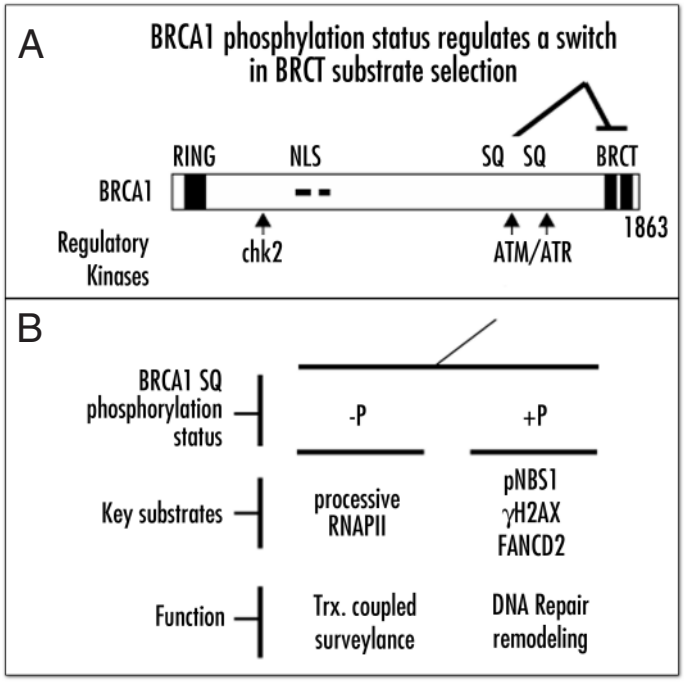

Figure 1. BRCA1 movement between processive RNAPII and the DNA repair complex is regulated by checkpoint and genotoxic stress-responsive kinases. BRCA1 binds to various phosphorylated proteins via its BRCT domains which comprise a phospho-protein binding module. Substrate selection is modified first by differential phosphorylation of the substrates and requires modulation of binding affinity by phosphorylation of BRCA1 itself. Phosphorylation of BRCA1 by ATM, ATR and potentially other kinases reduces contacts with phosphorylated RNAPII, allowing diffusion to sites of DNA/chromatin repair. RING, ring finger domain containing the E3 ligase domain; NLS, nuclear localization signal; SQ, clusters of serine glutamine pairs that are substrates for DNA-PK family kinases including ATM and ATR; BRCT, a domain containing tandem-BRCT motifs and shown to be a region of phospho-protein binding. Other symbols include: P, phosphate; arrows indicate recognition sites for chk2, ATM and ATR kinases. Other kinase recognition sites are not specified, but could play roles in target selection and protein stability/turnover.

known to colocalize following genotoxic stress.[88] In BRCA1 deficient cells, the XY body is not stably decorated with γH2AX suggesting a reciprocal stabilization of the complex.[89]

Following DNA damage, the BRCA1 protein becomes hyper-phosphorylated at multiple sites in the acidic pocket laying next to the tandem BRCT domains. The consequence of this phosphorylation is to change the affinity of BRCA1 for various substrates.[22,45,90] As reported by several groups, the BRCT domains show binding preferences for phospho-peptides.[91,94] BRCA1 is phosphorylated on serines 1387, 1423 and 1524, by ATM. Mutations in serines 1423 and 1524 block BRCA1-mediated G_2/M checkpoints [95] while mutation of serine 1387 disrupts the S-phase checkpoint.[96] BRCA1 is phosphorylated on serine 988 by chk2, a kinase mutated in subsets of Li-Fraumeni patients[97] and in nonBRCA associated familial breast cancer patients.[98] Mutation of serine 988 in BRCA1 prevents the dispersion of BRCA1 from nuclear foci following DNA damage[20] and abrogates BRCA1's ability to stimulate homologous recombination.[22]

The model presented here describes a mechanism that regulates BRCA1 binding to various bioactive complexes and suggests that BRCA1 binds processive RNAPII complex as part of a genome scanning function. This positioning of the BRCA1 protein places it in close proximity of the most sensitive regions of chromatin, those associated with transcription of active genes in any particular cell. Activation of the various genotoxic stress pathways (replicative stress, double stand breaks, and oxidative stress) have been shown to lead to BRCA1 phosphorylation and redistribution within the cell to sites of active repair. This model potentially accounts for cellular specificity of BRCA1 associated disease because individual cell types express differing amounts of BRCA1 and express different clusters of genes. Reduced or modest levels of BRCA1 in mammary, ovarian or prostate cells (known to be sites of tumor formation in BRCA1 mutation carriers), could predispose these tissues to genome instability phenotypes. In contrast, other proliferative tissues (notably thymus and spleen) express much higher levels of BRCA1 and may receive more protection from this type of disease.

The model shows that substrate recognition is governed by a phospho-peptide binding function within the BRCT domain of BRCA1 and that substrate selection is governed by the phosphorylation state of BRCA1, especially in the region of SQ clusters that are targets of ATM/ATR activity during response to replicative or genotoxic stress. The rational is to have DNA repair machinery, such as BRCA1, in close proximity to actively transcribed regions of the genome which are presumably the most critical in any given cell type.

References

1. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, et al. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. Science 1994; 266:66-71.
2. Xu C-F, Chambers JA, Nicolai H, Brown MA, Jujeirat Y, Mohammed S, et al. Mutations and alternative splicing of the *BRCA1* gene in UK breast/ovarian cancer families. Genes Chromosomes Cancer 1997; 18:102-10.
3. Brody LC, Biesecker BB. Breast cancer susceptibility genes *BRCA1* and *BRCA2*. Baltimore: Medicine 1998:77:208-26.
4. Narod SA, Boyd J. Current understanding of the epidemiology and clinical implications of BRCA1 and BRCA2 mutations for ovarian cancer. Curr Opin Obstet Gynecol 2002; 14:19-26.
5. Dasika GK, Lee EY-HP. DNA damage-induced cell cycle checkpoints and DNA strand break repair in development and tumorigenesis. Oncogene 1999; 18:7883-99.
6. Jasin M. Homologous repair of DNA damage and tumorigenesis: The BRCA connection. Oncogene 2002; 21:8981-93.
7. Deng CX, Wang RH. Roles of BRCA1 in DNA damage repair: A link between development and cancer. Hum Mol Genet 2003; 12:R113-23.
8. Venkitaraman AR. Tracing the network connecting BRCA and fanconi anaemia proteins. Nat Rev Cancer 2004; 4:266-76.
9. Crook T, Brooks LA, Crossland S, Osin P, Barker KT, Waller JPE, et al. p53 mutation with frequent novel codons but not a mutator phenotype in BRCA1- and BRCA2-associated breast tumours. Oncogene 1998; 17:1681-9.
10. Phillips KA, Nichol K, Ozcelik H, Knight J, Done SJ, Goodwin PJ, et al. Frequency of p53 mutations in breast carcinomas from Ashkenazi Jewish carriers of BRCA1 mutations. J Natl Cancer Inst 1999; 469-73.
11. Hakem R, de la Pompa JL, Elia A, Potter J, Mak TW. Partial rescue of Brca1 (5-6) early embryonic lethality by p53 or p21 null mutation. Nat Genet 1997; 16:298-302.
12. Ludwig T, Chapman DL, Papaioannou VE, Efstratiadis A. Targeted mutations of breast cancer susceptibility gene homologs in mice: Lethal phenotypes of Brca1, Brca2, Brca1/Brca2, Brca1/p53, and Brca2/p53 nullizygous embryos. Genes Dev 1997; 11:1226-41.
13. Xu X, Qiao W, Linke SP, Cao L, Li WM, Furth PA, et al. Genetic interactions between tumor suppressors Brca1 and p53 in apoptosis, cell cycle and tumorigenesis. Nat Genet 2001; 28:266-71.
14. Brodie SG, Xu X, Qiao W, Li WM, Cao L, Deng CX. Multiple genetic changes are associated with mammary tumorigenesis in Brca1 conditional knockout mice. Oncogene 2001; 20:7514-23.
15. Ongusaha PP, Ouchi T, Kim KT, Nytko E, Kwak JC, Duda RB, et al. BRCA1 shifts p53-mediated cellular outcomes towards irreversible growth arrest. Oncogene 2003; 22:3749-58.
16. Rosen EM, Fan S, Pestell RG, Goldberg ID. *BRCA1* gene in breast cancer. J Cell Physiol 2003; 196:19-41.
17. Kitagawa R, Bakkenist CJ, McKinnon PJ, Kastan MB. Phosphorylation of SMC1 is a critical downstream event in the ATM-NBS1-BRCA1 pathway. Genes Dev 2004; 18:1423-38.
18. Wang Y, Cortez D, Yazdi P, Neff N, Elledge SJ, Qin J. BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. Genes Dev 2000; 14:927-39.

19. Luo CM, Tang KW, Mekeel L, DeFrank JS, Anne PR, Powell SN. High frequency and error-prone DNA recombination in ataxia telangiectasia cell lines. J Biol Chem 1996; 271:4497-503.
20. Lee JS, Collins KM, Brown AL, Lee CH, Chung JH. hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response. Nature 2000; 404:201-4.
21. Lee SB, Kim SH, Bell DW, Wahrer DC, Schiripo TA, Jorczak MM, et al. Destabilization of CHK2 by a missense mutation associated with Li-Fraumeni Syndrome. Cancer Res 2001; 61:8062-7.
22. Zhang J, Willers H, Feng Z, Ghosh JC, Kim S, Weaver DT, et al. Chk2 phosphorylation of BRCA1 regulates DNA double-strand break repair. Mol Cell Biol 2004; 24:708-18.
23. Celeste A, Fernandez-Capetillo O, Kruhlak MJ, Pilch DR, Staudt DW, Lee A, et al. Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. Nat Cell Biol 2003; 5:675-9.
24. Fernandez-Capetillo O, Mahadevaiah SK, Celeste A, Romanienko PJ, Camerini-Otero RD, Bonner WM, et al. H2AX is required for chromatin remodeling and inactivation of sex chromosomes in male mouse meiosis. Dev Cell 2003; 4:497-508.
25. Bassing CH, Chua KF, Sekiguchi J, Suh H, Whitlow SR, Fleming JC, et al. Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX. Proc Natl Acad Sci USA 2002; 99:8173-8.
26. Scully R, Chen J, Ochs RL, Keegan K, Hoekstra M, Feunteun J, et al. Dynamic changes of BRCA1 subnuclear location and phosphorylation state are initiated by DNA damage. Cell 1997; 90:425-35.
27. Scully R, Anderson SF, Chao DM, Ye L, Young RA, Livingston DM, et al. BRCA1 is a component of the RNA polymerase II holoenzyme. Proc Natl Acad Sci USA 1997; 94:5605-10.
28. Krum SA, Miranda GA, Lin C, Lane TF. BRCA1 associates with processive RNA polymerase II. J Biol Chem 2003; 278:52012-20.
29. Anderson SF, Schlegal BP, Nakajima T, Wolpin ES, Parvin JD. BRCA1 protein is linked to the RNA polymerase II holoenzyme complex via RNA helicase A. Nature Genetics 1998; 19:254-6.
30. Schlegel BP, Starita LM, Parvin JD. Overexpression of a protein fragment of RNA helicase A causes inhibition of endogenous BRCA1 function and defects in ploidy and cytokinesis in mammary epithelial cells. Oncogene 2003; 22:983-91.
31. Cabart P, Chew HK, Murphy S. BRCA1 cooperates with NUFIP and P-TEFb to activate transcription by RNA polymerase II. Oncogene 2004; Epub ahead of print.
32. Ramanathan Y, Rajpara SM, Reza SM, Lees E, Shuman S, Mathews MB, et al. Three RNA polymerase II carboxyl-terminal domain kinases display distinct substrate preferences. J Biol Chem 2001; 276:10913-20.
33. Starita LM, Parvin JD. The multiple nuclear functions of BRCA1: Transcription, ubiquitination and DNA repair. Curr Opin Cell Biol 2003; 15:345-50.
34. Schlegel BP, Green VJ, Ladias JA, Parvin JD. BRCA1 interaction with RNA polymerase II reveals a role for hRPB2 and hRPB10α in activated transcription. Proc Natl Acad Sci USA 2000; 97:3148-53.
35. Moynahan ME, Chiu JW, Koller BH, Jasin M. BRCA1 controls homology-directed DNA repair. Molecular Cell 1999; 4:511-8.
36. Scully R, Livingston DM. In search of the tumour-suppressor functions of BRCA1 and BRCA2. Nature 2000; 408:429-32.
37. Deng CX. Roles of BRCA1 in centrosome duplication. Oncogene 2002; 21:6222-7.
38. Bochar DA, Shiekhattar R. BRCA1 is associated with a human SWI/SNF-related complex: Linking chromatin remodeling to breast cancer. Cell 2000; 102:257-65.
39. Ye Q, Hu YF, Zhong Nye AC, Belmont AS, Li R. BRCA1-induced large-scale chromatin unfolding and allele-specific effects of cancer-predisposing mutations. J Biol Chem 2001; 155:911-21.
40. Ganesan S, Silver DP, Greenberg RA, Avni D, Drapkin R, Miron A, et al. BRCA1 supports XIST RNA concentration on the inactive X chromosome. Cell 2002; 111:393-405.
41. Hashizume R, Fukuda M, Maeda I, Yabuki Y, Ogata H, Ohta T. The ring heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a brest cancer-derived mutation. J Biol Chem 2001; 276:14537-40.
42. Ruffner H, Joazeiro CA, Hemmati D, Hunter T, Verma IM. Cancer-predisposing mutations within the RING domain of BRCA1: Loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. Proc Natl Acad Sci USA 2001; 98:5134-9.
43. Mallery DL, Vandenberg CJ, Hiom K. Activation of the E3 ligase function of the BRCA1/BARD1 complex by polyubiquitin chains. EMBO J 2002; 21:6755-62.
44. Brzovic PS, Keeffe JR, Nishikawa H, Miyamoto K, Fox IIIrd D, Fukuda M, et al. Binding and recognition in the assembly of an active BRCA1/BARD1 ubiquitin-ligase complex. Proc Natl Acad Sci USA 2003; 100:5646-51.
45. Xu B, O'Donnell AH, Kim ST, Kastan MB. Phosphorylation of serine 1387 in Brca1 is specifically required for the atm-mediated S-phase checkpoint after ionizing irradiation. Cancer Res 2002; 62:4588-91.
46. Krum SA, Womack JE, Lane TF. Bovine BRCA1 shows classic responses to genotoxic stress but low in vitro transcriptional activation activity. Oncogene 2003; 22:6032-44.
47. Monteiro AN. Participation of BRCA1 in the DNA repair response via transcription. Cancer Biol Ther 2002; 1:187-8.
48. El-Deiry WS. Transactivation of repair genes by BRCA1. Cancer Biol Ther 2002; 1:490-1.
49. Chapman MS, Verma IM. Transcriptional activation by BRCA1. Nature 1996; 382:678-9.
50. Monteiro ANA, August A, Hanafusa H. Evidence for a transcriptional activation function of BRCA1 C-terminal region. Proc Natl Acad Sci USA 1996; 93:13595-9.
51. Billack B, Monteiro AN. Methods to classify BRCA1 variants of uncertain clinical significance: The more the merrier. Cancer Biol Ther 2004; 3:458-9.
52. Chiba N, Parvin JD. The BRCA1 and BARD1 association with the RNA polymerase II holoenzyme. Cancer Res 2002; 62:4222-8.
53. Monteiro AN. BRCA1: Exploring the links to transcription. Trends Biochem Sci 2000; 25:469-74.
54. Fan W, Jin S, Tong T, Zhao H, Fan F, Antinore MJ, et al. BRCA1 regulates GADD45 through its interactions with the OCT-1 and CAAT motifs. J Biol Chem 2002; 277:8061-7.
55. 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; 97:1020-5.
56. Fan S, Wang J, Yuan R, Ma Y, Meng Q, Erdos MR, et al. BRCA1 inhibition of estrogen receptor signaling in transfected cells. Science 1999; 284:1354-6.
57. Zheng L, Pan H, Li S, Flesken-Nikitin A, Chen PL, Boyer TG, et al. Sequence-specific transcriptional corepressor function for BRCA1 through a novel zinc finger protein, ZBRK1. Mol Cell 2000; 6:757-68.
58. Baker KM, Wei G, Schaffner AE, Ostrowski MC. Ets-2 and components of mammalian SWI/SNF form a repressor complex that negatively regulates the BRCA1 promoter. J Biol Chem 2003; 278:17876-84.
59. Peng H, Zheng L, Lee WH, JJ R, Rauscher IIIrd FJ. A common DNA-binding site for SZF1 and the BRCA1-associated zinc finger protein, ZBRK1. Cancer Res 2002; 62:3773-81.
60. Kleiman FE, Manley JL. The BARD1-CstF-50 interaction links mRNA 3' end formation to DNA damage and tumor suppression. Cell 2001; 104:743-53.
61. Kleiman FE, Manley JL. Functional interaction of BRCA1-associated BARD1 with polyadenylation factor CstF-50. Science 1999; 285:1576-9.
62. Harkin DP, Bean JM, Miklos D, Song YH, Truong VB, Englert C, et al. Induction of GADD45 and JNK/SAPK-dependent apoptosis following inducible expression of BRCA1. Cell 1999; 97:575-86.
63. Welcsh PL, Lee MK, Gonzalez-Hernandez RM, Black DJ, Mahadevappa M, Swisher EM, et al. BRCA1 transcriptionally regulates genes involved in breast tumorigenesis. Proc Natl Acad Sci USA 2002; 99:7560-5.
64. Jin S, Zhao H, Fan F, Blanck P, Fan W, Colchagie AB, et al. BRCA1 activation of the GADD45 promoter. Oncogene 2000; 19:4050-7.
65. Mullan PB, McWilliams S, Quinn J, Andrews H, Gilmore P, McCabe N, et al. Uncovering BRCA1-regulated signalling pathways by microarray-based expression profiling. Biochem Soc Trans 2001; 29:678-83.
66. Chen Y, Lee W-H, Chew HK. Emerging roles of BRCA1 in transcriptional regulation and DNA repair. J Cell Physiol 1999; 181:385-92.
67. Ma J, Ptashne M. A new class of yeast transcriptional activators. Cell 1987; 51:113-9.
68. Roberts SG, Ha I, Maldonado E, Reinberg D, Green MR. Interaction between an acidic activator and transcription factor TFIIB is required for transcriptional activation. Nature 1993; 363:741-4.
69. Benuck ML, Li Z, Childs G. Mutations that increase acidity enhance the transcriptional activity of the glutamine-rich activation domain in stage-specific activator protein. J Biol Chem 1999; 274:25419-25.
70. Ruffner H, Verma IM. BRCA1 is a cell cycle-regulated nuclear phosphoprotein. Proc Natl Acad Sci USA 1997; 94:7138-43.
71. Chodosh LA. Expression of BRCA1 and BRCA2 in normal and neoplastic cells. J Mammary Gland Biol Neoplasia 1998; 3:389-402.
72. Chen Y, Farmer AA, Chen CF, Jones DC, Chen PL, Lee WH. BRCA1 is a 220-kDa nuclear phosphoprotein that is expressed and phosphorylated in a cell cycle-dependent manner. Cancer Res 1996; 56:3168-72.
73. Gudas JM, Li T, Nguyen H, Jensen D, Rauscher IIIrd FJ, Cowan KH. Cell cycle regulation of BRCA1 messenger RNA in human breast epithelial cells. Cell Growth Differ 1996; 7:717-23.
74. Rajan JV, Wang M, Marquis ST, Chodosh LA. Brca2 is coordinately regulated with Brca1 during proliferation and differentiation in mammary epithelial cells. Proc Natl Acad Sci USA 1996; 93:13078-83.
75. Vaughn JP, Davis PL, Jarboe MD, Huper G, Evans AC, Wiseman RW, et al. BRCA1 expression is induced before DNA synthesis in both normal and tumor-derived breast cells. Cell Growth Differ 1996; 7:711-5.
76. Arizti P, Fang L, Park I, Yin Y, Solomon E, Ouchi T, et al. Tumor suppressor p53 is required to modulate BRCA1 expression. Mol Cell Biol 2000; 20:7450-9.
77. Lane TF, Lin C, Brown MA, Solomon E, Leder P. Gene replacement with the human BRCA1 locus: Tissue specific expression and rescue of embryonic lethality in mice. Oncogene 2000; 19:4085-90.
78. Chandler J, Hohenstein P, Swing D, Tessarollo L, Sharan S. Human *BRCA1* gene rescues the embryonic lethality of Brca1 mutant mice. Genesis 2001; 29:72-7.
79. Somasundaram K, Zhang H, Zeng Y-X, Houvras Y, Peng Y, Zhang H, et al. Arrest of the cell cycle by the tumour-suppressor BRCA1 requires the CDK-inhibitor $p21^{WAF1/CiP1}$. Nature 1997; 389:187-90.
80. Williamson EA, Dadmanesh F, Koeffler HP. BRCA1 transactivates the cyclin-dependent kinase inhibitor p27Kip1. Oncogene 2002; 21:3199-206.
81. MacLachlan TK, Takimoto R, El-Deiry WS. BRCA1 directs a selective p53-dependent transcriptional response towards growth arrest and DNA repair targets. Mol Cell Biol 2002; 22:4280-92.

82. Lane TF, Deng CX, 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; 9:2712-22.
83. Marquis ST, Rajan JV, Wynshaw-Boris A, Xu J, Yin G-Y, Abel KJ, et al. The developmental pattern of Brca1 expression implies a role in differentiation of the breast and other tissue. Nat Genet 1995; 11:17-26.
84. Zhang H, Somasundaram K, Peng Y, Tian H, Bi D, Weber BL, et al. BRCA1 physically associates with p53 and stimulates its transcriptional activity. Oncogene 1998; 16:1713-21.
85. Somasundaram K, MacLachlan TK, Burns TF, Sgagias M, Cowan KH, Weber BL, et al. BRCA1 signals ARF-dependent stabilization and coactivation of p53. Oncogene 1999; 18:6605-14.
86. MacLachlan TK, Somasundaram K, Sgagias M, Shifman Y, Muschel RJ, Cowan KH, et al. BRCA1 effects on the cell cycle and the DNA damage response are linked to altered gene expression. J Biol Chem 2000; 275:2777-85.
87. Aprelikova O, Pace AJ, Fang B, Koller BH, Liu ET. BRCA1 is a selective coactivator of 14-3-3σ gene transcription in mouse embryonic stem cells. J Biol Chem 2001; 276:25647-50.
88. Paull TT, Rogakou EP, Yamazaki V, Kirchgessner CU, Gellert M, Bonner WM. A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. Current Biology 2000; 10:886-95.
89. Xu X, Aprelikova O, Moens P, Deng CX, Furth PA. Impaired meiotic DNA-damage repair and lack of crossing-over during spermatogenesis in BRCA1 full-length isoform deficient mice. Development 2003; 130:2001-12.
90. Kim ST, Xu B, Kastan MB. Involvement of the cohesin protein, Smc1, in Atm-dependent and independent responses to DNA damage. Genes Dev 2002; 16:560-70.
91. Rodriguez M, Yu X, Chen J, Songyang Z. Phosphopeptide binding specificities of BRCA1 COOH-terminal (BRCT) domains. J Biol Chem 2003; 278:52914-8.
92. Manke IA, Lowery DM, Nguyen A, Yaffe MB. BRCT repeats as phosphopeptide-binding modules involved in protein targeting. Science 2003; 302:636-9.
93. Yu X, Chini CC, He M, Mer G, Chen J. The BRCT domain is a phospho-protein binding domain. Science 2003; 302:639-42.
94. Clapperton JA, Manke IA, Lowery DM, Ho T, Haire LF, Yaffe MB, et al. Structure and mechanism of BRCA1 BRCT domain recognition of phosphorylated BACH1 with implications for cancer. Nat Struct Mol Biol 2004; 11:512-8.
95. Xu B, Kim S, Kastan MB. Involvement of Brca1 in S-phase and G_2-phase checkpoints after ionizing irradiation. Mol Cell Biol 2001; 21:3445–50.
96. Xu B, Kim ST, Lim DS, Kastan MB. Two molecularly distinct G_2/M checkpoints are induced by ionizing irradiation. Mol Cell Biol 2002; 22:1049–59.
97. Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DCR, Shannon KE, et al. Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. Science 1999; 286:2528-31.
98. Meijers-Heijboer H, van den Ouweland A, Klijn J, Wasielewski M, de Snoo A, Oldenburg R, et al. Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. Nat Genet 2002; 31:55-9.