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Cancer Letters xx (2005) 1–11
CANCER Letters
www.elsevier.com/locate/canlet

Mini-review

BRCA1 regulation of transcription

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Received 18 April 2005; accepted 24 April 2005

Abstract

BRCA1, a tumor suppressor gene on chromosome 17q21, was identified in 1994 based on its linkage to hereditary breast and ovarian cancer syndromes. The BRCA1 gene encodes a 220 kDa nuclear phosphoprotein. Studies aimed at elucidating the mechanisms of its tumor suppressor activity have revealed, in part, that BRCA1 participates in the DNA damage response and acts to maintain the integrity of the genome. This activity is generic and does not account for the propensity of *BRCA1* mutation carriers to develop specific tumor types rather than a broad spectrum of cancers. In addition to genome maintenance, BRCA1 has been found to broadly regulate gene transcription, even though it is not itself a sequence-specific DNA-binding transcription factor. The ability of BRCA1 to function as a coregulator of transcription may underlie some of its tumor suppressor activity and may explain the tissue-specific nature of this activity. This review will focus on how BRCA1 selectively regulates transcription and how this regulatory function may relate to tumor suppression.

Keywords: Breast cancer susceptibility gene 1 (BRCA1); Transcription; RNA polymerase II; BARD1; Estrogen receptor (ER-α); ZBRK1

Abbreviations AD1, activation domain 1 of BRCA1; AR, androgen receptor; ATF1, activating transcription factor 1; ATM, ataxia-telangiectase mutated protein; ATR, ATM and rad3-related protein; BACH1, BRCA1-associated C-terminal helicase 1 (also called BRCA1-interacting protein 1, BRIP1); BAP1, BRCA1-associated protein 1; BARD1, BRCA1-associated RING domain 1 protein; BRCA1, breast cancer susceptibility gene 1 protein product; BRCA2, breast cancer susceptibility gene 2 protein product; BRCT, BRCA1 C-terminal domain; c-Abl, v-abl Abelson murine leukemia viral oncogene homolog 1; CAK, cyclin dependent kinase (CDK) activating kinase; CBP, CREB (cyclic AMP response element binding protein)-binding protein; CHK2, CHK2 checkpoint homolog (S. pombe); COACTIV, coactivator; COBRA1, cofactor of BRCA1 (also called negative elongation factor B, NELF-B); CtBP, C-terminal binding protein; CtIP, C-terminal interacting protein; ER-α, estrogen receptor-alpha; GST, glutathione sulfotransferase; HDAC1/2, histone deaceylase-1/2; hTERT, human telomerase reverse transcriptase (catalytic subunit of telomerase holoenzyme); IGF1R, insulin-like growth factor 1 (IGF1) receptor; IIO, RNA polymerase II, 220 kDa subunit (also called POLR2A); LMO4, LIM domain only 4 protein; LXCXE, consensus retinoblastoma family binding motif; NBS1, Nijmegen breakage syndrome 1 protein (p95); NES, nuclear export signal; NFE2L2, nuclear factor (erythroid derived 2)-like 2 (antioxidant response transcription factor); NLS1, nuclear localization signal 1; NUFIP, nuclear FRMP (fragile X mental retardation protein)-interacting protein; P-TEFb, positive transcription elongation factor b (also called cyclin T2, CCNT2); RB1, retinoblastoma susceptibility gene 1 protein product; RbAp46/48, RB1 associated proteins 46 and 48; RHA, RNA helicase A; RNA Pol II, RNA polymerase II holoenzyme; STAT1, signal transducer and activator of transcription 1; SWI/SNF, an ATP-dependent chromatin remodeling complex; TAD, C-terminal transcriptional activation domain of BRCA1; TF, transcription factor; TRAP220, thyroid hormone receptor-associated protein complex 220 kDa component (also DRIP205); TSS, transcription start site; ZBRK1, zinc finger and BRCA1-interacting protein with a KRAB domain 1.

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0304-3835/$ - see front matter © 2005 Published by Elsevier Ireland Ltd.
doi:10.1016/j.canlet.2005.04.037

1. Introduction

Women who inherit a mutation of the *BRCA1* gene are at high risk for the development of breast and ovarian cancers, with the relative risk of these tumor types dependent upon the site of the mutation [1–3]. In addition to breast and ovarian cancers, *BRCA1* mutation carriers are at increased risk for several other tumor types, including cancers of the pancreas, uterus, cervix, and prostate (especially in men younger than age 65) [4,5]. The predilection of *BRCA1* carriers for specific tumor types rather than a broad spectrum of cancers must be considered in understanding the function of the *BRCA1* gene. The finding that *BRCA1*-linked breast and ovarian cancers nearly always exhibit loss of the wild-type *BRCA1* allele [6,7] suggests that it functions as a tumor suppressor, consistent with the Knudsen two-hit hypothesis [8]. Thus, in terms of inheritance of cancer risk, *BRCA1* mutations act as an autosomal dominant with high penetrance; but in terms of cancer etiology, *BRCA1* acts recessively.

In the past 10 years, much has been learned about the function of the BRCA1 protein, based initially on sequence homology of several domains and subsequently on experiments in which BRCA1 was over-expressed, under-expressed, deleted, or mutated (reviewed in [9]). Early research revealed that BRCA1 contains a RING domain (a module that mediates protein interactions and exhibits an E3 ubiquitin ligase activity) at its N-terminus (amino acids 20–64) and a C-terminal acidic transcriptional activation domain (TAD, amino acids 1560–1863) [1,10]. A second transactivation domain (designated AD1, amino acids 1293–1560) can independently activate transcription or synergistically stimulate transcription with the C-terminal TAD [11] (see Fig. 1). The AD1 transcriptional activity of BRCA1 depends upon an interaction with Jun family proteins and upon the presence of the JunB protein. Numerous studies have identified roles for BRCA1 in regulation of the DNA damage response, genome integrity, cell cycle progression, apoptosis susceptibility, and transcription. We will review the evidence that BRCA1 regulates transcription and examine how this may relate to BRCA1 function and the tissue-specific nature of BRCA1 tumor suppression.

2. BRCA1 interaction with basal transcriptional machinery

Mammalian gene transcription is a complex process involving several high molecular weight

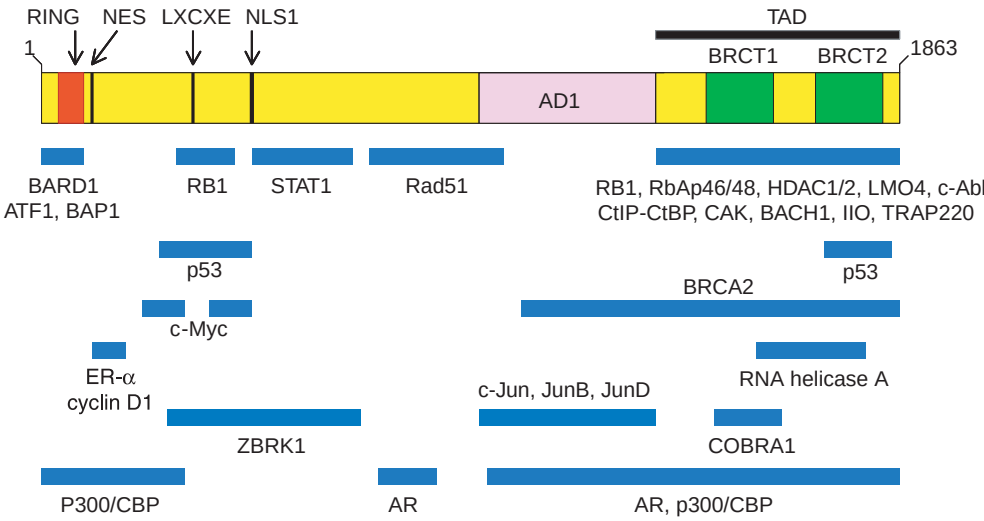

Fig. 1. BRCA1 domain structure and sites of protein interactions. The approximate locations of various protein-binding sites are shown with respect to the domain structure of BRCA1.

protein complexes that carry out distinct functions [12]. Transcription is initiated through the RNA polymerase II (pol II) complex, a multi-subunit enzyme that includes general transcription factors and is assembled at the site of a minimal promoter (the TATA box or related sequences) upstream of the transcription start site. In addition to general transcription factors, gene-specific transcription factors modulate transcription through the assembly of coactivator or corepressor complexes usually located upstream of the TATA box. Transcriptional coregulators mediate stimulatory or inhibitory contacts between gene-specific transcription factors and the general transcriptional machinery. Several studies have identified BRCA1 within the RNA pol II complex, suggesting that it plays a role in the regulation of transcription [13–16]. BRCA1 is linked to RNA pol II, in part, through RNA helicase A, an enzyme that unwinds duplex RNA and DNA [14]. It was also found that BRCA1 binds preferentially to RNA pol II complexes containing a polyphosphorylated (catalytically active) p220 subunit [15] and that BRCA1 regulates the phosphorylation state of p220 through the CDK-activating kinase (CAK) [17]. BRCA1 also interacts and cooperates with two other proteins, NUFIP and P-TEFb (a positive elongation factor) to stimulate RNA pol II activity in a cyclin T1-dependent fashion [18].

The BRCA1-associated RING domain protein (BARD1), which binds to the BRCA1 N-terminal RING domain [19], was also identified in the RNA pol II complex [20]. The BRCA1:BARD1 heterodimer exhibits ubiquitin ligase activity [21–23], suggesting it may mediate ubiquitination events within the pol II complex. The precise function of BRCA1 in the RNA pol II complex is unclear. According to one model, the BRCA1: BARD1 complex within the pol II enzyme is activated at sites of transcriptional blockage due to DNA damage, leading to ubiquitination and degradation of components of the pol II enzyme, binding of BRCA1 to the damaged DNA, and recruitment of DNA repair factors [24] (Fig. 2). The

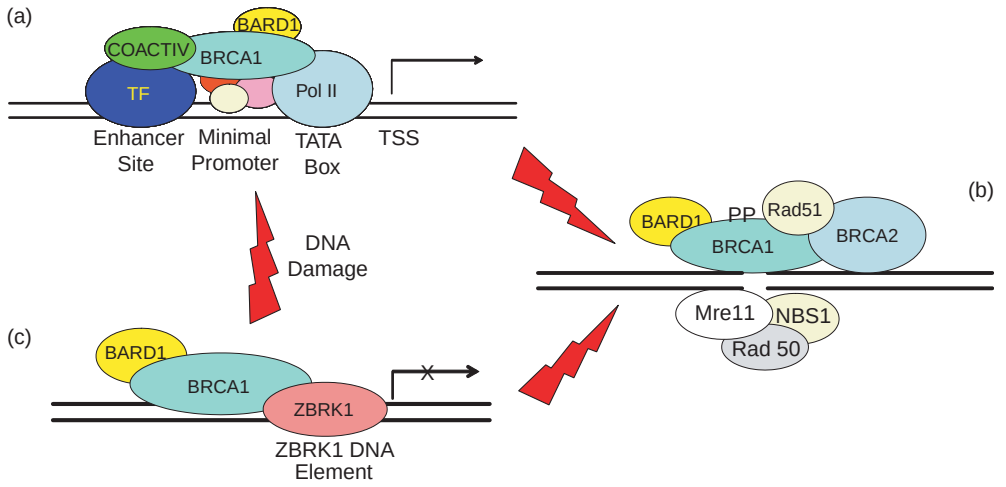

Fig. 2. BRCA1-mediated transcription in the absence vs presence of DNA damage. Panel a illustrates the potential role of BRCA1 as a coregulator of transcription, by linking a sequence-specific DNA binding transcription factor (TF) complex with the basal transcriptional machinery (RNA polymerase II). In one model, DNA damage causes BRCA1 phosphorylation (mediated by ATM, ATR, CHK2, and other kinases), activates the E3 ubiquitin ligase activity of the BRCA1/BARD1 dimer within the RNA Pol II complex, causing degradation of components of this complex and release of BRCA1 from the complex. The phosphorylated BRCA1 is then free to assemble a DNA repair complex at sites of damaged DNA (e.g. double strand breaks; panel b). In panel c, BRCA1 acts as a corepressor for ZBRK1, a repressor that functions to maintain low basal expression of DNA damage-inducible genes (e.g. Gadd45α) through binding to specific type of response element within the regulatory regions of these genes. DNA damage causes the ubiquitin-mediated degradation of ZBRK1, which releases BRCA1 to function within DNA repair complexes (panel b). Alternatively, the DNA damage-induced loss of ZBRK1 may allow BRCA1 to now function as a transcriptional coactivator for the previously repressed gene (panel a). See text for details and references.

BRCA1:BARD1 complex may suppress RNA processing in response to DNA damage, since DNA damage induces association of BRCA1/BARD1 with the RNA polyadenylation factor CstF and inhibition of mRNA polyadenylation [25].

BRCA1 is also present in a SWI/SNF-like chromatin-remodeling complex [26]. Thus, BRCA1 interacted directly with BRG1, an ATP-dependent SWI/SNF subunit; and a dominant negative BRG1 blocked the ability of BRCA1 to stimulate p53-mediated transcription (see below). In another study, BRCA1 was found to mediate a large-scale chromatin-unfolding activity through three domains, activation domain 1 (AD1) (see below) and the two C-terminal BRCA1 repeats (BRCTs) [27]. This unfolding activity did not involve histone acetylation and was mediated through the BRCA1-dependent recruitment of a cofactor of BRCA1 (COBRA1). COBRA1, also called NELF-B, is a component of the NELF (negative elongation factor) complex [28] that binds to estrogen receptor-alpha (ER-α) and represses its activity [29]. This interaction may contribute to BRCA1-mediated repression of ER-α activity (see below). A mediator (TRAP/DRIP) complex interacts with RNA pol II and regulates its activity, in part, through recruitment of chromatin-modifying cofactors [30]. This complex stimulates the transcriptional activity of nuclear receptors such as peroxisome proliferator activated receptors, thyroid hormone receptor, and others. BRCA1 may contribute to this mediator function through an interaction between the TRAP220 subunit and the BRCA1 BRCT domain [31].

Finally, the interaction of BRCA1 with various relatively generic transcriptional regulatory proteins may also contribute to its transcriptional regulatory activity, although the consequences of these interactions are not well defined. These proteins include general transcriptional coactivators (p300 and CBP) [32,33], the retinoblastoma susceptibility protein RB1 [34–36], RB1 binding proteins (RbAp46 and RbAp48), histone deacetylases (HDAC1 and HDAC2) [35], C-terminal interacting protein (CtIP) (which can recruit the repressor CtBP) [37], and the LIM-only protein LMO4, a repressor [38]. Most of these proteins interact with the C-terminus of BRCA1 (Fig. 1).

3. BRCA1 modulation of sequence-specific DNA binding transcription factors

While it has not been demonstrated that BRCA1 can interact directly with a specific sequence within undamaged DNA, it has been established that can bind to various sequence-specific DNA binding transcription factors to stimulate or inhibit transcription. Thus, BRCA1 may function as a selective coregulator, to drive transcription in specific directions. An example of this function is the BRCA1 interaction with tumor suppressor protein p53, which is mediated through sites in the N- and C-termini of BRCA1 [39–41]. BRCA1 both stabilizes p53 and stimulates its transcriptional activity, as demonstrated by an increase in p53-mediated activation of p53-responsive promoters [39–42]. Interestingly, the BRCA1 stabilization of p53 appears to induce a subset of p53-regulated genes involved in DNA repair and cell cycle arrest but *not* in apoptosis [43,44], suggesting that the BRCA1: p53 interaction may influence the 'cell fate' decision in the setting of DNA damage.

The transcription factor STAT1 transduces the cellular responses to interferon-γ (IFN-γ). It was found that a physical interaction between BRCA1 (amino acids 502–802) and the C-terminal activation domain of STAT1 induced a subset of IFN-γ responsive genes [45]. In addition to the synergy with IFN-γ in inducing gene expression, BRCA1 also potentiated IFN-γ mediated apoptosis [46]. Further evidence of a role for BRCA1 in immune regulation was the finding that BRCA1 binds to the p65/RelA subunit of NF-κB and stimulates the tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) induced transcription of NF-κB target promoters in an NF-κB dependent fashion [47]. While the significance of these findings for tumor suppression is not yet clear, we note that IFN-γ is thought to participate in a tumor immunosurveillance system [48].

Relative to cell cycle regulation, BRCA1 can stimulate the promoter activity and expression of growth inhibitory genes: e.g. $p21^{WAF1/Cip1}$, Gadd45α, and $p27^{Kip1}$ [49–53]. While p21 and Gadd45α are p53-regulated genes, BRCA1 up-regulated expression of these genes in p53 mutant cell lines, suggesting p53-independent and p53-dependent mechanisms of gene regulation. Thus, BRCA1 induction of Gadd45α

was mediated through the OCT-1 and CAAT motifs in the Gadd45α promoter; and BRCA1 was found to interact with transcription factors that bind to these motifs, Oct-1 and NF-YA, respectively [54]. Both BRCA1 and Gadd45α participate in the enforcement of a DNA damage-activated G2/M cell cycle checkpoint [55–57], but the dependence of BRCA1 on Gadd45α for activation of this checkpoint is unclear. BRCA1 also participates in another G2/M checkpoint that does not require Gadd45α or DNA damage, the decatenation checkpoint [58].

One transcriptional function of BRCA1 that may relate to the finding that *BRCA1* carriers develop specific types of cancer is the regulation of steroid hormone-dependent transcription. BRCA1 interacts directly with the estrogen (ER-α) and androgen (AR) receptors and represses ER-α [59–61] while stimulating AR activity [62,63]. These findings correlate with the epidemiologic data showing that *BRCA1* mutations confer an increased risk for several estrogen-responsive tumor types (breast, uterine, and cervical cancers) and an androgen-responsive tumor (prostate cancer) [4,5]. The role of BRCA1 in endocrine-responsive cancers was reviewed earlier [64,65] and will not be discussed exhaustively. However, we will point out several interesting facets of this subject.

Repression of ER-α signaling is mediated by a direct interaction between BRCA1 and ER-α that has been mapped at high resolution [60,61] and is dependent upon the coactivator p300 [33]. Overexpression of BRCA1 inhibits induction of 90% of the E2-inducible transcriptosome, including genes regulated by the membrane-localized ER-α, causing the inhibition of E2-stimulated cell growth [66,67]. The endogenous levels of BRCA1 are sufficient to inhibit ER-α activity, as evidenced by the findings that deletion of the BRCA1 gene or knockdown of BRCA1 expression allow activation of ER-α in the absence of ligand and further stimulate ER-α activity in the presence of estrogen [68,69]. Moreover, knockdown of endogenous BRCA1 by RNA interference enhanced the agonistic activity of the partial anti-estrogen Tamoxifen, a finding that correlates with the observation that administration of Tamoxifen to mice harboring a mammary-targeted deletion of Brca1 exon 11 and a heterozygous p53 mutation stimulated mammary tumorigenesis [69]. BRCA1 was found to interact with AR and its coactivator GRIP1 (glucocorticoid receptor interacting protein 1) and to stimulate AR activity. It also stimulated AR-dependent expression of $p21^{WAF1/Cip1}$ and apoptosis in PC-3 prostate cancer cells, suggesting that it may activate a subset of AR target genes relating to growth inhibition [62,63].

Recently, BRCA1 was found to interact with a zinc finger and KRAB domain protein, ZBRK1, that represses the Gadd45α gene through interaction with a specific DNA sequence of intron 3 [70]. This ZBRK1 response element also occurs in the regulatory regions of other DNA damage-inducible genes. ZBRK1 is degraded by the ubiquitin-proteasome pathway in response to DNA damage [71]. Further studies identify a BRCA1-dependent C-terminal repression domain in ZBRK1 that mediates BRCA1 binding, oligomerization of ZBRK1, and DNA binding [72,73]. These findings must be reconciled with studies cited above showing that BRCA1 *stimulates* Gadd45α promoter activity. One possibility is that the DNA damage-induced degradation of ZBRK1 unmasks BRCA1-mediated transcriptional activation of Gadd45α and other DNA damage-inducible genes. Here, over-expression of BRCA1 favors the BRCA1-dependent promoter activation over its augmentation of ZBRK1-mediated repression. Alternatively, over-expression of BRCA1 may indirectly derepress ZBRK1-repressed promoters by causing the removal of ZBRK1 or other corepressors from the promoter.

In addition to stimulating tumor suppressor and growth inhibitory pathways, BRCA1 can inhibit oncogene activity. Thus, BRCA1 binds to c-Myc, an oncoprotein that is amplified or over-expressed in many cancer types, and inhibit its transcriptional and transforming activity [74–76]. BRCA1 inhibits expression of the telomerase reverse transcriptase (TERT) and telomerase enzymatic activity, in part, by inhibiting c-Myc mediated transactivation of TERT [74,76]. The interaction of BRCA1 with oncogenic signaling pathways may be bi-directional. Thus, heregulin, through activation of the serine/threonine protein kinase c-Akt, can inactivate BRCA1 by causing mislocalization of BRCA1 to the cytoplasm or down-regulation of its expression [77,78].

Besides BARD1, the BRCA1 RING domain binds to ATF1, a member of the cAMP response element

(CRE)-binding protein/activating transcription factor (CREB/ATF) family, and stimulates CRE-dependent transcription [79]. Members of this family regulate expression of genes involved in development, stress response (including UV radiation damage and oxidative stress), cell survival, and apoptosis [80]. BRCA1 interacts with the four and one-half LIM only protein 2 (FHL2) and stimulates its transactivation function [81]. The significance of this observation is unclear, but FHL2 is a regulator of the Wnt/β-catenin signaling [82], a proliferative pathway that is activated in various types of cancers. BRCA1 has been linked to other transcriptional pathways, some of which are associated with a well-defined functional activity (see Section 4).

4. Regulation of the transcriptosome

Several functions of BRCA1 have been identified through DNA microarray analyses. For example, analyses of cancer cells over-expressing BRCA1 and mouse embryo fibroblasts with mutant *Brca1* revealed that BRCA1 stimulates the expression of various genes involved in the antioxidant response, including glutathione-S-transferases, oxidoreductases, and other antioxidant proteins [83]. Consistent with these findings, BRCA1 potentiated the activity of the antioxidant transcription factor NFE2L2 (Nrf2) and protected cells against oxidative stress [83]. Microarray analysis of *Brca1*-deficient mouse embryonic stem cells revealed down-regulation of a major G2/M checkpoint gene 14-3-3σ and a corresponding defect in the activation of this checkpoint in response to ionizing radiation [84]. The ability of BRCA1 to induce a subset of p53-regulated genes and to inhibit most of the E2-inducible transcriptosome was described earlier.

Although it has been difficult to identify unequivocal phenotypic alterations in cells heterozygous for a *BRCA1* mutation, microarray analysis following irradiation has identified gene expression differences in *BRCA1* heterozygous relative to control fibroblasts [85]. Several studies have examined the gene expression profiles of *BRCA1*-associated cancers. Two such studies identified distinct patterns of gene expression in *BRCA1* mutant vs *BRCA2* mutant vs sporadic breast [86] and ovarian [87] cancers. Gene expression analysis has allowed the categorization of *BRCA1*-mutant breast cancers into the basal phenotype [88]. This phenotype occurs in about 15% of breast cancers and is characterized by ER-α and HER2/Neu negativity and the expression of cytokeratins found in basal epithelial cells. It was also possible to identify gene expression 'signatures' that distinguish *BRCA1* and *BRCA2*-related cancers from familial non-*BRCA*-linked breast cancers [89]. These findings suggest that BRCA1 deficiency results in a pattern of transcriptional alterations that represent a unique molecular pathogenesis for *BRCA1* mutant cancers.

This hypothesis is consistent with the observation of a specific pathologic phenotype of *BRCA1* mutant breast cancers, characterized by a high incidence of p53 mutations, ER-α and progesterone receptor negativity, the absence of HER2/Neu or cyclin D1 amplification (both of which are common in sporadic cancers), frequent amplification of the proto-oncogene c-Myb, a high nuclear grade, and a high incidence of chromosomal aberrations (reviewed in [65]).

5. Role of BRCA1 in DNA repair: transcriptional vs non-transcriptional functions

BRCA1 transcriptionally regulates genes involved in the DNA damage response. It also transduces a DNA damage signal that stimulates DNA repair and cell cycle arrest. BRCA1 interacts with DNA repair proteins to form a BRCA1-associated genome surveillance complex (BASC) that contains proteins involved in mismatch repair (MSH2, MSH6, and MLH1), DNA double-strand break (DSB) repair (ATM and the Rad50-Mre11-p95^{NBS1} (RMN) complex), DNA replication (RFC), and recombination (BLM) [90]. In response to certain forms of DNA damage, BRCA1 is hyperphosphorylated and relocalizes to DNA replication complexes containing proliferating cell nuclear antigen (PCNA, a DNA processivity factor), Rad51 (a DNA recombinase), BARD1, and BRCA2 (which is also implicated in DNA repair and recombination) [91]. Subsequent studies showed that in response to different forms of DNA damage, BRCA1 is phosphorylated by ATM (ataxia-telangiectasia mutated), ATR (ATM and

Rad3-related), and/or the CHK2 kinase (reviewed in [9] and [92]). In response to DSBs, BRCA1 is phosphorylated by ATM and CHK2 and mediates homology-directed repair in cooperation with Rad51, BRCA2, and the RMN complex [92–95]. The ability of BRCA1 to form DSB-induced repair complexes is defective in the absence of histone H2AX, suggesting that H2AX facilitates assembly of BRCA1 repair complexes on chromatin and that BRCA1 participates in chromatin unfolding that is required for the accessibility of damaged DNA to repair proteins [27,96,97].

The precise contribution of transcriptional vs non-transcriptional events to DNA repair is unclear. The assembly of a DNA repair complex at sites of DNA damage is an extremely rapid BRCA1-dependent function that does not involve transcription. As noted earlier, it was hypothesized that DNA damage-induced activation of the BRCA1/BARD1 ubiquitin ligase activity may mediate degradation of components of the RNA pol II holoenzyme, which allows relocalization of BRCA1 from transcriptional to DNA repair related complexes [24]. Release of BRCA1 for DNA repair duties may also be mediated by DNA damage-induced degradation of the repressor ZBRK1 [71]. Fig. 2 shows a hypothetical scheme of BRCA1-related events that may transpire in response to DNA damage.

6. Conclusions and unanswered questions

Inherited mutations of *BRCA1*, a tumor suppressor, confer a high risk for breast cancer, ovarian cancer, and a few other tumor types. Functional activities attributed to BRCA1 include regulation of the DNA damage response, cell cycle progression, apoptosis, steroid hormone responses, and the maintenance of genomic integrity. The ability of BRCA1 to regulate gene transcription probably contributes to most or all of these activities. Several paradigms through which BRCA1 may regulate transcription have been identified. Thus, BRCA1 can function as a coregulator by binding to sequence-specific DNA binding transcription factors and stimulating (e.g. p53) or inhibiting (e.g. ER-α) their activity. BRCA1 is also a component of the RNA Pol II complex, suggesting that it may either modulate basal transcription and/or stimulate or inhibit enhancer driven transcription by mediating stimulatory or inhibitory contacts between sequence-specific transcription factors and the basal transcription factor. Some of the BRCA1-regulated transcriptional pathways that may contribute to tumor suppression are shown in Fig. 3. The predilection of *BRCA1* carriers to develop a specific set of tumor types suggests tissue-specific activities that may be due, in part, to tissue-specific transcriptional functions. Although a number of different BRCA1-

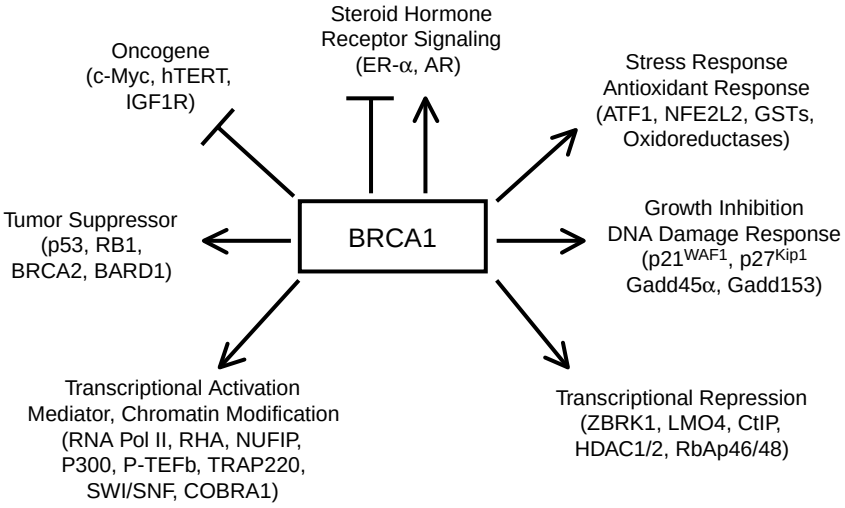

Fig. 3. Summary of transcription regulatory functions of BRCA1. This figure shows the range of transcriptional pathways and individual target genes that may be regulated, in part, through BRCA1. Some of these pathways may contribute to the tumor suppressor function of BRCA1. They may also relate to normal functions of the BRCA1 protein that are not directly linked to tumorigenesis.

mediated functions have been identified, the task remains to determine which of these are essential for its tumor suppressor activity, which are dispensible, and which may relate to normal functions of BRCA1 not linked to carcinogenesis.

Acknowledgements

Dr Rosen has been supported, in part, by United States Public Health Service grants R01-CA82599, R01-CA104546, and RO1-ES09169 and by a grant from the Susan G. Komen Breast Cancer Foundation (BCTR0201295).

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