

Review

Regulation of *BRCA1* expression and its relationship to sporadic breast cancerChristopher R Mueller¹ and Calvin D Roskelley²¹Cancer Research Laboratories, Queen's University, Kingston, Ontario, Canada²Department of Anatomy, University of British Columbia, Vancouver, British Columbia, CanadaCorresponding author: Christopher R Mueller (e-mail: muellerc@post.queensu.ca)

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Abstract

Germ-line mutations in the *BRCA1* tumour suppressor gene contribute to familial breast tumour formation, but there is no evidence for direct mutation of the *BRCA1* gene in the sporadic form of the disease. In contrast, decreased expression of the *BRCA1* gene has been shown to be common in sporadic tumours, and the magnitude of the decrease correlates with disease progression. *BRCA1* expression is also tightly regulated during normal breast development. Determining how these developmental regulators of *BRCA1* expression are co-opted during breast tumorigenesis could lead to a better understanding of sporadic breast cancer aetiology and the generation of novel therapeutic strategies aimed at preventing sporadic breast tumour progression.

Keywords: *BRCA1*, breast cancer, development, transcription, sporadic

Introduction

The cloning of the *BRCA1* gene, and later the *BRCA2* gene, were major breakthroughs in our understanding of breast cancer [1]. Both were isolated through traditional positional cloning approaches [2,3] and are probably responsible for the majority of familial breast cancer cases. Our understanding of the multifunctional protein products of the *BRCA1* gene and why defects in the gene result in breast and ovarian cancer is still in a state of flux. It appears to be involved in multiple processes in the nucleus, such as repair and recombination, as well as transcriptional regulation (for review [4]). Recently, *BRCA1* was reported to have the ability to bind directly to DNA, particularly branched structures [5], and regulates the key G2/M checkpoint protein Chk1 [6]. *BRCA1* has also been shown to induce large-scale chromatin unfolding [7], which may play a role in both transcription and repair. While the specific roles of *BRCA1* continue to be clarified, it is clear that functional *BRCA1* protein is required to prevent breast transformation. Selective inactivation of *BRCA1* in the breast results in blunted ductal development, breast hyperplasia and tumour formation [8].

Soon after its isolation, it became apparent that somatic mutations in the *BRCA1* gene do not play a major role in sporadic breast tumour development. Specifically, no coding region mutations have been found [9] and noncoding regulatory region mutations are extremely rare [10,11]. Because sporadic tumours account for more than 90% of the total breast cancer burden, these findings were initially a major disappointment. However, a key study suggested that *BRCA1* expression levels are reduced in sporadic tumours [12]. RNase A protection assays were used to examine mRNA levels in 19 sporadic tumour samples, and a consistent decrease in *BRCA1* mRNA levels was observed in invasive tumours as compared with either paired normal tissue or ductal carcinoma *in situ*. The significance of this finding was enhanced when it was shown that decreasing *BRCA1* levels increased the growth of tumour cells and that expression of *BRCA1* led to growth arrest and apoptosis. Since then a number of other studies have confirmed this correlation and extended the observation.

One of the largest studies used 142 sporadic tumour samples and found a general correlation between grade

CREB = cAMP responsive element binding; ECM = extracellular matrix; GABP = GA-binding protein; HLH = helix-loop-helix; ID4 = inhibitor of differentiation 4; LOH = loss of heterozygosity.

and decreased BRCA1 expression, with 20% of tumours showing complete loss of BRCA1 protein [13]. High-grade tumours generally have the lowest levels of BRCA1 [14] and exhibit higher proliferation rates [15]. Correlations between low BRCA1 levels, tumour grade, metastasis and prognostic markers such as oestrogen receptor status have also been made [16,17]. An inverse association with elevated c-erbB-2 levels has also been reported [18]. A recent study of 175 patients indicated an adverse effect on disease-free survival of low levels of BRCA1 [19]. Similar studies have correlated decreased BRCA1 levels with development of sporadic ovarian cancer [20]. This would be in accordance with the common function of BRCA1 as a tumour suppressor in both of these tissues. Overall, these findings suggest a model in which loss of BRCA1 activity, either by germ-line mutations or by down-regulation of gene expression, leads to tumour formation in appropriate target tissues.

The data generated from the clinical studies cited above strongly suggest that decreased BRCA1 expression contributes to sporadic breast tumour progression. The functional importance of such a decrease has been confirmed by experimental manipulations of BRCA1 expression in cultured cells [12]. Regulation of protein stability may affect BRCA1 levels in certain circumstances [21], but this aspect of BRCA1 regulation is outside the scope of the present review. Therefore, here we address the most likely mechanisms for permanent decrease in BRCA1 expression in sporadic breast tumours. We then review the factors and signalling pathways that may be responsible for the modulation of BRCA1 expression in various developmental contexts. Finally, we discuss how disruption of these pathways could contribute to development of sporadic breast cancer, and whether transcription factors that regulate BRCA1 are candidate sporadic breast cancer genes.

Loss of heterozygosity and methylation of the BRCA1 promoter

There are several potential mechanisms that could lead to a permanent decrease in BRCA1 levels in sporadic breast tumours. One such mechanism is allelic loss of heterozygosity (LOH), in the absence of mutations (Fig. 1a), which has been observed at the *BRCA1* locus in conjunction with a decrease in the steady-state levels of BRCA1 protein [22,23]. Normally, LOH alone would not contribute to a decrease in gene expression because the net result is two identical but genetically normal alleles. If one allele was subject to inactivation by methylation (see below), then LOH would lead to the presence of two methylated alleles and loss of expression. The observation of allele-specific expression of BRCA1 (Fig. 1b), in which examination of BRCA1 mRNA indicated that only one of the two genes was being expressed [24], could represent preliminary methylation of one allele. Currently, however, no

studies have clearly demonstrated a functional link between LOH and promoter methylation.

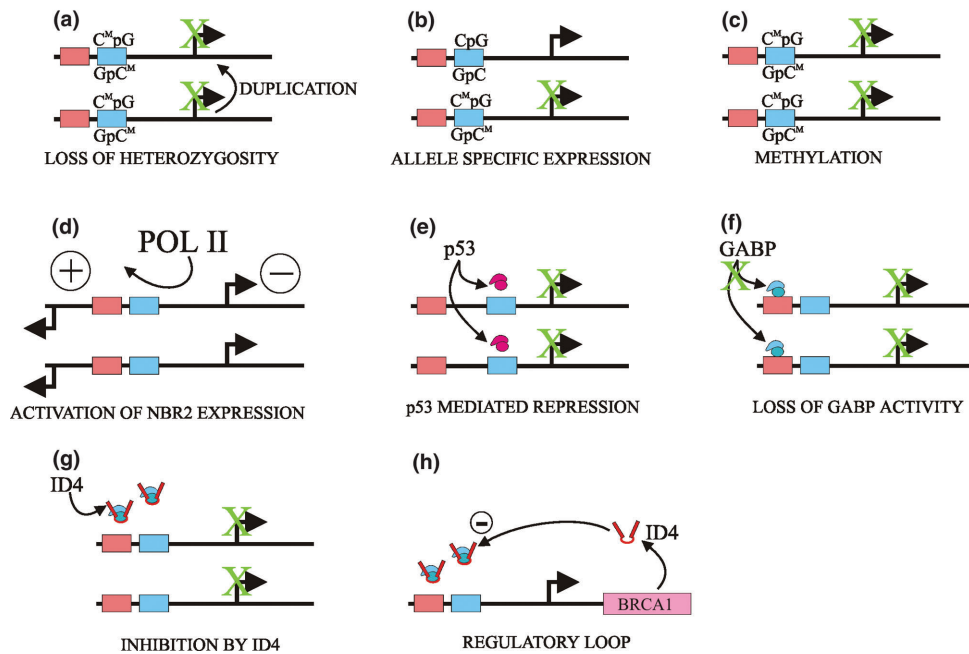
Methylation, which can be permanent and heritable, is associated with decreased tumour suppressor gene expression in a number of disease contexts [25]. Examination of methylation patterns in the *BRCA1* promoter region indicated that preferential methylation of some sites occurs (Fig. 1c), apparently only in tumours [26–30]. Moreover, promoter methylation may result in very low levels of BRCA1 [31]. Methylation is associated with greater chromatin compaction and lack of accessibility [32]. As with other systems, there is a question of whether methylation itself causes the initial decrease in BRCA1 expression or whether an initial transient decrease in transcriptional activity could have been the impetus for establishment of permanent methylation. Methylation appears to be a significant factor in BRCA1 regulation only in a small proportion of sporadic breast tumours [33]. Therefore, it is necessary to postulate that promoter silencing occurs by some other means in the majority of cases.

The BRCA1 promoter

The structure of the human *BRCA1* locus is complicated (Fig. 2) in that it includes a partial duplication that results in a pseudo-*BRCA1* gene and two distinct genes (i.e. *NBR1* and *NBR2*) [34,35] which are divergently transcribed from both the pseudo-gene and *BRCA1*. This structure is not found in the mouse [36], which may be partly responsible for the differences between human and mouse breast cancer models (i.e. heterozygous *brca1* knockout mice do not develop breast cancer, whereas human carriers do). The *BRCA1* and *NBR2* genes are separated by little more than 200 base pairs, and their transcription is divergent (Fig. 3). This region serves as the primary proximal promoter for the major breast-specific transcription start site located in exon 1a of *BRCA1* [37]. Several initial studies of the *BRCA1* promoter have been reported that have identified a possible upstream repressor element [38] and a positive element that is located some 200 base pairs upstream of this start site [39]. This region was able to direct expression in either the *BRCA1* or *NBR2* direction, suggesting that it functions as a bidirectional transcriptional element [40]. There is some evidence to suggest that the *NBR2* and *BRCA1* genes may actually be reciprocally regulated. In a number of cell lines, higher levels of *NBR2* expression are correlated with low *BRCA1* levels [41]. This may reflect competition between the promoters for RNA polymerase II; it also suggests that activation of the *NBR2* promoter could lead to decreased *BRCA1* expression (Fig. 1d).

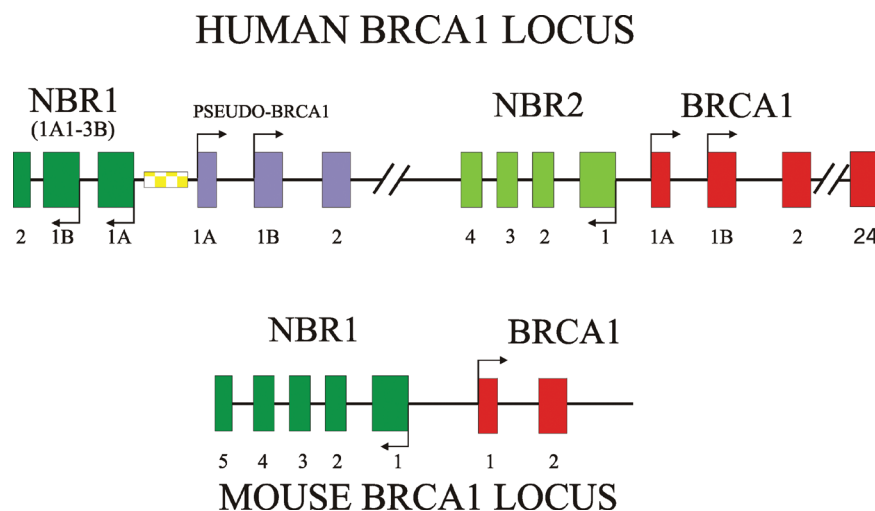
In culture *BRCA1* expression increases in replicating cells [42], and stimulation of *BRCA1* expression by oestrogen [43,44] appears to result from the effect of this hormone on breast cell growth [45]. This proliferation-mediated

Figure 1



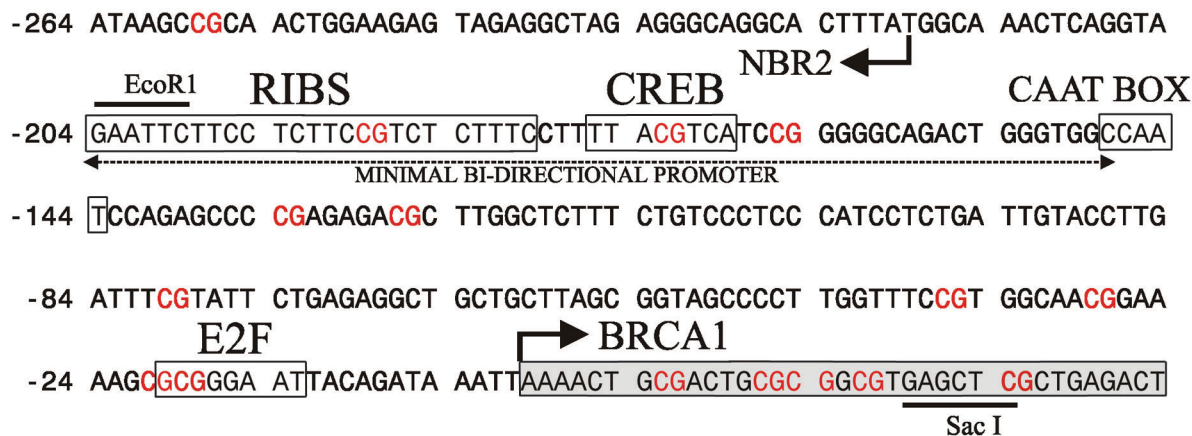
Potential mechanisms for the reducing BRCA1 expression. Schematics of the two BRCA1 alleles are shown. (a) Endo-reduplication of a methylated allele would lead to complete loss of expression. (b) Expression from only one allele could be indicative of methylation of one of the promoters. (c) Methylation of both promoters through an unidentified mechanism would lead to a dramatic decrease in expression. (d) Increased NBR2 expression could lead to decreased BRCA1 expression. (e) p53 could inhibit factors such as E2F. (f) Loss of DNA binding by the GABP- α/β complex leads to decreased expression. (g) ID4 expression leads to the down regulation of the BRCA1 promoter, presumably through interaction with a transcription factor that normally regulates the promoter. (h) BRCA1 and ID4 levels appear to be coregulated.

Figure 2



The structure of the human and mouse BRCA1 loci are shown. Transcription initiation sites are indicated by arrows. The general exon structure is indicated by the variously shaded boxes. The patterned box indicates the location of an acidic ribosomal phosphoprotein P1 pseudocopy [34], which appears to have been inserted into the region between NBR1 and the BRCA1 pseudo-gene. The BRCA1 gene appears to have two alternative first exons, although the 1A exon appears to be used much more frequently.

Figure 3



The sequence of the promoter region between the NBR2 and the BRCA1 genes is indicated. The transcriptional start sites are indicated by the left and right pointing arrows for the respective genes. Binding sites for transcription factors identified as being important for promoter activity are boxed. The minimal bidirectional promoter [40] is indicated by the dashed line. The first exon of the BRCA1 gene is indicated by the shaded box. CpG dinucleotides that are potentially methylated are shown in red. The location of an EcoRI and Sac I are indicated by thick lines.

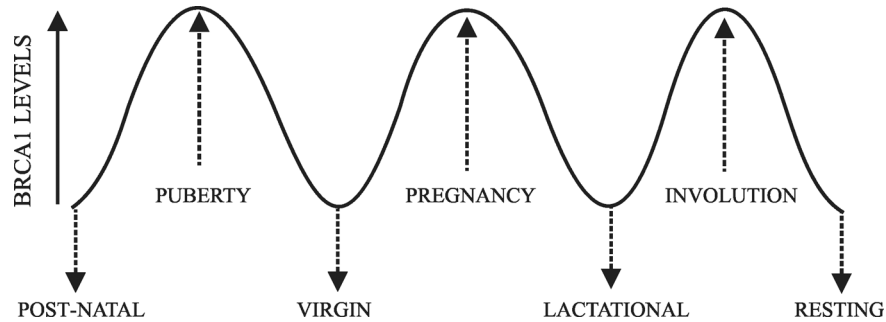
increase may act through an E2F site that has been characterized in the proximal promoter (Fig. 3) [46]. Conversely, p53 may inhibit BRCA1 expression by preventing E2F binding to this site (Fig. 1e) [47]. A negative regulatory site within the first intron has also been characterized, but the associated proteins have not been identified [48]. Studies of the methylation pattern of the promoter suggested that a potential cAMP-responsive element binding (CREB) protein site might be important for promoter function (Fig. 3) [26]. It was subsequently shown that this CREB site is a strong positive transcriptional element [49], although the site does not confer cAMP responsiveness on the promoter, as might be expected [50].

One transcription factor binding site has been identified that is critical for BRCA1 expression, but in a cell-line-specific manner. This RIBS element (Fig. 3) has been shown to bind the multisubunit *ets* protein GA-binding protein (GABP)- α/β and acts as an activator in MCF-7 cells but not in T47-D cells [51]. This site is within the positive element previously described [39] and is included within the minimal bidirectional promoter element (Fig. 3) [40]. GABP- α and GABP- β subunits have been shown to be present in both cell lines, but its DNA-binding activity was reduced in T47-D cells [51]. It is possible that mutation in one of the GABP subunits itself, or disruption of an upstream regulator, is responsible for loss of GABP-dependent positive transcriptional activity in these cells (Fig. 1f). An elegant selection screening system using a library of ribozymes was also able to identify a potential regulator of BRCA1 expression [52]. Inactivation of the helix-loop-helix (HLH) protein inhibitor of differentiation 4 (ID4) was able to increase expression of BRCA1 in MCF-7 cells (Fig. 1g). There appears to be a reciprocal relation-

ship between the levels of ID4 and BRCA1, and ID4 expression may be regulated by oestrogen or by oestrogen's ability to induce growth. An interesting correlation between ID4 and BRCA1 expression has also come from efforts to identify transcriptionally regulated targets of BRCA1. Microarray analysis revealed that ID4 mRNA levels were increased upon BRCA1 induction [53]. Furthermore, there was a general positive correlation between BRCA1 and ID4 levels in a variety of breast and ovarian tumours [53]. This is in contrast to the inverse relationship found by artificial manipulation of ID4 levels [51]. This suggests that ID4 may be involved in a developmental regulatory loop that is disrupted during sporadic tumourigenesis (Fig. 1h).

Developmental regulation of BRCA1 expression in mammary gland

The developmental regulation of BRCA1 expression has been most extensively studied in the mouse [54–58]. During early embryogenesis BRCA1 (denoted *brca1* in the mouse) is expressed diffusely in all three germ layers. Later, during foetal development, *brca1* expression is highest in condensed tissues undergoing considerable proliferative, differentiative and morphogenic changes. These include the following: spinal ganglia and the neuroepithelium of the eye (neuroectoderm derived); somites and cartilage (mesoderm derived); liver and lung (endoderm derived); lense of the eye; and basal proliferative layers of the epidermis (ectodermally derived). Although expression of *brca1* in the embryonic mammary gland has not been described, it is reasonable to suggest that its expression might parallel that seen in the epidermis because the glandular rudiment first forms as an invagination of this tissue.

Figure 4

Brca1 expression in the mouse mammary gland. The line represents relative brca1 expression levels and is regulated in periodic waves during mammary gland development. Levels are low in the quiescent periods before puberty, in the virgin adult gland and in the resting gland. Levels are also low during lactational terminal differentiation. Levels are high during the proliferative periods at puberty and pregnancy. Levels are also high during the apoptotic period of involution after weaning.

At birth the mouse mammary gland consists of a small number of epithelial ducts that radiate from the nipple a short distance into the underlying stromal fat pad. Before puberty the mammary ducts elongate into the fat pad at a rate that is similar to the overall growth of the animal. Further development of the gland takes place at puberty and during adult cycles of pregnancy, lactation and involution [59]. At puberty the ends of the ducts swell to form the proliferative terminal end-buds, which strongly express brca1 and initiate rapid ductal branching and elongation to the margins of the fat pad. After puberty, brca1 levels fall in the quiescent adult virgin gland. With the onset of pregnancy the terminal end-buds proliferate, branch and expand to form epithelial alveolar sacs. Throughout pregnancy brca1 is expressed at high levels in these developing alveoli. Later, during lactation, brca1 levels fall gradually as alveolar cells cease proliferating, terminally differentiate and produce milk. After weaning, milk production ceases, brca1 levels rise, and within a few days a massive apoptotic event is triggered. This drives involution and returns the gland to its quiescent state, whereupon brca1 levels once again fall. Therefore, in the postnatal mammary gland epithelial brca1 expression is induced in periodic waves that correspond to defined developmental periods in which intense proliferation, morphogenesis and/or apoptosis take place (Fig. 4). Given these observations, it is not unreasonable to expect that the molecular regulators of these developmental changes might also regulate brca1 expression.

Molecular regulators of mammary development

Mammary development is directed by at least three classes of molecular regulators. The first class consists of the systemic hormones, which are produced at distant sites but act within the mammary gland to initiate developmental change. Three such hormones, namely oestrogen

[43,44], progesterone [60] and prolactin [61], have all been shown to upregulate BRCA1 expression. In culture, this upregulation is manifested under conditions that are conducive to nondifferentiative cellular proliferation [45]. *In vivo*, this would occur during pregnancy, when all three hormones contribute to the massive proliferative burst before alveolar morphogenesis and differentiation [62]. Interestingly, when prolactin later acts as a lactogenic, differentiative agent, it no longer upregulates BRCA1 expression [56].

The second class of regulators consists of soluble microenvironmental factors. These molecules are produced within the mammary tissues proper and they act over very short distances, often in a paracrine manner, to mediate functional interactions between the stromal and epithelial compartments of the gland [63]. Two such factors are hepatocyte growth factor and neuregulin, which mediate ductal branching and alveolar morphogenesis, respectively [64]. The ability of these agents to regulate BRCA1 expression in an appropriate developmental context in culture has not yet been determined. However, neuregulin, which signals through the erbB tyrosine kinase receptors, is active *in vivo* at the transition to nonproliferative, terminal differentiation when BRCA1 levels fall dramatically. Interestingly, one of the known regulators of the BRCA1 promoter, namely GABP- α/β [51], is a downstream mediator of neuregulin signalling in nerve cells [65].

The third class of mammary-specific regulators are extracellular matrix (ECM) proteins. For example, laminin, a glycoprotein that is located in the specialized basement membrane ECM, binds to cell surface integrin receptors and generates signals within the mammary epithelium that are required for lactational differentiation [66,67]. Furthermore, a metalloproteinase-mediated destruction of laminin triggers alveolar cell apoptosis during involution [68,69].

Therefore, an intact laminin-containing basement membrane may be a negative regulator of BRCA1 expression in differentiated mammary epithelial cells, and that this negative regulation could be released during involution [70].

Conclusion

BRCA1 expression is clearly modulated by developmental regulators in the normal mammary gland. At the same time, decreased BRCA1 expression is an early event in the process of breast transformation, and progresses with the degree of malignancy. Given the known molecular functions of BRCA1, it is reasonable to assume that such decreases lead to a relaxation in genome surveillance. This relaxation would not be deleterious during normal development because BRCA1 levels are lowest when mammary epithelial cells are either quiescent or terminally differentiated. However, changes in BRCA1 levels may also play an active role in regulating developmental processes, such as inducing postlactational regression. A number of transcription factors have been identified that regulate BRCA1 expression. Positive regulators such as E2F and GABP- α/β , as well as repressors such as ID4, combine to produce the varied developmental expression that is observed *in vivo*. These factors presumably lie at the end of a variety of signalling pathways that are initiated by molecular regulators of developmental change in the mammary gland. Importantly, many of the signals initiated by these regulators have themselves been implicated in mammary oncogenesis. Thus, we propose that mutations in the transcription factors themselves or alterations to the developmentally regulated signalling pathways that modulate their activity contribute to the inappropriate repression of BRCA1 expression. Obviously, these two possibilities are not mutually exclusive and could be overlapping.

One key regulator of terminal differentiation and BRCA1 repression is the basement membrane ECM. Cross-talk occurs between basement membrane-mediated signalling, prolactin signalling and morphogenic erbB receptor signalling during development [67,66,71,72]. This would ensure that in quiescent cells the ECM signal prevents BRCA1 induction, but during growth this blockade is lifted, and prolactin and erbB signals, which are proliferative outside the context of morphogenesis and lactational differentiation, result in BRCA1 induction. Tumour cell survival presumably requires abrogation of this BRCA1 induction. There are observational and experimental data that indirectly support this tentative hypothesis. First, in sporadic tumours BRCA1 levels are most affected after progression from ductal carcinoma *in situ* [12], a time when the basement membrane is undergoing chronic remodelling. Second, mammary-specific expression of an activated metalloprotease that remodels the basement membrane [73,74] causes a morphogenic and stochastic tumour formation in transgenic mice that is reminiscent of the phenotype observed in the targeted BRCA1 knockout

[8]. Third, a developmentally inappropriate integrin signal switching that contributes to breast tumour progression causes erbB signalling to become oncogenic [75,76]. Fourth, oncogenic erbB signalling and BRCA1 expression are inversely correlated in tumour cells [18]. Finally, forced expression of ID1, which is closely related to the BRCA1 repressor ID4, causes mammary epithelial cells to proliferate rather than differentiate when they are exposed to a basement membrane ECM [77,78].

Transcription factors that regulate the BRCA1 promoter directly modulate the production of this tumour suppressor, and thus their loss or gain of function will have the same functional consequences as inactivation of BRCA1 itself. This raises the question of whether these factors act as sporadic breast cancer genes. Optimal BRCA1 expression requires the concerted action of a number of transcription factors, so that inactivation of any one protein will affect BRCA1 levels. Given a random distribution of sporadic mutations in the genome, it may be more likely that one of a number of different transcription factor genes would be a target, rather than the BRCA1 gene itself. In addition, loss of activity from a single allele of a transcription factor gene may be sufficient to decrease BRCA1 expression significantly, with functional consequences. This may explain why sporadic mutations in BRCA1 have not been observed, because these may be rarer events than transcription factor gene mutations. Furthermore, as discussed above, loss of transcriptional regulators of BRCA1 may not require actual mutational events. Disruption of the signalling pathways that regulate BRCA1 expression could have long-term consequences for the associated transcriptional regulators, which persist through subsequent progression events. In some cases, BRCA1 transcription is reduced sufficiently for methylation of the promoter to occur, creating a self-sustaining repressed state. It may be possible to restore BRCA1 expression in tumours. This could be achieved by the use of demethylating agents such as deazacytadine, inducing alternative pathways where mutations have blocked other factors, or restoring developmental signals that have been co-opted. Induction of BRCA1 expression has been shown to induce apoptosis or reduce growth in tumour cells [12], and offers an alternative therapeutic approach that may be tumour specific.

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