REVIEW ARTICLE

BRCA1: A REVIEW OF STRUCTURE AND PUTATIVE FUNCTIONS

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SUMMARY

BRCA1 is a complex gene implicated in familial breast and ovarian cancer. Although it is almost certainly a tumour suppressor, it is also essential for the normal growth and development of embryonic cells. BRCA1 is probably involved in DNA damage and repair, in cell cycle regulation, and in differentiation of cells. It remains to be established whether all these functions are subserved by single mechanism or pathway. Since the cloning of BRCA1 in 1994, much has been learned about the function of the gene. However, a great deal more still has to be uncovered. The size of the protein coded by the *BRCA1* gene and the variety of transcripts argues for a complexity of function and regulation that will provide intellectual and technical challenges for years to come.

KEY WORDS BRCA1 Cancer genetics Molecular structure Function DNA repair
Differentiation Cell cycle

BRCA1 DISCOVERY AND CANCER CONNECTIONS

BRCA1 has the characteristics of a tumour suppressor gene responsible for early onset breast and ovarian cancer in susceptible families. The gene, which maps to 17q21 (Bowcock *et al.*, 1993; Kelsell *et al.*, 1993; Neuhausen *et al.*, 1994), was cloned in 1994 (Miki *et al.*, 1994), and mutations were found to segregate with a cancer predisposing phenotype in BRCA1 linked families (Futreal *et al.*, 1994; Miki *et al.*, 1994). Familial cancers are thought to represent approximately 5–10% of all breast cancer cases, while BRCA1 is believed to account for approximately 40 to 50% of all familial cases (Szabo and King, 1995). BRCA2 (Wooster *et al.*, 1994), p53 (Lane, 1994), PTEN (Li *et al.*, 1997) and other, undiscovered, cancer predisposing genes are responsible for the rest.

The BRCA1 cDNA obtained by Miki and colleagues (1994) coded for a single long open reading frame of 1863 amino acids, with an amino terminal Cys₃-His-Cys₄ zinc-finger domain. The gene consists of 22 coding exons, distributed over 100kb of genomic DNA. Northern analysis revealed a single 7.8kb *BRCA1* transcript, abundant in testis and thymus, but also present in breast and ovary.

Families carrying BRCA1 germline mutations include those with an increased susceptibility to breast cancer alone, or to breast and ovarian cancer together (Szabo and

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King, 1995; Easton *et al.* 1993), and carriers have a slightly increased rate of both prostate and colon cancers (Ford *et al.* 1994). Male breast cancer is rarely a feature of these families. Breast and ovarian cancer susceptibility is inherited as an autosomal dominant trait, with high penetrance in affected families (Newman *et al.*, 1988). The lifetime risk for mutation carriers is generally quoted as 80–90% for breast cancer and nearly 50% for ovarian cancer (Ford *et al.*, 1994) though more recent data suggest the figures may be somewhat lower (Levy-Lahad *et al.*, 1997). The majority of disease causing mutations identified to date result in protein truncation, with only a few missense mutations that lead to amino acid substitutions (Couch *et al.*, 1996). These missense mutations cluster either to the amino terminal RING finger domain or the carboxyl terminal region (the BRCT domain discussed below), supporting the importance of these two structural elements in the function of the protein.

MOLECULAR ORGANISATION AND REGULATION

The promoter region of *BRCA1* is complex, with evidence for genetic duplication that has generated a *BRCA1* pseudogene and promoter shared with the *NBR2* gene (Barker *et al.*, 1996). Two separate promoters exist for *BRCA1*, generating transcripts that differ in the first exon (part of the untranslated region) (Xu *et al.*, 1995). Both promoters contain oestrogen response elements, suggesting that *BRCA1* may be involved in hormone signalling pathways (Xu *et al.*, 1997). *BRCA1* expression is responsive to oestrogen, although evidence suggests that this may be an indirect and nonspecific effect, related to changes in the cell cycle rather than to interactions between *BRCA1* control pathways and oestrogen (Spillman and Bowcock, 1996; Marks *et al.*, 1997; Gudas *et al.*, 1995). Variable methylation has also been proposed as a control mechanism for *BRCA1* in sporadic cancers (Dobrovic and Simpfendorfer, 1997), as have various post-translational modifications, such as proteolytic cleavage (Jensen *et al.*, 1996a), phosphorylation (Chen *et al.*, 1995; Chen *et al.*, 1996b; Zhang *et al.*, 1997; Scully *et al.*, 1997b), and differential sub-cellular localization (Chen *et al.*, 1995; Jensen *et al.*, 1996a; Thakur *et al.*, 1997; Wilson *et al.*, 1997; Wang *et al.*, 1997). Any of these may be operating independently, or several may act in concert to regulate *BRCA1* function.

A complex pattern of splicing of the mRNA was noted when *BRCA1* was first cloned (Miki *et al.*, 1994), and has been confirmed repeatedly (Xu *et al.*, 1995; Thakur *et al.*, 1997; Lu *et al.*, 1996; Wilson *et al.*, 1997; Wang *et al.*, 1997). The splice variants have yet to be assigned any biological function, although circumstantial evidence suggests they are important. It has been noted that overexpression of the full length *BRCA1* protein (i.e., the protein coded by the entire open reading frame) results in cellular toxicity, but a splice variant protein lacking most of exon 11 (*BRCA1*- Δ 11b) is not toxic (Wilson *et al.*, 1997). Furthermore, while the expression level of the full length protein was found to be similar in all cell lines tested, *BRCA1*- Δ 11b levels were decreased in the most aggressive cancer cell lines, suggesting that splice variants may modulate tumorigenicity. However, it is uncertain whether the decrease in expression of the splice variant is a cause or an effect of the increased tumorigenicity. Splice variants, normally cytosolic, become nuclear (presumably through an alternative nuclear localization pathway) when cells are serum starved (Wang *et al.*, 1997). Finally, the sheer number of reported splice variants, encompassing both the coding region and the 5' and 3' untranslated regions

(Miki *et al.*, 1994; Xu *et al.*, 1995; Thakur *et al.*, 1997; Lu *et al.*, 1996; Wilson *et al.*, 1997; Wang *et al.*, 1997), suggests that they must make significant contributions to overall BRCA1 function.

BRCA1 is not mutated in sporadic breast tumours and only rarely in sporadic ovarian cancers (Takahashi *et al.*, 1996; Foster *et al.*, 1996). Yet evidence for the involvement of BRCA1 in sporadic tumours is convincing (Cropp *et al.*, 1994; Borg *et al.*, 1994; Thompson *et al.*, 1995; Holt *et al.*, 1996; Dobrovic and Simpfendorfer, 1997). It is possible that DNA lesions, not detectable by standard mutation screening, may be responsible for the disruption of function of BRCA1 in sporadic tumours. Possible mechanisms include variable methylation (Dobrovic and Simpfendorfer, 1997), large deletion events (Puget *et al.*, 1997; Petrijbosch *et al.*, 1997), and downregulated expression (Thompson *et al.*, 1995). However, it is also possible that BRCA1 does not participate in the formation of sporadic breast cancers at all. BRCA1 has been found to have a role in embryonic development (at least in mice) (Gowen *et al.*, 1996; Haken *et al.*, 1996; Ludwig *et al.*, 1997) and in differentiation (Marquis *et al.*, 1995; Lane *et al.*, 1995; Rajan *et al.*, 1996), supporting a possible role in the very early initiation of tumorigenesis, for example, during development *in utero*.

Several different domains have been recognised in BRCA1 (Fig 1). A coding sequence for a RING finger zinc binding domain was identified in 5' end of the transcript (Miki *et al.*, 1994), as well as a carboxyl terminal BRCT (BRCA1 carboxyl terminal) domain of unknown function (Koonin *et al.*, 1996). Both regions share the highest level of conservation between species found anywhere in the coding sequence (Abel *et al.*, 1995; Sharan *et al.*, 1995; Chen *et al.*, 1996), implying some functional importance. Overall, BRCA1 shows very poor inter-species conservation. Other potentially functional regions identified in the BRCA1 coding sequence include two nuclear localization sequences (Chen *et al.*, 1996), a granin consensus sequence (Jensen *et al.*, 1996a), and a domain that interacts with Rad51 (Scully *et al.*, 1997c).

BRCA1 FUNCTION

Evidence to support the view that BRCA1 is a tumour suppressor was apparent even before the gene had been cloned. Loss of heterozygosity has been observed in both sporadic and familial tumours (Cropp *et al.*, 1994; Borg *et al.*, 1994; Cropp *et al.*, 1993; Lindblom *et al.*, 1993; Foulkes *et al.*, 1993; Kerangueven *et al.*, 1997; Sato *et al.*, 1991; Futreal *et al.*, 1992; Beckmann *et al.*, 1996; Munn *et al.*, 1996; Papp *et al.*, 1996) and, when linkage allowed determination of the disease causing allele, LOH was always found to affect the wild type allele in the familial tumours (Neuhausen and Marshall, 1994; Smith *et al.*, 1992). More concrete evidence for the importance of BRCA1 in preventing tumour growth emerged from the finding that overexpression of BRCA1 in cells in culture is lethal (Wilson *et al.*, 1997) while antisense inhibition of BRCA1 is reported to cause an increase in growth rates specific for breast and ovarian cells (Thompson *et al.*, 1995), although this has yet to be substantiated by other groups. Antisense inhibition of mouse NIH-3T3 fibroblasts increased growth rate, soft agar growth, and tumorigenicity (Rao *et al.*, 1996) further supporting a role for BRCA1 in tumour suppression.

The exact biochemical and cellular functions of BRCA1 remain elusive, as do some basic characteristics, such as the sub-cellular localization. The weight of evidence

Figure 1 - Representation of domains and interactions for BRCA1. BRCA1 is represented linearly, with exons indicated. Shaded boxes are all the domains that have been identified (named above). Below each domain its demonstrated or inferred functions are shown.

suggests that BRCA1 is localized to the nucleus (Chen *et al.*, 1995; Aprelikova *et al.*, 1996; Chen *et al.*, 1996a; Chen *et al.*, 1996b; Scully *et al.*, 1996; Shao *et al.*, 1996; Thomas *et al.*, 1996; Scully *et al.*, 1997c; Wilson *et al.*, 1997), with a nuclear punctate pattern apparent during S and M phases (Chen *et al.*, 1996b; Scully *et al.*, 1996; Scully *et al.*, 1997c). However, there is also a claim that BRCA1 may be a granin, initially targeted extracellularly and proteolytically cleaved to activate its function (Jensen *et al.*, 1996a). These extracellular protein fragments may become localized to the nucleus; however, this would be by a secondary mechanism involving transport, following binding to membrane receptors or other carrier molecules (Jensen *et al.*, 1996b).

BRCA1 probably subserves a complex set of functions in the cell that may or may not be linked to a common biochemical mechanism. Targeted disruption of *Brcal* in mice results in death *in utero*, with gross anatomical abnormalities and disruption of cell growth in *Brcal* null animals (Gowen *et al.*, 1996; Haken *et al.*, 1996; Ludwig *et al.*, 1997). The stage at which death occurs has been reported to be either before E7.5 (Haken *et al.*, 1996) or as late as E13 (Gowen *et al.*, 1996), suggesting that additional factors (such as the genetic background of the experimental strain or the precise position of the truncating mutation) may modify the effect of *Brcal* knockout. Mouse models do not shed much light on the effects of BRCA1 in normal adult mammary tissue; mice heterozygous for a *Brcal* mutation are viable and completely normal, without apparent susceptibility to any cancers. Although normal *Brcal* function is essential for early development in the mouse, it appears to be less so in humans. At least one developmentally normal individual (though with early onset breast cancer) has been reported who has inherited identical mutations (2800delAA) from both parents. (Boyd *et al.*, 1995). The data thus suggest that BRCA1 may serve functions both in development and in cancer progression. The differences between mice and humans highlight the difficulty of extrapolating from animal models to establish the function of specific genes; human BRCA1 mutation heterozygotes have greatly increased risk for breast cancer, while heterozygous mice apparently do not. These differences may have a trivial explanation (for example, mice may not live long enough to exhibit *Brcal*-associated mammary tumours) or they may reflect fundamental differences in the biology of the two species.

BRCA1 in DNA repair

The BRCT domain has been recognised as common to BRCA1, a p53 binding protein, 53BP1 and a number of other proteins (Koonin *et al.*, 1996). Further characterization of

the domain led to the identification of its presence in a considerable number of other genes, both characterized and uncharacterized (Bork *et al.*, 1997; Callebaut and Mornon, 1997). In most instances the BRCT domain has had no function directly attributed to it but it appears to participate in either DNA binding (for example in RFC1 and RAP1 (Bork *et al.*, 1997)) or in protein-protein interactions (for example, between XRCC4 and DNA Ligase IV (Critchlow *et al.*, 1997), PARP, DNA Ligase III/XRCC1 (Bork *et al.*, 1997), 53BP1 (Koonin *et al.* 1996), and BRCA1/RNA Polymerase II holoenzyme (Scully *et al.*, 1997a)). BRCT domains are thought not to participate directly in the enzymatic functions of the proteins that bear them, however, it has been suggested that enzyme activity may be controlled by the domain (for example in RAP1 (Bork *et al.*, 1997)) or that it may modify the specificity of the reaction (for example, in the TdT's) (Bork *et al.*, 1997). Such interactions have been proposed as a mechanism for recruitment of members of the DNA damage repair family to the site of repair, through BRCT domain interactions; for example XRCC4 recruitment of a suitable DNA ligase to DNA PK repair complexes (Critchlow *et al.*, 1997). The BRCT domain may be involved directly in DNA repair and metabolism since a high proportion of the characterized proteins that contain it participate in such functions (Callebaut and Mornon, 1997; Bork *et al.*, 1997; Critchlow *et al.*, 1997).

BRCA1 has been shown to have a specific interaction with Rad51 protein mediated through a BRCA1 exon 11 domain (Scully *et al.*, 1997c). Rad51, in mammals, is involved with the homologous pairing of DNA during the process of recombination and recombinational repair (Sung and Robberson, 1995; Baumann *et al.*, 1996), supporting the proposition that BRCA1 is involved in DNA repair processes. The interaction of Rad51 and BRCA1 was first identified through the observation of similar staining patterns for the two proteins (discrete nuclear punctate staining during S and M phases of the cell cycle) (Scully *et al.*, 1996; Scully *et al.*, 1997c). Although the interaction appears specific, its functional basis has not been proved. There are reports that BRCA1 undergoes dramatic changes in phosphorylation and subnuclear localization in response to DNA damage (Scully *et al.*, 1997b). The same changes in localization apply to Rad51, suggesting that they may have a physiological interaction that is triggered by DNA damage. This interaction with Rad51, however, cannot be the sole function for BRCA1; BRCA1 is highly expressed during spermiogenesis (Zabludoff *et al.*, 1996) when Rad51 expression is silenced. (Yamamoto *et al.*, 1996).

Both *Rad51* and *Brca1* nullizygous mice undergo severe disruption of embryogenesis and die *in utero*, suggesting that similar pathways may be implicated. *Brca1* and *Rad51* nullizygotes share other early developmental characteristics, including the inability of embryonic stem cells to survive in tissue culture and partial rescue of the phenotype in mice with p53 or p21^{Waf1/Cip1} null background (Lim and Hasty, 1996; Ludwig *et al.*, 1997; Hakem *et al.*, 1997). However, the phenotype is not precisely the same for both genes; *BRCA1* loss of function results in death near E7.5 (Haken *et al.*, 1996; Ludwig *et al.*, 1997) or even later at E11 (Gowen *et al.*, 1996), while *Rad51* nullizygosity results in death as early as the morula/blastocyst stage (Tsuzuki *et al.*, 1996; Lim and Hasty, 1996). For *BRCA1*, this apparent block on cell growth may apply only to fetal cells, as disruption of expression in adult cells is reported to increase cell growth (Thompson *et al.*, 1995) and tumorigenicity (Rao *et al.*, 1996).

The final evidence supporting the participation of BRCA1 in DNA damage response comes from interactions observed between BRCA1 and p53 pathways. As mentioned

above, a p53 null background partially rescues the Brca1-null lethal phenotype, extending the time of death from E7.5 up to E11 (Ludwig *et al.*, 1997; Hakem *et al.*, 1997). This suggests that BRCA1 may be responsible for DNA damage sensing and repair, so that and when its function is lost, DNA damage accumulates and p53 responsive pathways cause cellular arrest and apoptosis. Loss of p53 function in these cells then allows the cells to continue dividing with unrepaired damage until genomic stability is sufficiently compromised to result in cell death. Although this link between the pathways is tenuous, clinical observations support an association between BRCA1 associated tumorigenesis and p53. In a recent study, p53 was mutated in all the breast tumours of BRCA1 mutation carriers (Crook *et al.*, 1997), suggesting that loss of p53 function may be an absolute requirement for the progression of cancer. Furthermore, the spectrum of mutations was not as expected for breast cancers; mutations were mainly in exon 5 of p53 (the amino end of the DNA binding domain of p53), while specific mutations that are common in sporadic breast cancer were rare in BRCA1 associated cancers. In several cases, multiple mutations were identified in a single allele of p53, sometimes as many as four. This hypermutability of p53 did not appear to result from increased general mutation rates as other genes did not show increased mutability, nor did other regions of p53. The observation of multiple mutations within single alleles of p53 is curious; a single event is enough to impair p53 function, so multiple events should provide no further growth advantage to cells.

BRCA1 in Cell cycle control

In addition to p53 involvement, a recent report has shown p21^{Waf1/CIP1} activation by BRCA1 to induce a G₁ to S phase cell cycle block (Somasundaram *et al.*, 1997). This finding is consistent with observations of the cell cycle regulation of BRCA1; although expression seems maximal after the G₁/S checkpoint, it is induced in late G₁ (Chen *et al.*, 1996b; Gudas *et al.*, 1996; Vaughn *et al.*, 1996; Ruffner and Verma, 1997). The interaction with p21^{Waf1/CIP1} does suggest that a link exists between BRCA1 and cell cycle checkpoint control, implying that BRCA1 may be more than a simple DNA repair protein. It has been proposed that BRCA1 is a guardian of genomic integrity (Kinzler and Vogelstein, 1997), and such a role could explain why BRCA1 mutations are not found in sporadic cancers.

BRCA1 protein expression is closely linked to the cell cycle, both in terms of absolute level and of phosphorylation status (Chen *et al.*, 1996b; Gudas *et al.*, 1996; Vaughn *et al.*, 1996; Ruffner and Verma, 1997; Zhang *et al.*, 1997). Various cell cycle regulated and regulatory proteins, such as cdc2, cdk2, cdk4, cyclin A, cyclin B, cyclin B1, cyclin D, cyclin D1, and E2F-4, interact with BRCA1 (Chen *et al.*, 1996b; Wang *et al.*, 1997), mainly modifying the phosphorylation state. Furthermore, an association between BRCA1 and erbB/neu proteins (the EGFR family of receptor tyrosine kinases) has been proposed (Zhang *et al.*, 1997) such that activated tyrosine kinase receptors cause hypophosphorylation of BRCA1 at tyrosine residues, leading to functional inactivation of the protein. This hypophosphorylation of BRCA1 may induce G₂/M cell cycle arrest (Zhang *et al.*, 1997). The proposal for a G₂/M arrest has been supported by observations made with a BRCA1 mutant that abrogates such a checkpoint (Larson *et al.*, 1997). Several reports suggest that BRCA1 mediates a G₁/S phase checkpoint (Vaughn *et al.*, 1996; Somasundaram *et al.*, 1997). Despite the evidence for BRCA1/p53 interaction, the

G₁/S checkpoint may be activated directly through the transcriptional activation of p21^{Waf1/CIP1} (Somasundaram *et al.*, 1997) without participation of p53.

Further evidence for a role in control of the cell cycle is suggested by observations of changes in the subnuclear localization during the cell cycle. BRCA1 is detected as diffuse, weak, nuclear staining in cells in most stages of the cell cycle. However, during S and M phases, a nuclear punctate pattern appears (Scully *et al.*, 1996; Scully *et al.*, 1997c). This dramatic change in the localization is also modified by the presence of DNA damage (Scully *et al.*, 1997b), which results in the release of BRCA1 from the complexes with a concurrent change of phosphorylation state and the accumulation of BRCA1 on PCNA-containing structures. These findings seem to indicate a direct interaction between BRCA1 and damaged, replicating DNA. Evidence is strong that BRCA1 does affect the cell cycle; reduced expression is associated with highly proliferative cells *in vivo* (Sobol *et al.*, 1996) and low BRCA1 levels increase growth rates (Thompson *et al.*, 1995; Rao *et al.*, 1996); high expression of BRCA1 is toxic to cells (Holt *et al.*, 1996; Wilson *et al.*, 1997) and the effect is seen even in yeast cells (Humphrey *et al.*, 1997) which do not have any identifiable BRCA1 homologue. It has been proposed that BRCA1 is a DNA binding transcription activator (Miki *et al.*, 1994), as supported by the demonstration of transcriptional activation by GAL4/BRCA1 fusions (Chapman and Verma, 1996; Monteiro *et al.*, 1996) and the association of BRCA1 with the RNA polymerase II holoenzyme (Scully *et al.*, 1997a). The same (BRCT) domain, is both the transcriptional activator and the RNA polymerase II interacting domain, suggesting that BRCA1 may recruit the transcriptional machinery to target promoters through interaction with DNA or other proteins. Such evidence suggests a mechanism by which BRCA1 may mediate control over the cell cycle.

Development and Differentiation

BRCA1 appears to serve a role in development and differentiation. The nullizygous mouse models that have been developed show that such a function is essential for cellular survival during embryogenesis (Gowen *et al.*, 1996; Hakem *et al.*, 1996; Ludwig *et al.*, 1997; Hakem *et al.*, 1997). The protein is expressed throughout all tissues of the developing mouse, particularly those that are rapidly proliferating and undergoing differentiation (Marquis *et al.*, 1995). During pregnancy and lactation in the mouse, high levels of BRCA1 are expressed at the time of rapid, hormonally induced, growth associated with lactation and differentiation of the mammary gland (Marquis *et al.*, 1995; Lane *et al.*, 1995). Observations on the expression of BRCA1 in human spermatocytes support a corresponding role in differentiation in humans (Zabludoff *et al.*, 1996).

MEDICAL RELEVANCE OF BRCA1

Beyond screening individuals from families with high risk of cancer, BRCA1 may have some potential for use in therapy. Given the cellular toxicity accompanying over-expression of BRCA1 (Holt *et al.*, 1996; Wilson *et al.*, 1997), clinical trials have been undertaken to introduce wild type BRCA1 to ovarian tumours using a retroviral vector (Tait *et al.*, 1997). Limitations of the delivery systems available are likely, however, to restrict this approach to tumours with a large accessible surface, which therefore would exclude most breast cancers. Furthermore, if BRCA1 is fundamentally a mediator of genomic stability (Kinzler and Vogelstein, 1997), this treatment strategy may be ill-

advised. By stabilizing the tumour genome after a high proliferation rate has been established, many cells could be protected from the accumulation of lethal damage.

Quite apart from attempts at direct gene therapy, careful study of BRCA1 and tumour phenotypes may lead to improvements in traditional treatment of BRCA1 associated tumours. Mutation status of *BRCA1* has been found to correlate with several histopathological parameters that collectively determine the grade of the tumour (Goldstein *et al.*, 1987; Eisinger *et al.*, 1996; Rubin *et al.*, 1996; Lakhani *et al.*, 1997). BRCA1-associated tumours tend to be poorly differentiated and highly proliferative. Such changes are intuitively associated with a worse prognosis, however, BRCA1 tumours may be more drug-responsive than their unmutated counterparts (Spillman and Bowcock, 1996; Marks *et al.*, 1997; Phillips *et al.*, 1997). Enhanced drug sensitivity may be a result of increased genomic instability or may simply reflect higher growth rates. Further study should help to clarify this, and contribute to the development of better treatment strategies. In addition, BRCA1 status appears to influence the clinical outcome of patients (Porter *et al.*, 1993; Malone *et al.*, 1996; Marcus *et al.*, 1996; Rubin *et al.*, 1996; Brunet *et al.*, 1997; Burk, 1997; Cannistra, 1997; Johannsson *et al.*, 1997; Modan, 1997; Rubin, 1997; Whitmore, 1997). Many BRCA1-linked familial cancers evidently carry a better prognosis than their sporadic counterparts. If true, the reason is still unclear and may be a direct result of the way that BRCA1 contributes to tumorigenesis, at the molecular level, or a less direct consequence of the tumour phenotype associated with germline BRCA1 mutation.

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