ROLE OF BRCA1 AND BRCA2 GENES IN CELLULAR METABOLISM AND BREAST CANCER

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ABSTRACT

BRCA1 and BRCA2 are tumour suppressor genes, their mutations may lead to breast and ovarian cancers. It was shown that BRCA proteins are involved in a multitude of pivotal cellular processes. Both genes contribute to DNA repair and transcriptional regulation in response to DNA damage. BRCA proteins are required for maintenance of chromosomal stability, thereby protecting the genome from damage. The reasons why mutations in BRCA genes lead to the development of breast and ovarian cancers are not clearly understood. Genetic testing for BRCA mutations is expanding in clinical oncology centres worldwide. Testing may help target unaffected high-risk women for prevention and/or close surveillance and may also help affected women choose the best chemotherapy. It is important to have an understanding of the pathologic features and the natural history of BRCA-associated breast cancers in order that personalized treatments can be developed and delivered. The goals of treatment for a woman with a BRCA-associated breast cancer should be to prevent recurrence of the initial cancer and to prevent second primary breast and ovarian cancers. Women with breast cancer and a BRCA1 mutation may benefit from tailored treatments. Mutations in BRCA1 are distributed in populations throughout the world.

KEY WORDS: BRCA1; BRCA2; Mutation, Breast Cancer.

INTRODUCTION

Breast cancer is the development of cancer from breast tissue. Symptoms of breast cancer may be a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, or a red scaly patch of skin. In those with distant spread of the disease, there...
may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin (Saunders et al., 2009). Breast cancer is one of the most frequent malignancies affecting women. The cumulative life time risk of a female for the development of this disease is about 10% (Claus et al., 1991). Risk factors for developing breast cancer include obesity, lack of physical exercise, drinking alcohol, hormone replacement therapy during menopause, ionizing radiation, early age at first menstruation, and having children late (Stewart and Wild, 2014). About 5-10% of cases are due to genes inherited from a person's parents, including BRCA (BReast-CAncer susceptibility gene) 1 and BRCA2 among others. Approximately 5% of breast cancers show a familiar pattern of occurrence (Rosen et al., 2003). Discovery of the genes conferring susceptibility to familial breast cancer and determination of their functional mechanisms would considerably enhance our understanding of the etiology and progression of breast tumours. In 1990, genetic studies provided initial evidence that the risk of breast cancer in some families is linked to chromosome 17q21 (Hall et al., 1990). This 17q-associated syndrome was characterized by autosomal dominant inheritance with incomplete penetrance. In fact, loss of heterozygosity (LOH) at 17q was found in most familial breast and ovarian tumours, suggesting the involvement of tumour suppressor genes (Smith et al., 1992; Neuhausen and Marshall, 1994). In 1994, the breast-cancer susceptibility gene, BRCA1, was identified by positional cloning; subsequently, this gene has been the subject of intensive research effort (Miki et al., 1994) (Fig. 1). BRCA1 is composed of 22 coding exons distributed over 100 kb of genomic DNA. This gene encodes 1863 amino acids, and more than 200 different germline mutations associated with cancer susceptibility have been identified. Many disease-predisposing alleles of BRCA1 have loss-of-function mutations, the majority of which result in premature truncation of the protein. Because only 45% of familial breast cancers showed evidence of linkage to BRCA1, the search for a second breast cancer susceptibility gene continued. In 1995, the BRCA2 gene was identified at chromosome 13q12.3 (Wooster et al., 1994; Wooster et al., 1995) (Fig. 1). Mutations in BRCA1 and BRCA2 are not simply associated with an elevated risk of breast cancer (Rahman and Stratton, 1998). Mutation carriers also have increased susceptibility to ovarian, pancreatic, prostatic, and male breast cancers. Surprisingly, despite the inherited predisposition to cancer associated with BRCA1 and BRCA2, somatic disease-causing mutations in either of these genes are extremely rare in sporadic breast cancers (Futreal et al., 1994; Lancaster et al., 1996). Research on the functions of BRCA proteins revealed that BRCA proteins interact with a number of regulatory proteins (Table 1). Much has been learned about the structures, functions, and unique features of BRCA gene products.
Fig. 1: Features of the BRCA proteins.

(Source: Yoshida and Miki, 2004).

Table. 1: Proteins interacting with BRCA1.

<table>
<thead>
<tr>
<th>DNA repair</th>
<th>ATM, CHK2, ATR, BRCA2, RAD51, RAD50/MRE11/NBS1, BASC, PCNA, H2AX, e-Abl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcription</td>
<td>RNA polymerase II holoenzyme (RNA helicase A, RPB2, RPB10 α), HDAC1, HDAC2, E2F, CBP/p300,</td>
</tr>
<tr>
<td>Cell Cycle</td>
<td>RB, CDK2, p21, p27, BARD1</td>
</tr>
<tr>
<td>Others</td>
<td>BAP1, BIP1, BRAP2, importin α</td>
</tr>
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(Source: Yoshida and Miki, 2004).

Functions of BRCA1 and BRCA2

BRCA1 and BRCA2 are tumour suppressors which are essential for the faithful repair of double-strand DNA breaks by homologous recombination (Narod and Foulkes, 2004). However BRCA1 also participates in several other cellular functions which are important in maintaining genomic integrity, including the assembly of the mitotic spindle, centrosome duplication, cell-cycle control, and chromatin remodeling at sites of double-strand DNA breaks. The role of BRCA2 appears primarily to regulate RAD51 filament formation - this is a critical step in catalyzing strand invasion and initiating homologous recombination. Cells lacking BRCA1 or BRCA2 are unable to repair double strand breaks by homologous recombination and therefore repair proceeds through error-prone pathways, such as non-
homologous end-joining. These cells may incur mutations during strand repair and often accumulate chromosomal rearrangements during successive rounds of cell division (Narod and Foulkes, 2004). While the majority of these rearrangements result in cell death, in some cases the mutant daughter cells are stable and these lead to the emergence of a dominant cell lineage that acquires the capabilities of autonomous cell division and of metastatic potential, two of the hallmarks of cancer. BRCA1 has also been shown to be required for the activation of both S- and G2/M-phase cell-cycle arrest after DNA damage, the latter being dependent on prior phosphorylation of BRCA1 by the master checkpoint kinase ATM (ataxia telangiectasia) mutated (Xu et al., 2001). BRCA1 has been shown to interact with multiple DNA repair/recombination proteins, including Rad51, the Rad50/MRE11/Nibrin complex, Bloom’s helicase, and the Fanconi D2 protein (Yun and Hiom, 2009). Furthermore, the role for BRCA1 in transcriptional regulation and proliferation are mediated through associations with CTIP, ZBRK, p300, estrogen receptor (ER), HDAC, Rb, p53, RNA polymerase II holoenzyme, cyclin D1, c-myc, and at least one member of the Swi/Snf complex (Narod and Foulkes, 2004).

Livingston and colleagues proposed that BRCA1 protein is implicated in genetic silencing of the X-chromosome. They proposed that direct binding of BRCA1 with XIST (X-inactive specific transcript) is critical to keep the X-chromosome silent (Ganesan et al., 2002). This relationship between the BRCA1 and XIST, the main mediator of X-chromosome inactivation, remains controversial (Vincent-Salomon et al., 2007). Some have speculated that this effect of BRCA1 could be part of a broader effect on heterochromatin maintenance and/or gene regulation (Pageau et al., 2007). In support of this hypothesis, Verma and colleagues argue that no unifying framework can link all of the reported biochemical activities of BRCA1 to its tumour suppressor function and they propose that many of the tumour suppressor functions of BRCA1 are due to its maintenance of global heterochromatin integrity (Zhu et al., 2011). Although there is no consensus yet on mechanism, loss of BRCA1 or BRCA2 functions in cell lineages is a critical step in breast and ovarian carcinogenesis among women with a mutation.

**ROLE IN DNA REPAIR**

1) BRCA1

Initial evidence suggesting a role of BRCA1 in the repair of damaged DNA was derived from the observation that BRCA1 is hyperphosphorylated in response to DNA damage and
relocated to sites of replication forks marked by proliferating cell nuclear antigen (PCNA) (Scully et al., 1997; Thomas et al., 1997). In response to ionizing radiation, BRCA1 is bound and phosphorylated by an ataxia-telangiectasia mutated (ATM) kinase (Cortez et al., 1999; Gatei et al., 2000) (Fig. 1). The major target for ATM phosphorylation after ionizing radiation is Ser1387 of BRCA1. In response to ultraviolet irradiation, Ser1457 is primarily phosphorylated, mainly by ATM-related kinase (ATR) (Gatei et al., 2001). The G2/M control kinase, CHK2, has been shown to phosphorylate BRCA1 at Ser988 on exposure to ionizing radiation (Chaturvedi et al., 1999; Bell et al., 1999) (Fig. 1). Other sites of BRCA1 that are phosphorylated in response to DNA damage, such as Ser1423 and Ser1524, have been reported (Cortez et al., 1999; Gatei et al., 2000). It is likely, therefore, that BRCA1 is phosphorylated at multiple residues by different kinases after DNA damage (Fig. 2). However, how each type of phosphorylation affects the functions of BRCA1 remains obscure. Subsequent studies demonstrated the involvement of BRCA1 and BRCA2 in complexes that activate the repair of double strand breaks (DSBs) and initiate homologous recombination (HR), linking the maintenance of genomic integrity to tumour suppression.

BRCA1 and BRCA2 co-localize with Rad51 to form complexes (Scully et al., 1997; et al., 1998). Eukaryotic Rad51 proteins are homologues of bacterial RecA and are required for recombination during mitosis and meiosis, as well as for HR repair of DSBs (Shinohara et al., 1992). Rad51 coats single-stranded DNA to form a nucleoprotein filament that invades and pairs with a homologous region in duplex DNA, and then activates strand exchange to generate a crossover between the juxtaposed DNA (Sung, 1994; Baumann et al., 1996). Co-localization of BRCAs with Rad51 at sites of recombination and DNA damage induced foci strongly suggests that BRCAs have a role in both the detection and the repair of DSBs (Scully et al., 1997) (Fig. 3). In this regard, focus formation of Rad51 is reduced after treatment with DNA-damaging agents and is deficient during repair of DSBs by HR in BRCA1-deficient cells (Scully et al., 1999; Moynahan et al., 2001). However, accumulating evidence suggests that BRCA1 might not directly regulate Rad51, since interactions between BRCA1 and Rad51 are indirect and stoichiometrically negligible (Venkitaraman, 2001).
On DNA damage, BRCA proteins interact with numerous other proteins to modulate DNA repair, transcription, and the cell cycle.

Other studies have shown that BRCA1 co-localizes and co-immunoprecipitates with Rad50, together with its partners Mre11 and NBS1 (Zhong et al., 1999; Wang et al., 2000). BRCA1 apparently functions as a regulator of the Rad50-Mre11-NBS1 complex (Wu et al., 2000). Mre11 encodes nuclease activity, which resects flush ends of DSBs to generate ssDNA tracts (Haber, 1998). BRCA1 binds DNA directly and inhibits this Mre11 activity, regulating the length and the persistence of ss-DNA generation at sites of DNA damage (Paull et al., 2001) (Fig. 3). Since ss-DNA is a substrate for DNA repair by HR, BRCA1 might play an essential role in HR-mediated repair of DSBs by inactivating Mre11. Indeed, HR is defective in BRCA1-deficient cells (Moynahan et al., 1999). Studies have shown that BRCA1 co-localizes with phosphorylated H2AX (γ-H2AX) in response to DNA damage. DSBs promote an extensive response in chromatin, demonstrated by the phosphorylation of Ser139 at the C-terminus of H2AX (Rogakou et al., 1998; Rogakou et al., 1999). This event extends for thousands of bases around a DSB and can be mediated by DNA damage signaling. γ-H2AX forms discrete foci within 10 min after DNA damage, and BRCA1 is detectable in these foci 30 min thereafter (Paull et al., 2000). Importantly, in H2AX-deficient cells, BRCA1 fails to form DNA damage-induced foci, suggesting that at least part of the BRCA1 response to
DSBs takes place on chromatin (Celeste et al., 2002). Forced entrapment of BRCA1 in chromatin causes phosphorylation of H2AX by co-localization with BRCA1 in a DNA damage-independent manner. BRCA1 might therefore recruit kinases responsible for H2AX phosphorylation to DNA lesions and nucleate repair foci (Ye et al., 2001). A recent study has revealed that BRCA1 contributes to the regulation of c-Abl activity (Foray et al., 2002). c-Abl tyrosine kinase is ubiquitously expressed and localized in the cytoplasm and nucleus. Nuclear c-Abl is activated by diverse genotoxic agents and induces apoptosis mediated by p73 or Rad9 (Yuan et al., 1999; Yoshida et al., 2002). c-Abl is also implicated as a regulator of transcription and DNA repair. BRCA1 and c-Abl form a complex constitutively, and exposure to ionizing radiation triggers an ATM-dependent disruption of this BRCA1-c-Abl complex, coinciding with the activation of c-Abl kinase activity (Foray et al., 2002). Loss of BRCA1 results in constitutively elevated c-Abl kinase activity, suggesting that BRCA1 is involved in the control of such activity. These findings suggest a route by which BRCA1 affects cellular responses to DNA damage, distinct from a direct role in DNA repair or a role in cell cycle checkpoint control.

BRCA1 is phosphorylated by ATM in response to DSBs. Phosphorylated BRCA1 activates DNA repair through HR, in cooperation with BRCA2 and Rad51. BRCA1 also recruits Rad50-Mre11-NBS complex to the sites of DNA damage.

2) BRCA2
The roles played by BRCA1 and BRCA2 in the repair of DSBs by HR appear to differ. Available evidence indicates a more direct role of BRCA2. BRCA2-deficient cells exhibit
increased sensitivity to ionizing radiation, indicative of a defect in DSB repair, whereas the cell cycle checkpoint and apoptotic responses to DNA damage remain intact (Yu et al., 2000; Moynahan et al., 2001). In addition, BRCA2-deficient cells accumulate chromosomal breaks and aberrant mitotic exchanges during culture. Rad51-deficient cells show similar phenotypes, providing genetic evidence that interactions of BRCA2 with Rad51 are fundamental for the maintenance of cell division and chromosome structure. Physiologically, interactions between BRCA2 and Rad51 are mediated by the BRC repeat and an unrelated domain located at the C-terminus (Fig. 1). Recent studies have shown that BRCA2 regulates the intracellular localization and function of Rad51 (Davies et al., 2002). In BRCA2-deficient cells, nuclear transport of Rad51 is impaired, suggesting that BRCA2 moves Rad51 from the site of synthesis to the site of DNA damage processing (Davies et al., 2002). These in vitro findings led to the hypothesis that BRCA2 plays an essential role in the repair of DSBs in vivo. One possible model is that the BRCA2-Rad51 complex resides in two states in vivo: an inactive state, which prevents the binding of single-strand DNA by Rad51, and an active state, in which Rad51 forms nucleoprotein filaments to be transferred to sites of DNA damage by BRCA2 (Fig. 3). Transition from the inactive to the active state is speculated to involve post-translational modification, such as phosphorylation induced by DNA damage, triggering a substantial structural change in the BRCA2-Rad51 complex to release Rad51 from BRCA2. Whether this model, based on in vitro biochemical observations, is relevant to the cellular function of full-length BRCA2 remains to be clarified. Structural characterization of the BRCA2-Rad51 complex might provide clues to this question.

ROLE IN TRANSCRIPTIONAL RESPONSE TO DNA DAMAGE

1) BRCA1

BRCA1 has been implicated in the transcriptional regulation of several genes activated in response to DNA damage. The first line of evidence came from an observation that the C-terminus of BRCA1 binds and activates the basal transcriptional machinery (Chapman and Verma, 1996; Monteiro et al., 1996). A subsequent series of studies demonstrated that the C-terminus of human BRCA1 (amino acids 1528–1863) complexes with RNA polymerase II through RNA helicase A (Scully et al., 1997). This interaction appears to involve several proteins associated with the core polymerase complex. In fact, BRCA1 protein is a component of the RNA polymerase II holoenzyme, and deletion of the C-terminal 11 amino acids of BRCA1 attenuates the association with this holoenzyme. BRCA1 was also shown to regulate transcription in a purified in vitro system. More recently, other regions of BRCA1
have also been shown to contribute to transcriptional regulation in concert with RNA polymerase II holoenzyme (Anderson et al., 1998). It is now clear that BRCA1 bound to holoenzyme is present as a heterodimer with BARD1 (BRCA1-associated RING domain protein 1), suggesting that the N-terminal RING-finger domain of BRCA1 provides another pool for the holoenzyme component (Chiba and Parvin, 2002). The internal portion of BRCA1 binds to a large number of transcriptional factors, which may mediate signals to RNA polymerase II. Indeed, transcriptional activation by BRCA1 is supported by its ability to interact directly or indirectly with several transcriptional factors.

Finding target genes regulated by BRCA1 would shed considerable light on the transcriptional role of BRCA1. Studies using microarray technology have shown that p53-responsive cell cycle progression inhibitor and stress-response factors such as p21 and GADD45 are stimulated by BRCA1 over-expression (MacLachlan et al., 2002) (Fig. 4). Subsequent investigations have revealed that BRCA1 serves as a co-activator for p53 (Zhang et al., 1998). Co-immunoprecipitation experiments have also demonstrated that BRCA1 interacts with p53. Deletion of the N-terminus (amino acids 224–500) impairs in vitro interactions with p53. Furthermore, a truncated mutant of BRCA1 that retains the p53-binding site exhibits a dominant negative effect in p53-mediated transcription, thereby substantiating a pivotal role for interactions of BRCA1 and p53 in vivo. A recent study shows that p53 is stabilized by overexpression of BRCA1, suggesting that BRCA1 functions to stimulate p53 pathways (MacLachlan et al., 2002).

![Fig. 4: Role of BRCA1 in transcriptional regulation after exposure to ionizing radiation (IR)](source: Yoshida and Miki, 2004)
ATM is activated by IR and phosphorylates CtIP to disrupt the CtIP-CtBP-BRCA1 complexes. BRCA1 is then released and activates p21 and GADD45. Activation of cell cycle checkpoints induces replication arrest to allow repair of DNA damage.

BRCA1 also binds to ZBRK1, a transcriptional factor binding specifically to the DNA sequence GGGXXxCAGXXXTTT (Zheng et al., 2000). This binding motif is present in the promoter region of many transcriptional targets for BRCA1, such as p21, GADD45, and EGR1. Indeed, coexpression of BRCA1 and ZBRK1 was found to repress GADD45 promoter, contrary to previous findings that BRCA1 activates GADD45 expression. One explanation for this discrepancy is that overexpression of BRCA1 may titrate ZBRK1 away from the promoter, allowing transcription to occur. Phosphorylation induced by DNA damage and binding of BRCA1 to other repressors such as CtIP may modify this regulatory mechanism. Previous studies indicated that BRCA1 activation is attenuated by the CtIP-CtBP complex, which binds to the BRCT domain of BRCA1. The interaction of BRCA1 with CtIP is partially abrogated by DNA-damage-induced, ATM-dependent phosphorylation of CtIP, thus relieving suppression of the transactivation potential of BRCA1 (Li et al., 2000) (Fig. 4).

It was reported that BRCA1 and STAT1 co-operate to regulate p21. BRCA1 binds to the transcriptional activation domain of STAT1 (Ouchi et al., 2000). The binding of BRCA1 to STAT1 leads to the induction of a subset of IFN-γ regulated genes. STAT1-mediated transcriptional activation by UV radiation depends on Ser727 phosphorylation by p38/MAPK, and BRCA1 mainly associates with Ser727-phosphorylated STAT1. These findings raise the intriguing possibility that BRCA1 functions as a bridging protein, connecting DNA damage and stress response pathways to execute specific cellular responses, such as cell cycle arrest or apoptosis.

2) BRCA2

The possible function of BRCA2 as a transcriptional regulator is far less certain. Available evidence suggests that the product of BRCA2 exon 3 (amino acids 23–105) activates transcription and that a missense mutation (Tyr42Cys) of BRCA2 reduces the transactivation potential. The basis for this mutation and its relevance to carcinogenesis remain to be defined. Other studies have shown that overexpression of BRCA2 is associated with down-regulation of basal p53 transcriptional activity. In contrast, BRCA2 might activate transcription by modulating histone acetylation. BRCA2 interacts with the transcriptional co-activator protein P/CAF (p300/CBP-associated factor) and its associated p300/CBP, both of which possess
histone acetylase activity. BRCA2 might recruit these histone modifiers to the transcription complex to induce transcriptional activity.

Recent work has demonstrated that a novel protein, EMSY, binds to exon 3 of BRCA2 (Hughes-Davies et al., 2003). EMSY is amplified in some sporadic breast cancers and appears to negatively regulate BRCA2 function in transcriptional activation. A role for EMSY in the DNA damage response is supported by its co-localization with γ-H2AX and BRCA2 in irradiated cells. EMSY also has a basal promoter suppressive function, suggesting that it functions as a general transcriptional repressor. Moreover, EMSY is implicated in DNA repair and chromatin remodeling. In sporadic breast cancer, EMSY amplification correlates with a poorer outcome, specifically for node-negative breast cancer. In addition, overexpression of EMSY is associated with high-grade sporadic ovarian carcinomas, suggesting involvement of the BRCA2 pathway in sporadic breast and ovarian cancers. Although the mechanistic implications of BRCA2-EMSY interactions remain to be fully delineated, further analysis of this novel BRCA2-associated protein might provide important insights into pathways that are disrupted in sporadic breast and ovarian cancers.

ROLE IN DNA DAMAGE-RESPONSIVE CELL CYCLE CHECKPOINTS

1) BRCA 1

Cell cycle checkpoints play an essential role in cell survival by preventing the propagation of DNA damage through cell cycle progression before DNA repair. Recent studies using cells defective for different DNA damage-responsive proteins have demonstrated that both ATM and BRCA1 are required for effective S-phase and G2/M-phase checkpoints. Expression of BRCA1 variants defective for ATM-mediated phosphorylation is associated with a defect in G2/M arrest, suggesting that BRCA1 phosphorylation by ATM is indispensable for G2/M checkpoints in the DNA damage response (Cortez et al., 1999) (Fig. 4). Other work has indicated that BRCA1 regulates G2/M DNA damage induced checkpoints through its ability to activate Chk1 kinase and thereby induce signaling cascades downstream of Chk1 (Yarden et al., 2002) In this context, the finding that BRCA1-deficient cells exhibit defective G2/M arrest in response to ionizing radiation further supports a role of BRCA1 in the regulation of G2/M check-points.

BRCA1 functions as a co-activator of p53-mediated gene transcription. In BRCA1-deficient cells, the expression of 14-3-3σ, which is regulated by p53, is significantly diminished (Aprelikova et al., 2002). Since 14-3-3σ is a major G2/M check-point control gene, 14-3-3σ
induction by BRCA1 may also be involved in BRCA1-mediated G2/M checkpoints. Other studies have shown that overexpression of BRCA1 results in the transcriptional activation of GADD45 in a p53-dependent manner (Harkin et al., 1999; MacLachlan et al., 2000). As GADD45 has been implicated in G2/M check-points, BRCA1 may in part activate G2/M checkpoints by induction of GADD45 protein (Fig. 4). Interestingly, another p53 target gene, G1 cyclin-dependent kinase inhibitor p21, is also transactivated by exogenous expression of BRCA1 to block S-phase entry in a p53-independent manner (Somasundaram et al., 1997) (Fig. 4). Importantly, cancer-associated mutant BRCA1 failed to activate the p21 promoter. BRCA1 has also been found to transactivate the cyclin-dependent kinase inhibitor p27KIP1 (Williamson et al., 2002). The induction of G1 arrest by exogenous BRCA1 expression is likely to be associated with activation of p27KIP1.

2) BRCA2
It remains unclear whether BRCA2 participates directly in cell cycle regulation or checkpoint functions. Available evidence suggests that BRCA2 mediates G2/M-phase control by interacting with a novel protein, BRCA2-associated factor 35 (BRAF35), which binds to branched DNA structures (Marmorstein et al., 2001).

Nuclear staining has revealed a close association of BRAF35/BRCA2 complex with condensed chromatin, coincident with histone H3 phosphorylation. Importantly, antibody microinjection experiments suggest a role of BRCA2/BRAF35 complex in modulation of metaphase progression (Marmorstein et al., 2001). However, it is premature to conclude that BRCA2 is directly involved in mitotic progression. Since BRCA2 has a major role in DNA repair, its suppression is thought to induce unrepaired DNA lesions, which cause cell cycle arrest by activating checkpoint signaling, including mitotic progression.

Another line of evidence suggests that genetic instability caused by loss of BRCA2 function could trigger mutations, including those in checkpoint genes such as p53. (Lee et al., 1999). Furthermore, previous work has shown that tumours from BRCA2-deficient animals exhibit dysfunction of the spindle assembly check-point, accompanied by mutations in p53 (Greenblatt et al., 2001). In this context, dysfunction of p53 as a result of mutation leads to uncontrolled cell cycle checkpoints, inducing uncontrolled proliferation and invasive growth. These observations clearly indicate an indirect role of BRCA2 in cell cycle regulation. Obviously, more work will be required to determine whether BRCA2 regulates cell cycle control, as distinct from its role in DNA repair.
Mutations in BRCA1/BRCA2

Genetic testing is simple and inexpensive in populations where founder mutations are present. In these populations, a relatively small number of mutations account for the majority of the mutations. Surprisingly, the country with the highest known frequency of BRCA1 mutations among breast cancer cases is the Bahamas (23%) (Donenberg et al., 2011). In Canada (an ethnically-mixed population with few founder mutations) the corresponding figure is about one percent. Founder mutations have also been seen in other island populations, including Iceland (Tulinius et al., 2002), Greenland (Harboe et al., 2009), and Cyprus (Loizidou et al., 2007), and it is believed that these founder mutations reflect the geographic isolation of these islands. Populations may also be ethnically distinct due to social factors and marriage preferences. In the Ashkenazi Jewish population, two mutations in BRCA1 (185delAG and 5382insC) and one mutation in BRCA2 (6174delT) account for the majority of BRCA mutations (>90%) (Phelan et al., 2002). In Jewish women, approximately 12% of all breast cancers and 35% of all ovarian cancers are due to a founder mutation (Warner et al., 1999; Moslehi et al., 2000). Other populations with important founder mutations include ethnic isolates such as the Dutch (Verhoog et al., 2001) and French-Canadians (Ghadirian et al., 2009) and many countries of Eastern Europe with Slavic origins, including Poland (Gorski et al., 2000), Russia (Sokolenko et al., 2007), Belarus (Uglanitsa et al., 2010), and the Baltic states (Elsakov et al., 2010; Tikhomrova et al., 2010). In countries with founder mutations it becomes reasonable to test all breast cancer patients for mutations. In Ontario, it has been proposed that all Jewish women be eligible for genetic testing, regardless of their personal or family history of cancer (Metcalf et al., 2010).

RISKS ASSOCIATED WITH A MUTATION

The lifetime risk of breast cancer in women with a BRCA1 or BRCA2 mutation is approximately 75%. For BRCA1, there is little evidence that the risk varies for different mutations; however, this is not the case for BRCA2. The risk of breast cancer for women with the common Ashkenazi founder mutation BRCA2 6714delT is approximately half that of women with other BRCA2 mutations. There is also a common truncating variant in the terminal exon 27 of BRCA2 that does not appear to increase the risk of breast cancer (Mazoyer et al., 1996), although it may increase the risk of pancreatic cancer (Martin et al., 2005) and esophageal cancer (Akbari et al., 2008).
There have been many studies of potential modifiers of risk of BRCA1 and BRCA2. Penetrance may vary by geographic region; perhaps this reflects the underlying cancer rates in the general population. For example, the risk of breast cancer for women in Poland with a BRCA1 mutation appears to be much lower than that for women in North America (Lubinski et al., 2010). The difference could not be explained by mutation type, by reproductive factors, exogenous hormones, body-mass index (BMI), or by screening intensity. In this study, the difference was attributable to different risks early in life. In North America, the annual risk of breast cancer for women ages 25-39 with a BRCA1 mutation was 3.8% and in Poland it was 1.4% (p < 0.001). It has also been suggested that the risk of breast cancer in BRCA1 carriers is increasing with recent year of birth (Litton et al., 2011). Several lines of evidence support this hypothesis (Narod, 2011). The reason for this possible cohort effect is unclear, but one contributing factor might be a declining age of menarche.

In women at increased risk for breast and ovarian cancer, the identification of a BRCA1/2 mutation has important implications for screening and prevention counseling. Uncertainty regarding the role of BRCA1/2 testing in high-risk women from diverse ancestral backgrounds exists due to variability in prevalence estimates of deleterious (disease-associated) mutations in non-White populations. We examined the prevalence of BRCA1/2 mutations in an ethnically diverse group of women referred for genetic testing. A cross-sectional analysis was carried out to assess the prevalence of BRCA1/2 mutations in a group of non-Ashkenazi Jewish women undergoing genetic testing. From 1996-2006, 46,276 women meeting study criteria underwent DNA full-sequence analysis of the BRCA1 and BRCA2 genes. Deleterious mutations were identified in 12.5% of subjects, and recurrent deleterious mutations (prevalence > 2%) were identified in all ancestral groups. Women of non-European descent were younger (45.9 yrs, SD11.6) than European (50.0 yrs, SD11.9)(p<0.001). Women of African (15.6%)[OR 1.3(1.1-1.5)] and Latin American (14.8%)[OR 1.2(1.1-1.4)] ancestries had a significantly higher prevalence of deleterious BRCA1/2 mutations compared to women of Western European ancestry (12.1%), primarily due to an increased prevalence of BRCA1 mutations in these two groups. Non-European ethnicity was strongly associated with having a variant of uncertain significance; however, re-classification decreased variant reporting (12.8%→5.9%), with women of African ancestry experiencing the largest decline (58%). Mutation prevalence is high among women referred for clinical BRCA1/2 testing, and risk is similar across diverse
ethnicities. BRCA1/2 testing is integral to cancer risk assessment in all high-risk women (Hall et al., 2009).

**BRCA1 Mutation Analysis**

BRCA1 gene encodes a nuclear phosphoprotein that plays a role in maintaining genomic stability, and it also acts as a tumour suppressor. The encoded protein combines with other tumour suppressors, DNA damage sensors, and signal transducers to form a large multi-subunit protein complex known as the BRCA1-associated genome surveillance complex (BASC). This gene product associates with RNA polymerase II, and through the C-terminal domain, also interacts with histone deacetylase complexes. This protein thus plays a role in transcription, DNA repair of double-stranded breaks, and recombination. Mutations in this gene are responsible for approximately 40% of inherited breast cancers and more than 80% of inherited breast and ovarian cancers. Alternative splicing plays a role in modulating the subcellular localization and physiological function of this gene. Many alternatively spliced transcript variants, some of which are disease-associated mutations, have been described for this gene, but the full-length natures of only some of these variants has been described. A related pseudogene, which is also located on chromosome 17, has been identified. Germ line mutations of the BRCA1 gene confer a high risk of breast cancer and ovarian cancer to female mutation carriers. The BRCA1 protein is involved in the regulation of DNA repair. How specific tumour-associated mutations affect the molecular function of BRCA1, however, awaits further elucidation. Cell lines that harbor BRCA1 gene mutations are invaluable tools for such functional studies. Up to now, the HCC1937 cell line was the only human breast cancer cell line with an identified BRCA1 mutation. In this study, we identified three other BRCA1 mutants from among 41 human breast cancer cell lines by sequencing of the complete coding sequence of BRCA1. Cell line MDA-MB-436 had the 5396 + 1G>A mutation in the splice donor site of exon 20. Cell line SUM149PT carried the 2288delT mutation and SUM1315MO2 carried the 185delAG mutation. All three mutations were accompanied by loss of the other BRCA1 allele. The 185delAG and 5396 + 1G>A mutations are both classified as pathogenic mutations. In contrast with wild-type cell lines, none of the BRCA1 mutants expressed nuclear BRCA1 proteins as detected with Ab-1 and Ab-2 anti-BRCA1 monoclonal antibodies. These three new human BRCA1 mutant cell lines thus seem to be representative breast cancer models that could aid in further unraveling of the function of BRCA1 (Elstrodt et al., 2006).
Clinical and pathologic differences between BRCA1, BRCA2, and non-BRCA associated breast cancers

Mutations in BRCA1 and BRCA2 confer an increased risk to breast and other cancers, but to date there have only been limited numbers of studies of BRCA1- and BRCA2-associated cancers among Asians. Malaysia is a multiracial country with three main races: Malays, Chinese, Indians. We determined whether tumour pathologic features and clinical features differ in patients with and without BRCA mutations in this Asian population. A retrospective review of the medical records of 152 women with breast cancer who underwent genetic testing for BRCA mutations. The patients self-reported ethnicity, age at onset, and clinical stage at diagnosis and tumour pathology were reviewed. A total of 31 patients carried germline deleterious mutations (16 BRCA1, 15 BRCA2). We found that tumours in BRCA1 carriers were more likely to be estrogen receptor (ER)-negative and progesterone receptor (PR)-negative. HER2 was more likely to be negative in both BRCA1 and BRCA2 subjects compared with non-BRCA subjects. We found a strong association between triple-negative status and BRCA1 carriers. In addition, tumours in BRCA1 carriers were more likely to be higher grade than those in BRCA2 and non-BRCA carriers; but the difference was not statistically significant. These results suggest that tumours associated with BRCA1 mutations are distinct from those of BRCA2-associated and non-BRCA-associated breast cancers, and that the tumours associated with BRCA2 mutations are similar to the non-BRCA-associated breast cancers. Further studies are required to determine if the prognosis is different in each of these groups and the best management strategy for each group (Yip et al., 2009).

Pathology of BRCA-associated Breast Cancer

The distribution of histologic types of BRCA1-associated breast cancers differs from sporadic breast cancers in various aspects, but BRCA2-associated breast cancers do not appear to exhibit a specific pathologic phenotype (Da Silva and Lakhani 2010). The majority of the BRCA1 associated tumours are invasive ductal carcinomas, but approximately 15% are classified as medullary and are grade 3 and they often show lymphocytic infiltration and ‘pushing’ margins (Lakhani et al., 2005). The majority of BRCA1-associated breast cancers are ER-negative, PR-negative, and ERBB2-negative (i.e., triple-negative; Foulkes et al, 2003) but only about 10% of early-onset triple-negative breast cancers are BRCA1-positive (Young et al., 2009). Among triple-negative breast cancers, those that express epithelial keratins ck5 or ck6 have been referred to as the ‘basal’ phenotype. Foulkes et al., (2003) demonstrated
that the basal phenotype was over-represented among breast cancers that were associated with \textit{BRCA1} mutations compared to other types.

**Other Cancers**

Women with a \textit{BRCA1} mutation are also at risk for cancer of the fallopian tube, ovarian cancer, primary peritoneal cancer, and pancreatic cancer (Finch \textit{et al.}, 2006). There is no conclusive evidence that they are at increased risk for endometrial cancer, colon cancer, melanoma, or other forms of cancer (Thompson \textit{et al.}, 2002). The clinical expression of \textit{BRCA2} is wider and includes melanoma and pancreatic cancer (Breast Cancer Linkage Consortium, 1999).

**Cancer in Men**

Men who carry a \textit{BRCA1} or \textit{BRCA2} mutation are at increased risk for male breast cancer, although the lifetime risk for this is low (Liede \textit{et al.}, 2004). Among \textit{BRCA2} carriers, men are at increased risk of pancreatic cancer, melanoma, and an aggressive form of prostate cancer (Breast Cancer Linkage Consortium, 1999). The risk is less for men with a \textit{BRCA1} mutation; although they appear to be at higher risk than expected for pancreatic cancer, there is little evidence that the risk of prostate, colon, or other cancers is increased.

**Risk Factors for Breast Cancer among Mutation Carriers**

Several case-control and cohort studies have been conducted on samples of women with \textit{BRCA1} and \textit{BRCA2} mutations, but to date, few risk factors have been firmly established. Among \textit{BRCA1} carriers, breast cancer risk is influenced by age of menarche (Kotsopoulos \textit{et al.}, 2005), by breastfeeding (Jernstrom \textit{et al.}, 2004), and by family history of breast cancer (Metcalfe \textit{et al.}, 2010). A late age of menarche is protective; compared to carriers whose age at menarche was less than or equal to 11 years, women with an age of menarche between 14 and 15 years old had a 54% reduction in risk (OR = 0.46; 95% CI 0.30 to 0.69; Kotsopoulos \textit{et al.}, 2005). A similar effect is not seen for \textit{BRCA2} carriers. Breastfeeding is also protective for \textit{BRCA1} carriers, but not for \textit{BRCA2} carriers (Jernstrom \textit{et al.}, 2004). Women with a \textit{BRCA1} mutation who breast-fed for more than one year were less likely to have breast cancer than those who never breast-fed (OR = 0.55, 95% CI = 0.38 to 0.80). The third important risk factor is a family history of breast cancer, in particular the number of first-degree relatives with breast cancer diagnosed before age 50. In a prospective study of women with a \textit{BRCA1} mutation, the risk of breast cancer increased by 1.2-fold for each first-degree relative with breast cancer before age 50 years (HR = 1.2; 95% CI 0.94 to 1.57; Metcalfe \textit{et al.}, 2010).
Among women with a BRCA2 mutation, the effect was much stronger. The risk of breast cancer increased by 1.7-fold for each first-degree relative younger than 50 years diagnosed with breast cancer (HR = 1.67; 95% CI = 1.04 to 2.07).

Screening for Hereditary Breast Cancer
Screening for breast cancer in BRCA1 and BRCA2 carriers should ideally include an annual magnetic resonance imaging (MRI) examination. MRI has been consistently found to be more sensitive than mammography (Kriege et al., 2004; Leach et al., 2005). In a recent prospective study, the incidence of advanced breast cancer (2 cm or more or node-positive) was decreased by 70% in the six-year follow-up period in women with a BRCA mutation who underwent regular MRI screening, compared to those who had conventional mammography (Warner et al., 2011). MRI screening should be conducted annually from age 25 to 65. Mammography is a much less expensive alternative, but in cohort studies of BRCA carriers who are screened by mammography alone, the rate of interval cancers is unacceptably high. It has not been demonstrated that, when annual MRI is done, there is an incremental benefit to adding mammography.

Prevention of Hereditary Breast Cancer
The ideal prevention strategy will be a short-term intervention which offers long term-protection. Prevention strategies can be divided into those that offer transient protection and those that offer lifelong protection. Preventive mastectomy is a one-time intervention that offers almost complete protection against breast cancer. In women who choose not to have a preventive mastectomy, chemoprevention should be considered. For pre-menopausal women, tamoxifen is the only currently approved chemopreventive drug. For post-menopausal woman raloxifene and exemestane are alternatives to tamoxifen (Vogel et al., 2010; Goss et al., 2011). Tamoxifen has been associated with a 50-70% reduction in contralateral breast cancer in both BRCA1 and BRCA2 carriers (Gronwald et al., 2006). It may be equally effective in the primary prevention of breast cancer as well, but this is as yet unproven. We have recently reported that, in the context of contralateral breast cancer, one year of tamoxifen was as effective (and possibly more effective) compared to the conventional five-year course. Many women choose not to take tamoxifen over the concern of acute and long-term side effects. Serious side effects of tamoxifen are rare in young women (Iqbal et al., 2011). Alternatives to tamoxifen for post-menopausal women (including women after preventive oophorectomy) are raloxifene and exemestane, but data on these drugs specifically
in BRCA carriers have not been published. In a recent analysis of the STAR trial, the protective effect of tamoxifen exceeded that of raloxifene by approximately 25% in women in the general population (Vogel et al., 2010). Raloxifene and tamoxifen are selective estrogen receptor modulators (SERMs) and target the estrogen receptor, but exemestane is an aromatase inhibitor and inhibits the conversion of androgen to estrogen. Recently, exemestane was shown to be highly effective for breast cancer prevention in the non-BRCA setting; 4,560 postmenopausal women (median age of 62.5 years) were randomized to receive exemestane or placebo (Goss et al., 2011). After 35 months of follow-up, a 65% reduction was observed in the annual incidence of invasive breast cancer (HR = 0.35; 95% CI, 0.18 to 0.70; P = 0.002). Other potential agents for chemoprevention include poly (ADP-ribose) polymerase (PARP) inhibitors (Hay et al., 2009) and the RANKL inhibitor denusomab (Body et al., 2006; Schramek et al., 2010). The target of the PARP inhibitors will be small cancers or pre-neoplastic lesions that have undergone loss of heterozygosity of BRCA1. The target of denusomab is the RANK ligand (RANKL) which is central to transmitting the progesterone signal in the regulation of the mammary stem cell population (Schramek et al., 2010).

Oophorectomy
Oophorectomy is associated with reductions in the risks of both ovarian and breast cancer in BRCA carriers (Eisen et al., 2005; Rebbeck et al., 2009; Finch et al., 2006). Approximately one-half of breast cancers occur in BRCA1 mutation carriers before the age of 40 and therefore the earlier the oophorectomy, the greater the potential for prevention. An oophorectomy before age 40 was associated with a reduction in the risk of breast cancer of 64% in women with a BRCA1 mutation and 31% in women with a BRCA2 mutation (Eisen et al., 2005). It is not known yet if the oophorectomy offers lifelong protection or if a post-menopausal oophorectomy is also protective for breast cancer. In the case-control study of Eisen et al. (2005), no protective effect was observed for an oophorectomy done 15 or more years prior to diagnosis. Some women are reluctant to undergo oophorectomy at age 35 because of a desire to preserve fertility and most are concerned about the acute effects of surgical menopause. Several of the symptoms of menopause can be mitigated by hormone replacement therapy, but the quality of life does not return to pre-surgical levels (Madalinska et al., 2006; Finch et al., 2011). Two studies in BRCA carriers reported that hormone replacement therapy did not increase the subsequent risk of breast cancer (Rebbeck et al., 2005; Eisen et al., 2008).
Natural History of Hereditary Breast Cancer

Breast cancers in women with a BRCA1 mutation are typically diagnosed at a young age and are triple-negative (i.e., ER-negative, PR-negative, HER2-negative; Lakhani et al., 2005). To a large extent, the natural history of cancer in carrier women is similar to that of young women with triple-negative breast cancer. The salient features of triple-negative cancers include a propensity to early distant recurrence (commonly within one to three years) and an attenuated relationship between tumour size, lymph node status, and survival (Dent et al., 2007). In a historical cohort study from Israel, Rennert and colleagues found that the ten-year survival of women with a BRCA1 mutation (49%) or a BRCA2 mutation (48%) was similar to that of non-carriers (51%), but tumour size did not predict survival in the BRCA1-positive cohort (Rennert et al., 2007). Women with small cancers (< 2cm) did relatively poorly and women with large cancers (> 2cm) did comparatively well. There are few comparable data for BRCA2 carriers.

Treatment of Hereditary Breast Cancer

In choosing treatment for a BRCA-positive woman with breast cancer, several factors should be taken into consideration, including the risk of recurrence and the risks of contralateral breast cancer and ovarian cancer. The response to treatment of BRCA1-associated cancers is related to the underlying genetic defect and this may differ from other cancers with a similar histologic appearance. Based on pre-clinical models, it was predicted that BRCA1-associated breast cancers would be sensitive to DNA-breaking agents, such as mitomycin C and cis-platinum (reviewed in Michalak and Jonkers, 2011).

A study was performed on Ashkenazi Jewish cohort with early-stage breast cancers. It was found that women with a BRCA1 mutation who did not receive chemotherapy did worse than women with non-hereditary breast cancers of similar size, but the adverse effect of mutation status on prognosis was neutralized if chemotherapy was given (Robson et al., 2004). Similarly, an enhanced benefit of chemotherapy for BRCA1 carriers was seen (Rennert et al., 2007). BRCA1 carriers were highly sensitive to cis-platinum, given as neo-adjuvant chemotherapy. Of 12 women who were given cis-platinum, ten (83%) experienced a pathologic complete response, compared to 13 of 76 women (17%) who received an anthracycline-containing regimen (Byrski et al., 2010). It has been proposed that the responsiveness of these patients may relate to the triple-negative phenotype, and not the mutation status per se, but early studies have not supported this model. The degree of
response to cis-platinum among BRCA1 carriers appears to exceed that of non-carrier women with triple-negative cancer. 4 of 26 women with non-BRCA1 triple-negative breast cancer had a pathologic complete response after cis-platinum treatment. In the same study, 2 of 2 women with a BRCA1 mutation and triple-negative breast cancer had a complete response. The rate of pathologic complete response (pCR) in BRCA1 and BRCA2 carriers was estimated and compared these with women without a mutation. Forty six percent of 53 BRCA1 carriers had a pCR, compared to only 13% of 23 patients with a BRCA2 mutation and 22% of 237 non-carriers (Silver et al., 2010). Among BRCA1 carriers who experienced a pCR, the survival rate at five years was 100%. The survival rate at five years for the BRCA2 carriers was also very high (100%) despite the low pCR rate, but this likely reflects on the small number of BRCA2 carriers and the short follow-up time (median 3.2 years) (Arun et al., 2010).

Recently, there has been much optimism expressed regarding a new class of drugs call poly (ADP-ribose) polymerase inhibitors (PARP inhibitors; McCabe et al., 2006). Members of this class of drugs have shown effectiveness against BRCA1-positive and BRCA2-positive breast cancer cells in preclinical models and phase II studies (McCabe et al., 2006; Farmer et al., 2005). Poly (ADP-ribose) polymerase is involved in the repair of single strand DNA breaks, and inhibition of the enzyme results in an impairment of DNA repair and an augmentation in the number of double-strand DNA breaks. This phenotype is particularly detrimental to cells with no intact BRCA1 or BRCA2 protein (such as breast cancer cells in mutation carriers, which have undergone loss of heterozygosity) and results in cell death. Single agent olaparib (a PARP inhibitor) has shown effectiveness in treating metastatic hereditary breast cancer (Fong et al., 2009; Tutt et al., 2010) and it is hoped that this drug, or others in its class, will be used to potentiate the effect of other chemotherapies, such as cis-platinum.

BRCA Database
University of Utah, Huntsman Cancer Institute Collection maintains BRCA Resources database. In BRCA1 Database there are 1168 total entries. BRCA1 in silico Prediction covers 5,592 bases encoding 1,863 amino acids. Of the total number of variants identified so far, 92% are definitely pathogenenic while 7% are not pathogenic. (Tavtigian and Best, 2014). In BRCA2 Database there are 1159 total entries. BRCA2 in silico Prediction covers 10,296 bases encoding 3,419 amino acids. Of the total number of variants identified so far, 91% are definitely pathogenenic while 6% are not pathogenic. (Tavtigian and Mao, 2014).
CONCLUSION
BRCA1 was identified about 10 years ago. Mutations in BRCA genes have been noticed to predispose women to breast and ovarian cancers, the endpoint of BRCA protein dysfunction. Although previous studies have implicated both BRCA1 and BRCA2 in the cellular response to DNA damage, little is known about the mechanism by which BRCA proteins modulate this response. Research revealed that BRCA proteins bind and interact with a number of regulatory proteins. Evidences from research suggest that BRCA1 and BRCA2 participate in multiple functions, including DNA repair, transcription, and cell cycle control. It is unclear why a BRCA-related predisposition to cancer is apparently site-specific, affecting the breast and ovary, despite the fact that the known functions of BRCA proteins are essential to all cell types. A possible explanation is that breast or ovarian epithelia are particularly vulnerable to transformation when heterozygous for BRCA gene mutations. This increased vulnerability could be attributed to tissue-specific effects of the haploinsufficiency involved in the hormone-responsive proliferative changes unique to these cells. At present, however, the roles of BRCA proteins in epithelial cell biology and transformation remain uncertain.

Our knowledge of the clinical manifestations of hereditary breast cancer continues to expand. Genetic testing can be offered to high risk women in order to establish whether or not they carry a mutation in BRCA1 or BRCA2. Those who test positive can be offered preventive mastectomy, annual screening with MRI, or chemoprevention with tamoxifen or another drug. Genetic testing may also be useful at the point of diagnosis. Women who test positive may benefit from bilateral mastectomy and oophorectomy. Furthermore chemotherapy options should include cis-platinum and a PARP inhibitor.

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