Individuals carrying germline mutations in one allele of the \textit{BRCA1} or \textit{BRCA2} genes are at significantly increased risk of developing cancer. Although the increased risk of breast cancer is often highlighted, cancer at several other sites is also considerably more common in these individuals. Here, we discuss existing knowledge of the role of \textit{BRCA1} and \textit{BRCA2} mutation in pre-disposition to ovarian cancer. The risk of an individual with a mutation developing cancer of the ovary appears to be influenced by the position of the mutation within the \textit{BRCA} gene, the presence of allelic variants of modifying genes and the hormonal exposure of the carrier. Once cancer has developed, the pathology and clinical behaviour of \textit{BRCA}-associated tumours is distinct from sporadic cases. Comparison of the pathogenesis of breast and ovarian cancers caused by \textit{BRCA} mutation provides insight into the function of \textit{BRCA} proteins as tumour suppressors in different cellular environments.

**Introduction**

Epithelial ovarian cancer is a frequently lethal gynaecological malignancy, and the poor survival of patients developing this disease is, in part, attributable to the difficulties in diagnosis at an early stage. Approximately 10\% of invasive ovarian cancers have a hereditary basis (1,2), and many of these cases are caused by mutations in the \textit{BRCA1} and \textit{BRCA2} genes (3). Women inheriting a mutated copy of \textit{BRCA1} or \textit{BRCA2} have a ~40 or ~10\% risk, respectively, of developing ovarian cancer by the age of 70 (4). Tumours arising as a result of a \textit{BRCA} mutation generally show loss-of-heterozygosity of the wild-type allele and retention of the mutated, disease-predisposing allele, validating \textit{BRCA1} and \textit{BRCA2} as \textit{bona fide} tumour suppressor genes.

Both \textit{BRCA1} and \textit{BRCA2} encode proteins that are involved in the repair of double-stranded DNA breaks (DSBs) by homologous recombination (HR) (5). HR is a potentially error-free mechanism of DNA damage repair that requires RAD51 localization to DSBs; this can be observed as foci of RAD51 localization within the nucleus. \textit{BRCA2} interacts directly with RAD51 and is required for the formation of RAD51 foci, as is a \textit{BRCA2}-associated protein called DSS1 (6,7). \textit{BRCA1} is also required for the formation of RAD51 foci, perhaps through direct or indirect interaction with RAD51 or BRCA2. \textit{BRCA2} has also been identified as the \textit{FANCD1} gene, a member of the Fanconi anaemia complex of proteins, and cells deficient in Fanconi anaemia proteins have a similar phenotype to those deficient in \textit{BRCA}. Cells lacking functional \textit{BRCA1} or \textit{BRCA2} are unable to repair their DNA conservatively by HR, and instead utilize error-prone pathways such as non-homologous end joining or single-strand annealing. This results in chromosomal abnormalities and genetic instability, both hallmarks of cancer cells. A function in HR is the best documented role of \textit{BRCA2}, but many other cellular functions have been proposed for \textit{BRCA1} including in nucleotide excision repair, cell cycle checkpoint control, ubiquitylation of proteins, chromatin remodelling and the transcriptional activation of genes required for repair of oxidative damage (8–10).

Deleting \textit{BRCA1} or \textit{BRCA2} specifically in mammary epithelial cells of the mouse does not result in animals more prone to mammary gland cancer and it is thought that failure to repair DNA damage activates a p53-dependent cell cycle checkpoint leading to growth arrest or apoptosis. Bypassing the p53 pathway allows \textit{BRCA}-null cells to continue proliferating unchecked, and gives rise to genetically unstable breast tumours reminiscent of those seen in human \textit{BRCA} mutation carriers (11). As most \textit{BRCA}-associated cancers have a disrupted p53 pathway, it is probable that loss-of-heterozygosity of the wild-type \textit{BRCA1} or \textit{BRCA2} allele only leads to tumour formation if previous or concurrent inactivation of other genes occurs.

The risk of developing \textit{BRCA}-associated ovarian cancer is modified by several factors such as the reproductive history and hormonal exposure of an affected individual, and the co-inheritance of modifying genes. Here, we discuss the similarities and differences between the pathogenesis of \textit{BRCA}-associated and sporadic ovarian tumours.

**Why do mutations in \textit{BRCA} genes predispose to ovarian cancer?**

Both \textit{BRCA1} and \textit{BRCA2} proteins appear to be widely expressed and have roles in processes common to many cell types. Hence, it is not obvious why loss-of-function of proteins required for general cellular functions should be associated with a disease-causing phenotype in some tissues but not others, and even less clear why some specific mutations favour a disposition to one type of cancer over another. Mutations in \textit{BRCA1} are mainly associated with female breast cancer and cancer of the ovary, fallopian tube and peritoneum ( Müllerian duct-derived organs), whereas \textit{BRCA2} mutation carriers are also at risk for a spectrum of cancers at several other sites (12). A number of theories have been put forward to explain the cancer susceptibility of \textit{BRCA1} mutation carriers. Some of these posit that the properties of the breast and the ovarian tissue microenvironments are crucial for pathogenesis. The most common types of \textit{BRCA}-associated ovarian cancers...
derive from the ovarian surface epithelium (OSE), specifically when it has been internalized to form inclusion cysts. In adults epithelial cell proliferation is not common and, in females, occurs cyclically in the ovary and endometrium during the menstrual cycle, the ducts of the mammary gland during puberty, pregnancy and lactation and in the gut throughout life. Acquisition of an ordered set of genetic changes might be facilitated by bursts of proliferation and it is, perhaps, notable that the resulting cells are retained within breast lobules and ovarian inclusion cysts, but shed from the endometrium and gut lining. This may lead to a situation where susceptible cells are exposed to a breast or ovarian-specific microenvironment, and altered BRCA1 protein function may be more permissive to cell survival in one or other of the tissues (Figure 1).

The positive correlation between estrogen levels and breast cancer is striking, and evidence suggests that estrogens can also promote tumour initiation and promotion in OSE cells. The levels of estrogen in the ovarian stroma and follicular fluid to which the OSE and inclusion cysts are exposed are at least 100-fold greater than circulating levels (13), and both estrogen and estrone are mitogenic to OSE cells in vitro (14). Furthermore, estrogen may cause genotoxic damage to cells, and this could be less effectively repaired in cells lacking BRCA1 or BRCA2 (Figure 1). As well as this, it has been suggested that BRCA1 may be involved in regulating estrogen receptor activity. One study showed that depleting BRCA1 in an estrogen-dependent ovarian cancer cell line increases survival on estrogen withdrawal (15). Since most BRCA1-associated breast tumours are estrogen receptor negative, it is possible that one of the relevant functions of BRCA1 in breast and ovarian cells is to prevent estrogen-independent proliferation. Supporting this theory is the suggestion that BRCA1 inhibits ligand-independent transcriptional activation of the estrogen receptor (16), and that BRCA1 and ER-α interact directly (17). The use of estrogen-containing oral contraceptives decreases rather than increases the risk of developing ovarian cancer, both in BRCA mutation carriers and non-carriers. This is presumably because ovulation is prevented, and the lack of local estrogen release from the ruptured follicle and post-ovulation wound healing of the OSE has a protective effect (see below). As yet, there are no molecular studies that have linked BRCA2 function to hormone-induced proliferation.

The site of mutation in BRCA1 or BRCA2 may influence tumour tropism

BRCA1 and BRCA2 are both large genes encoding proteins of 1863 and 3418 amino acids, respectively (5). Pathogenic mutations occur throughout the BRCA1 and BRCA2 genes and include small insertions or deletions, or point mutations that give rise to a nonsense codon. Many of these mutations are predicted to give rise to truncated proteins lacking a functional domain; one caveat is that it is unknown how many of these mutant mRNAs are destroyed by nonsense-mediated degradation mechanisms. Truncated proteins could be partially active, but may have lost the ability to perform an important tissue-specific role. Several studies have shown that a cluster of mutations within nt 4075–6503 in exon 11 of the BRCA2 gene are associated with increased ovarian cancer risk and decreased breast cancer risk [(18–20); Figure 2A]. This region is known as the ovarian cancer cluster region. Mutations occurring in this region are predicted to retain one or more BRC motifs and, therefore, have at least some potential to bind to RAD51. However, it could be that proteins truncated...
in this way may have dominant-negative effects on HR by inhibiting RAD51 function.

The evidence of a genotype–phenotype correlation with breast and ovarian cancer for mutations in \textit{BRCA1} is weaker than that for \textit{BRCA2}. However, mutations in the C-terminal region of the \textit{BRCA1} protein, which is required for DNA repair and transcriptional activation, appear to be associated with breast cancer and mutations towards the N-terminus with ovarian cancer \cite{21; Figure 2B}. The N-terminus of \textit{BRCA1} contains a RING-finger domain, which binds \textit{BRCA1}-associated RING domain 1, and a commonly inherited mutation of this region appears to alter the response of OSE cells to apoptotic stimuli \cite{22}. A recent study has identified an alternative, shortened splice variant of \textit{BRCA1}, \textit{BRCA1-IRIS} that has a distinct expression profile to full-length \textit{BRCA1} in the adult \cite{23,24}. This protein appears to function in DNA replication, and is expressed in both proliferating and quiescent cells, whereas the full-length protein is expressed only in proliferating cells. Mutations in the 5' end of \textit{BRCA1} are predicted to lead to loss of both \textit{BRCA1-IRIS} and full-length \textit{BRCA1}, whereas mutations in the 3' retain expression of \textit{BRCA1-IRIS}. However, exactly how this would lead to an increased risk of ovarian cancer rather than breast cancer is not clear. Perhaps of relevance is the observation that introduction of a transcript of \textit{BRCA1} mutated at the 3' end into ovarian cancer cells inhibited growth to the same extent as full-length \textit{BRCA1}, but had no effect on the growth inhibition of breast cancer cells \cite{25}. However, whether this result is of physiological relevance is questionable and the observation remains unconfirmed.

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
& \textbf{Risk of breast cancer} & \textbf{Risk of ovarian cancer} \\
\hline
\textbf{Inherited factors} & \textit{BRCA1} & \textit{BRCA2} & \textit{BRCA1} & \textit{BRCA2} \\
\hline
Active AR variant & Decrease & No effect & Increase & No effect \\
SNP in RAD51 & No effect & Increase & ? & ? \\
Rare HRAS1 allele & No effect & No effect & Increase & No effect \\
Lifestyle factors & & & & \\
Oral contraceptives & Increase? & Increase? & Decrease & Decrease \\
Pregnancy & Increase & Increase & Decrease & Decrease \\
Breast-feeding & Decrease & No effect & No effect & No effect \\
Tubal ligation & No effect & No effect & Decrease & Decrease \\
Tamoxifen & Decrease & Decrease & No effect & No effect \\
\hline
\end{tabular}
\caption{Inherited and lifestyle modifying factors have differential effects on the risk of \textit{BRCA1} mutation carriers developing breast and ovarian cancer}
\end{table}

Inherited factors appear to have effects specific to \textit{BRCA} mutation carriers, whereas lifestyle modifiers exaggerate a trend already seen amongst the general population.

\textbf{Factors modifying penetrance of \textit{BRCA} mutation in ovarian cancer}

The differential effects of inherited and lifestyle factors on breast and ovarian cancer risk for \textit{BRCA} mutation carriers are summarized in Table I, although, it should be noted that many of these observations are preliminary. The penetrance of a \textit{BRCA1} mutation for ovarian or breast cancer appears to be modified by the co-inheritance of allelic variants of other genes. \textit{BRCA1} may be a co-activator of the androgen receptor (AR), and women who inherit a shorter, more active allelic
variant of the AR gene as well as a mutated copy of BRCA1 develop ovarian cancer at an earlier age (26). In contrast, another study showed that BRCA1 patients with the longer, less active AR variant were diagnosed with breast cancer earlier (27). These data are in keeping with the theory that, at least in BRCA1 mutation carriers, androgens have a protective effect on breast tissue but induce proliferation of OSE. The presence of rare, genetically inherited variants of the HRAS proto-oncogene has also been associated with increased risk of ovarian but not breast cancer in BRCA1 mutation carriers (28), but the biological basis for this difference is not understood. Little is known about the genetic interaction of BRCA2 mutation with other genes and how this affects ovarian or breast cancer risk.

A major concern for BRCA mutation carriers has been the apparently opposing effects of oral contraception on the risk of developing breast or ovarian cancer. Three studies have reported an up to 56% reduction in ovarian cancer risk associated with oral contraceptive use in BRCA1 or BRCA2 mutation carriers (29–31); a similar protective effect is seen in non-mutation carriers. However, oral contraceptives appear to increase the breast cancer risk for BRCA1 mutation carriers to a considerably higher level than that seen in the general population (32). The risk may depend on the type of oral contraceptive used, since there appears to be no increase in risk for women who used more modern versions of these agents. The biological basis of this difference in risk may lie in the biology of the OSE. OSE is thought to be prone to cancerous changes after ovulation, when it proliferates in response to wound healing factors to repair the damage caused by the rupture of the follicle. Oral contraceptives prevent ovulation, and so the OSE remains in a quiescent state. In contrast, oral contraceptives do not have an antiproliferative effect on breast epithelium, and the levels of supplementary estrogen in older generation oral contraceptives may cause the increased risk of breast cancer. Since pregnancy also prevents ovulation, it also has a protective effect against developing ovarian cancer, both in BRCA mutation carriers and the general population (33,34).

Pregnancy increases the risk of developing early-onset breast cancer (before the age of 40) in the general population as well as in BRCA1 and BRCA2 mutation carriers, presumably due to the dramatic rise in the levels of circulatory estrogens that are mitogenic to occult tumours (34). Since early-onset breast tumours are characteristic of BRCA1-associated disease, the increase in risk appears much higher in this group. On the other hand, breast-feeding for at least a year appears to reduce the breast cancer risk in BRCA1 patients more so than in the general population; the same study did not find a protective effect in BRCA2 mutation carriers, however (34). Breast differentiation occurs during pregnancy and lactation, and it has been suggested that the population of cancer-prone cells in the breast are reduced as a result. Perhaps surprisingly, given the suppressive effect of breast-feeding on ovulation, no protective effect has been observed for ovarian cancer. Tubal ligation has been shown to decrease the risk of ovarian cancer in the general population and BRCA1, but not BRCA2 mutation carriers (30); the reason for this discrepancy is also unclear. As expected, tubal ligation has no effect on breast cancer risk. The use of the anti-estrogen drug tamoxifen has been linked to breast cancer risk reduction in the general population, as well as in BRCA mutation carriers, but seems to have no effect on preventing ovarian cancer (35). In conclusion, it appears that estrogen is the most important tumour-promoting factor in BRCA-associated breast cancer, whereas OSE proliferation post-ovulation is more relevant to the risk of BRCA-associated ovarian cancer.

### Molecular pathological features of BRCA-associated cancers

The histopathological characteristics and gene expression profiles of BRCA1 and 2-mutated breast and ovarian cancers have been compared with those of their sporadic counterparts (36–38). In both breast and ovarian cancer, BRCA1-associated tumours have phenotypes that are readily distinguishable from those of sporadic tumours. Preliminary indications are that this is also true of BRCA2-associated ovarian carcinomas but less so for BRCA2-associated breast cancers. Phenotypic differences between BRCA-associated and sporadic tumours are summarized in Table II (breast tumours) and Table III (ovarian tumours).

BRCA-associated ovarian carcinomas tend to be predominantly serous adenocarcinoma, with mucinous carcinomas under-represented compared with sporadic ovarian cancer groups. Mutations in BRCA1/2 do not predispose carriers with borderline ovarian tumours, which are rarely detected in this group. P53 dysregulation is more common in BRCA1-associated ovarian tumours than sporadic (39,40), in keeping with the

| Table II. Comparison of the phenotypes of BRCA-associated breast tumours with sporadic tumours |
|----------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Phenoype (compared with sporadic)             | BRCA1-associated                                | BRCA2-associated                                |
| Morphology                                    | Trend to invasive ductal                       | Similar                                         |
| Grade                                         | Higher (more grade 3)                          | Higher (more grade 2)                           |
| Tubule formation                              | Lower                                           | Similar                                         |
| Nuclear polymorphism                          | Higher                                          | Similar                                         |
| DCIS-association                              | Rarer                                           | Similar                                         |
| PFC                                           | Lower                                           | Similar                                         |
| ER expression                                 | Lower                                           | Similar                                         |
| PR expression                                 | Lower                                           | Similar                                         |
| ERBB2 expression                              | Higher                                          | Similar                                         |
| p53 dysfunction                               | Higher                                          | Similar                                         |
| Cyclin D1 expression                          | Lower                                           | Similar                                         |
| MYC amplification                             | Higher                                          | Higher                                          |

BRCA1-associated breast tumours are more phenotypically different to sporadic tumours than are BRCA2-associated breast tumours. PFC, proliferative fibrocystic changes.

| Table III. Comparison of the phenotypes of BRCA1-associated ovarian tumours with sporadic ovarian tumours |
|---------------------------------------------------------------|---------------------------------------------------------------|
| Phenoype (compared with sporadic)                             | BRCA1-associated                                | BRCA2-associated                                |
| Morphology                                                    | Trend to more                                              | Trend to more                                  |
| Grade                                                         | serous carcinoma,                                         | serous carcinoma,                              |
| Borderline tumours                                            | less mucinous carcinoma                                         |
| ERBB2 expression                                              | Higher                                           | Higher                                          |
| p53 dysfunction                                               | Similar                                         | Similar                                         |
| MYC mutation                                                  | Similar                                         | Similar                                         |

BRCA1- and BRCA2-associated ovarian tumours are both phenotypically different to sporadic tumours.
theory that BRCA-deficient cells need to by-pass the p53 apoptotic pathway in order to survive and proliferate. Unlike sporadic tumours, BRCA-linked ovarian tumours do not frequently carry mutations in RAS or amplifications of the MYC gene (41). BRCA1- and BRCA2-associated ovarian tumours seem to have similar phenotypes; this is distinct from the situation in breast tumours where BRCA1 and BRCA2 phenotypes are markedly different.

Recent gene expression microarray analyses have shown that BRCA1- and BRCA2-associated tumours have profiles that are distinct from each other as well as sporadic tumours. This is true of both ovarian and breast cancers, although where comparison was possible, only a few genes were identified as being commonly deregulated in BRCA1-associated breast cancer and BRCA1-associated ovarian cancer (36,38). As with sporadic breast cancer, it seems that a subset of sporadic ovarian cancers share expression profiles with those occurring in BRCA mutation carriers and may, therefore, have hallmarks of ‘BRCAness’ (42).

Differences in clinical behaviour of BRCA-associated cancers

Opinions differ as to the relationship between BRCA1/2 mutation status and survival after developing ovarian epithelial cancer. Three early studies following the survival of ovarian cancer patients with BRCA1 mutations compared with a population-based control group found a highly significant survival advantage (43,44) and an initial survival advantage that disappeared over time in the BRCA1 mutant groups (33). In contrast, a more recent study found that, survival in familial ovarian cases was significantly worse for this group as a whole than for population controls, but that BRCA1/2 mutation status was not predictive of worse outcome compared with non-mutation carriers (45). The discrepancies between these studies may be due to the insensitivity of BRCA mutation testing, difficulties in the selection of control groups and differences between the stage of disease compared. However, more recent prospective studies have lead to a consensus of opinion that the presence of a germline BRCA1 mutation does improve survival of ovarian cancer patients, despite their having an earlier age of diagnosis (46–48). The relationship between BRCA2 mutation status and survival has been less intensively studied, partly due to the decreased frequency of the mutation in ovarian cancer compared with BRCA1. However, two studies did find that age at diagnosis between BRCA2 mutation carriers and sporadic cancer groups did not significantly differ, but that there was a trend towards better survival (46,48).

The improved survival of BRCA-mutated ovarian cancer patients compared with those with sporadic tumours can be explained in terms of the therapy used to treat ovarian cancer. BRCA-associated ovarian cancer appears to respond better to combination platinum-based chemotherapy, independently of characteristics of tumour aggression (46,48). This agrees with findings in vitro suggesting that BRCA-deficient ovarian cancer cells have a greater sensitivity to specific cytotoxic agents through their inability to repair certain types of DNA damage. In support of this, depleting BRCA1 protein in a chemotherapy-resistant ovarian cell line restored sensitivity to cisplatin (49). Human breast cancer cells null for the BRCA1 and BRCA2 genes also show increased sensitivity to cisplatin and carboplatin (50), but since platinum salts are not used as part of the standard breast cancer chemotherapy regime, it is not yet known whether it is a more effective treatment for BRCA1-associated breast cancer. However, a clinical trial is underway to test this hypothesis (www.breakthroughcentre.org.uk).

Conclusions

The BRCA1 and BRCA2 proteins are ubiquitously expressed and have significant roles in general cellular functions including DNA repair, but their dysfunction is associated with tissue-specific carcinogenesis. Both the nature of the mutation in the BRCA gene and variants in other genes can affect the preponderance of breast and ovarian cancer. Additionally, lifestyle and reproductive history can affect the risk of cancer. Importantly, some factors, such as oral contraceptives can have opposing effects on the risk of breast and ovarian cancer. Ovarian tumours associated with BRCA1 and BRCA2 mutations have distinctive features compared with sporadic carcinomas. These features probably underline the preliminary observations of the better response of BRCA mutation associated ovarian cancers to certain chemotherapeutic drugs. In conclusion, it is critical that we understand the functional differences between the BRCA proteins, and how the interplay with genetic and tissue-specific factors influences their tumour suppressor activity. This will have implications for counselling BRCA mutation carriers on strategies to reduce their risk of breast and ovarian tumours. Finally, it will inform choice of the optimal treatment for those who do develop cancer.

Conflict of Interest Statement: None declared.

References


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