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Translational Challenges and Therapeutic Opportunities in BRCA1-Related Breast Cancer

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Abstract

Although significant progress has been made in the management of the hereditary cancer syndrome related to mutations of BRCA1, two fundamental and clinically relevant questions regarding BRCA1-related cancer syndrome remain unresolved: (1) What factors account for the tissue specificity of the BRCA1-related cancer risk? (2) How does a mutation or loss of BRCA1 lead to the basal-like phenotype of breast cancer? This review focuses on recent studies in BRCA1-related pathways that lead to specific characteristics of the hereditary cancer syndrome and discusses the current translational evidence for exploiting these pathways in new therapeutic strategies. Mounting evidence suggests that estrogen signaling and metabolism, oxidative stress, specific secondary mutations, and regulation of specific progenitor cells and transcriptional programs are critical in BRCA1-associated breast cancer. Strategies geared toward estrogen reduction may play a role in treatment and prevention. Therapies aimed at mitigating oxidative stress may be a strategy for risk reduction, while cancer-cell-specific sensitivity to oxidative stress may also be an opportunity for specific targeting. BRCA1-related transcriptional regulation and signaling provide a number of therapeutic targets, including the PI3-AKT and Notch pathways. Thus, significant opportunities exist in translational and clinical research for developing the treatment strategies for the management of BRCA1-related breast cancer.

Keywords: BRCA1, basal-like breast cancer, DNA repair, estrogen, reactive oxygen species

1. Introduction

In 1866, Pierre Paul Broca described the remarkable pedigree of his wife's family in his treatise *Traité des Tumeurs*: Of 19 women from five generations who lived to the age of 30 years, cancer developed in 14 women, including nine cases of breast cancer [1]. The French physician and surgeon observed: "This deadly predisposition, impossible to foretell, impossible to escape, inaccessible to surgery, and until now even inaccessible to internal or medical treatment, is an indication of a general state which precedes each local manifestation ... in certain cases this predisposition transmits itself by heredity through several generations" [1]. Progress was slow for the next 100 years. Further identification of family pedigrees suggested the hereditary passage of breast cancer, but this remained controversial [2]. With the second half of the twentieth century came the recognition of a familial breast cancer syndrome that in some cases could be associated with an autosomal dominant allele encoding a tumor suppressor gene [3]. In 1990, linkage analysis mapped the putative allele to chromosome 17q21, and the gene, BRCA1, was finally cloned in 1994 [4, 5]. The identification of BRCA2 followed a similar trajectory, with its localization to 13q12-13 in 1994 and cloning the following year [6, 7]. In addition to BRCA1/2, a number of genes have been implicated in familial breast cancer with varying degrees of conferred risk that are generally inversely related to population allele frequency; BRCA1, for example, has rare mutations with high penetrance [8]. Since that time, significant advances have been made in understanding the risks and mechanisms of BRCA1/2-related carcinogenesis. For patients with BRCA1/2 mutations, estimates of lifetime cancer risk widely vary due to different studies of cohorts and varying penetrance attributed to different mutation-related phenotypes, family/genetic history, and environmental exposures. Cumulative risk by 70 years of age for patients with germline BRCA1 mutations ranges between 46% and 87% for breast cancer and between 27% and 63% for ovarian cancer, with the low-risk and general population studies falling in the lower range of the estimate and high-risk families in the upper range. For BRCA2 mutations, the risks are 31–56% and up to 11%, respectively [9]. Beyond female breast and ovarian cancer, BRCA1 mutations may also be associated with the risk for melanoma, whereas BRCA2 is associated with male breast cancer, pancreatic cancer, and prostate cancer [10].

A number of therapeutic strategies have been developed to manage hereditary breast cancer. The National Comprehensive Cancer Network evidence-based guidelines provide an algorithm for the management of hereditary breast cancer, including identification of high-risk individuals and families, genetic testing, cancer screening, risk mitigation through prophylactic salpingo-oophorectomy and possible mastectomy, locoregional and systemic treatment, and ongoing surveillance [11]. Newer therapies and combination strategies are being designed to target more specific features of the cancer genotype and phenotype. The use of PARP inhibitors in the model of synthetic lethality to exploit deficiency in homologous repair in BRCA1/2 deficient cells is one such example [12–15]. This targets one aspect of BRCA1/2 function, but the development of further strategies is desirable, especially as BRCA1-related cancer and cancer risk have a more complicated etiology than defective homologous repair alone. The BRCA1 cancer syndrome may be related to the myriad cellular processes in which BRCA1 is involved, including recognition and repair of genomic damage, regulation of

chromosome sorting and mitosis, control of cell-cycle checkpoints, protein ubiquitination, cell signaling, and transcriptional regulation [16–20]. Further, abrogation of BRCA1 function can be the result of a multitude of lesions and processes with variable penetrance and relative hypomorphism, including mutations resulting in a premature stop codon with or without mRNA transcription and expression of a truncated protein, mutations resulting in loss of the function of particular functional domains, intronic mutations resulting in splice variants, large rearrangements, variation in mRNA splicing, methylation and silencing of the gene, and regulation by microRNA [21–23]. Although most BRCA1 mutant mRNAs have shortened half-life, some BRCA1 mutant proteins are translated [24]. However, characterizing cellular localization and function of these proteins is difficult. Staining for intracellular BRCA1 in mutants is inconsistent, as patterns vary with different antibodies, fixation methods, and methods of exposing epitopes, which may lead to a lack of correlation between BRCA1 staining and qPCR levels [25, 26]. Despite nearly two decades of genetic testing, there remain a significant number of variants of uncertain significance. Even when a mutation is established as pathogenic, targeted therapies against DNA repair may not always be effective, as preclinical data suggest in the case of at least one common pathogenic mutation, C61G [27].

Out of this complicated picture, two fundamental questions arise regarding specific characteristics of the BRCA1-related cancer syndrome, both with the potential to guide cancer prevention and therapy: (1) What accounts for the tissue specificity of the BRCA1-related cancer risk? (2) How does mutation or loss of BRCA1 lead to the basal-like phenotype of breast cancer, as opposed to the luminal phenotype of BRCA2 mutations? We searched PubMed for English-language studies and reviews related to BRCA1 function, estrogen metabolism, oxidative stress, basal-like breast cancer, and BRCA1-related therapy. Reference lists of selected articles were searched to track the provenience of key ideas and findings.

2. BRCA1/2 and carcinogenesis

The best known and perhaps dominant roles for the BRCA proteins in tumor suppression lie in the maintenance of chromosomal stability, a role played in nearly all tissues [28]. Both BRCA1 and BRCA2 are essential for homologous repair, a high-fidelity repair mechanism for double-stranded breaks and daughter strand gaps, lesions that can arise from DNA damage and at stalled replication forks [29, 30]. Lack of BRCA proteins results in these lesions being shunted into error-prone repair pathways resulting in chromosomal rearrangements and deletions [30–32]. Both BRCA1/2 have additional roles in chromosomal stability: BRCA1 functions in the recognition of DNA damage and the recruitment and assembly of protein complexes for repair of lesions, and BRCA2 stabilizes stalled replication forks to allow for repair rather than degradation and prevents spontaneous hyperrecombination [28, 33, 34]. BRCA1 also heterodimerizes with BARD1 at the N-terminal ring domain, conferring E3 ubiquitin ligase activity and regulating mitotic spindle assembly; loss of this interaction also results in loss of tumor suppressor activity [27, 35]. Dysregulation of the mitotic spindle assembly as well as centrosome amplification, along with failure of the G2-M checkpoint, leads

to defects in chromosome segregation, abnormal division, and aneuploidy [35, 36]. BRCA2 may also have a role in cell-cycle checkpoint control [37].

Maintenance of chromosomal stability alone cannot explain comprehensively the related cancer syndrome beyond an increased risk of carcinogenesis. Other factors are needed to account for the tissue tropism of BRCA1/2-related cancers as well as the particular phenotype of BRCA1-related cancer: basal subtype cancer developing specifically in the epithelium of the breast and papillary serous cancer developing most likely in the fimbria of the fallopian tube – cancers that have similar mutational profiles and likely similar early driving events in carcinogenesis [38]. Several explanations may account for the tissue specificity of the carcinogenic potential. First, breast and fallopian tube cells are subject to a unique exposure resulting in accumulating mutations and genomic damage, possibly related to the genotoxic effects of estrogen metabolism and the generation of reactive oxygen species (ROS), or related to abrogation of normal cell-cycle control in tissues periodically undergoing multiple cycles of rapid proliferation. These tissues may provide an environment that is permissive for, or even driving, cell survival and proliferation despite mounting genomic damage. Further, functions of BRCA1 unrelated to genomic stability may contribute both to the tissue-specific risk as well as the particular phenotype including transcription-related roles in the regulation of mammary progenitor cells and moderation of the proliferative effects of estrogen signaling.

2.1. BRCA1 cancer tissue specificity: estrogen and oxidative stress

Maintenance of the genome alone cannot explain the tissue-specific nature of BRCA1-related cancer risk. It is well documented that cumulative estradiol exposure is linked to lifetime risk of the development of breast cancer [39]. Estrogen-linked carcinogenesis could be related to the transcriptional program of estrogen signaling, which promotes cell proliferation, or to the toxic side effects of estrogen metabolism. BRCA1 interacts with the classical estrogen signaling pathway in combination with BARD1 by repressing ER α -related transcription through ubiquitination, a function lost with deleterious mutations of the BRCA1 RING domain [40, 41]. However, estrogen signaling is not restricted to the nuclear receptors ER α and ER β and likely plays a role in estrogen receptor negative cancers. Recent studies have shown an alternative mechanism of BRCA1 cell survival based on nonclassical binding of estrogen to cytoplasmic and membrane-associated proteins with downstream effects preventing damage from oxidative stress [42]. Gorrini et al. showed that both oxidative stress and estrogen induced the expression of NRF2, a master regulator of antioxidant capacities, through the PI3K-AKT pathway, and that NRF2 induced by estrogen was crucial for cell survival. They also showed that apoptosis may be prevented in BRCA1 knockdown mammary epithelial cells with exposure to estrogen [43, 44]. This is consistent with clinical observations that reduction in estrogen load reduces risk of cancer in women carrying a BRCA mutation, even if BRCA1-related cells are estrogen receptor negative [45].

BRCA1 mutants may be particularly sensitive to estrogen metabolites, and the early risk of developing cancer reflects the rapid, uncorrected accumulation of genotoxic damage from exposure to both estrogen metabolites and reactive oxygen species produced by oxidative metabolism of estrogen through the catechol pathway, a topic reviewed by Yager and David-

son [39]. Metabolism of estrogen by cytochrome P-450 enzymes, including some tissue-specific enzymes, leads to the formation of estrogen-3,4-quinone, which can form stable DNA adducts and depurinating DNA adducts resulting in mutagenesis. Reduction of oxidized estrogen metabolites leads to reactive oxygen species, which may further damage DNA, proteins, and lipids [39]. Recently, Santen et al. have shown in ER knockout mice the dose-dependent accumulation of toxic estrogen metabolites and concordant rates of tumor formation along with mitigation by estrogen reduction via oophorectomy or aromatase inhibitor treatment [46]. Further, Savage et al. showed that treatment with the estrogen metabolites, 2-hydroxyestradiol and 4-hydroxyestradiol, resulted in double-strand breaks, produced primarily during S-phase, and that BRCA1 deficiency, including both heterozygous and homozygous mutants, led to increased double-strand breaks and loss of efficient repair [47]. Further, wild-type BRCA1 represses the expression of estrogen metabolizing genes, resulting in decreased damage to DNA [47].

Reactive oxygen species (ROS) are produced during normal aerobic metabolism and multiple other cellular processes. ROS and the redox state of a cell are also essential components in cell signaling and homeostasis. Imbalance of pro- and antioxidant factors, whether from endogenous sources or exogenous sources (e.g., UV radiation and tobacco), results in oxidative damage to nucleic acids, amino acids, and fatty acids, and contributes to a number of disease processes. Among the most common DNA lesions resulting from oxidative stress is 8-oxoguanine, which results in a mutagenic template during DNA replication resulting in base pair substitutions and stalling of RNA polymerase II at the site of the lesion with inhibition of nucleotide excision repair [48, 49]. Bae et al. showed that BRCA1 has a role in the response to oxidative stress by upregulating expression of antioxidant genes and enhancing the activity of NRF2 [50]. Further, BRCA1 maintains balance of the cellular redox state, making cells more resistant to exogenous oxidative stress. BRCA1 overexpression and deficiency result in increased and decreased resistance to exogenous oxidative stress, respectively [50]. Besides activating cellular defenses to oxidative stress, BRCA1/2 also mediate repair of DNA damage resulting from oxidative stress. Le Page et al. showed that BRCA1- and BRCA2-deficient cells are unable to repair 8-oxoG lesions and that reconstitution of wild-type BRCA proteins leads to recovery of the transcription-coupled repair mechanism [49]. These studies suggest that BRCA1 tumor suppression involves mitigating the damage of oxidative stress before it is required to repair the resulting DNA lesions.

2.2. BRCA1 cancer phenotype: progenitor cells and transcriptional regulation

Tumors arising in the setting of a germline BRCA1 mutation share common features from the level of genomic alterations, gene expression, histologic phenotype, clinical behavior and prognosis, and response to therapy. Histologically, they are high grade with high mitotic index, pushing tumor margins, central necrosis, and a lymphocytic infiltrate [51]. A subset of sporadic tumors, often demonstrating a relative decrease in BRCA expression through mechanisms other than germline loss, and arising in the same tissues as germline mutants, appears to share this constellation of traits [52]. Turner et al. coined the term BRCAness to identify “the phenotypes that some sporadic tumors share with familial-BRCA cancers” [52]. This is in

contrast to BRCA2-related breast cancer, which has a significantly different gene expression profile, and which is more typically lower grade, more differentiated, appearing later in life, and of the luminal/ER-positive subtype [51, 53]. Interestingly, the relative risk profiles for BRCA1 and BRCA2 mutations are also different, further suggesting different etiologic mechanisms. Compared to the aged-matched general population, the relative risk for BRCA1 mutation carriers is greatest in the young population and approaches the population risk in the later decades of life; the relative risk for BRCA2 remains constantly elevated over the population risk throughout the patient's lifetime [54].

BRCAness may be a feature related to the progenitor cell of origin from which these cancers arise. Foulkes hypothesized that BRCA1 acting as a regulator of mammary stem cell function may drive the phenotype of BRCA1-related cancers [55]. In this model, immortal mammary stem cells absent a BRCA1 signal would maintain a relatively undifferentiated, proliferative phenotype that would require very few additional genomic "hits" in order to become malignant; genomic instability conferred by loss of BRCA1-mediated DNA repair functions would account for the proclivity for malignant derangement and the early age of presentation. The general model is appealing, although it appears more likely that BRCA1-associated cancer arises from luminal epithelial progenitors, not mammary stem cells [56].

The mammary epithelium can be sorted into subgroups representing different stages of the differentiation from multipotent mammary stem cell to mature luminal epithelium, which requires BRCA1 for proper development [57]. Depletion of BRCA1 results in failure of mammary cells to differentiate and form acini in culture, but increases cell proliferation [58]. Liu et al. showed that BRCA1 knockdown increases stem/progenitor cell population while preventing mammosphere formation [59]. Furthermore, in human mammary tissue, BRCA1 heterozygotes showed lobules comprised of ALDH1 (stem cell marker) positive cells with minimal ER expression and evidence of BRCA1 loss of heterozygosity. These lobules occurred in normal tissue, showing that mammary progenitor cells can survive without BRCA1 expression and create atypical, nonmalignant lobules. This suggests that the tissue tropism of BRCA1-related tumors is due to a permissive environment, possibly due to release of paracrine factors from luminal epithelium, for the survival of BRCA1 negative cells [60].

Lim et al. [62] demonstrated that normal mammary cells sorted by basal and epithelial markers showed varied potency and clonogenic activity *in vivo* corresponding to bipotent progenitor, committed luminal progenitor, and mature luminal cells. These may represent the cells of origin of the different subtypes of mammary epithelial tumors that can be segregated by gene expression [53, 61]. Comparison of gene expression profiles between normal mammary subpopulations of mammary stem cells, luminal progenitor cells, and mature luminal cells showed significant associations with, respectively, claudin-low, basal, and luminal A and B cancer cell populations [62]. Further analysis of these subtypes reveals few somatic mutations common to all breast cancers, but within the well-defined subtypes, genetic and epigenetic changes give rise to their common phenotypes [38]. These tumor subtypes can also be segregated by clinical behavior, prognosis, and response to treatment [63].

Gene-expression profiles of BRCA1-associated tumors correlated most closely with luminal progenitor cells and loss of BRCA1 in a mouse luminal breast cancer model leads to epithelial-

mesenchymal transition (EMT), dedifferentiation, and basal tumor development [62, 64, 65]. BRCA1 transcriptionally regulates a number of genes associated with basal-like breast cancer, including Notch ligands and receptors, with loss of BRCA1 associated with decreased luminal differentiation and ER- α signaling and also with increased basal-like and proliferation markers [66]. Increased Notch signaling due to BRCA1 loss may contribute to the basal-like phenotype as well as suppression of apoptosis [67]. Wild-type BRCA1 represses expression of a number of genes associated with basal-like and BRCA1-related cancers, including FOXC1, *p*-Cadherin, and CK5 and 17 [68, 69]. The luminal progenitor, as the cell of origin for BRCA1-related cancer, is consistent with an important role for ROS as mammary stem cells and multipotent progenitor cells have lower concentrations of ROS than more mature progenitor cells [70].

Around 80–90% of BRCA1 tumors are basal, as opposed to 10–15% of all tumors, although may also sort with the claudin-low subtype [38, 71, 72]. Conversely, around 20% of basal tumors show germline or somatic BRCA1/2 mutation [38]. BRCA1-related cancers exhibit common mutational profiles. A total of 81–89% of BRCA1 tumors, both ER+ and ER–, have a loss of heterozygosity of the wild-type allele, which is correlated with higher grade and increased proliferation [73]. For cells lacking functional BRCA1, cell survival is generally dependent on secondary mutations. BRCA1 tumors commonly show mutations of PTEN and TP53 allowing cell proliferation to continue in spite of mounting genomic irregularities. These mutations appear to follow a general evolutionary pattern preceding the loss of the WT BRCA allele [74]. This is significantly different than tumorigenesis in luminal cancers, with rare PTEN mutations and late loss of TP53. Even without loss of wild-type allele, heterozygotes display altered gene expression, including in genes related to cell differentiation and proliferation [75].

3. Developments in targeted therapy

Therapy for BRCA1/2 related tumors involves surgery, radiation, and systemic chemotherapy and endocrine therapy. Specific treatment for cancer developing in the setting of BRCA1/2 has targeted deficient DNA repair. Platinum-based chemotherapy creates intra- and interstrand DNA crosslinks resulting in double-stranded breaks. In the absence of homologous repair, the accumulation of genomic damage results in cell death. Pegylated liposomal doxorubicin has also been shown to have a survival advantage [76].

PARP inhibitors have been used to exploit the DNA repair defect [13]. PARP-1 binds to DNA strand breaks and signals DNA damage by hydrolyzing NAD⁺ to form poly(ADP-ribosyl) tails on histones and itself, resulting in the recruitment of the protein machinery for repair [77]. PARP inhibitors include a nicotinamide moiety that competes with NAD⁺, inhibiting the enzymatic function of PARP, and trapping the PARP enzyme at the site of DNA damage, preventing repair [78]. Loss of PARP-mediated regulation and repair of single strand breaks leads to stalled replication forks, and double strand breaks develop, which leads to cell death in a process referred to as synthetic lethality. Loss of BRCA1 prevents homologous repair from occurring; loss of PARP function results in loss of regulation of nonhomologous end joining, which leads to error-prone repair, genomic instability, and cell death [79]. A number of clinical

trials assessing the efficacy of PARP inhibitors in BRCA-mutated breast cancer are currently underway, in metastatic disease as well as in the adjuvant and neoadjuvant setting (<http://www.cancer.gov/about-cancer/treatment/clinical-trials>).

However, not all BRCA1-related tumors are sensitive to DNA damaging agents and PARP inhibitors. BRCA1 deleterious mutations in the RING finger domain lose tumor suppressor function related to loss of interaction with PALB but retain some homologous repair activity, rendering them less responsive to PARP inhibitors and platinum chemotherapy [27]. There is also evidence that BRCA1/2-associated tumors gain resistance to platinum and PARP inhibitor therapy through mutations resulting in reversion to the wild-type sequence or other restoration of the open reading frame [80]. Lord and Ashworth also review preclinical data suggesting that loss of 53BP1 or the related RAP1-interacting factor 1 (RIF1), proteins involved in nonhomologous end joining, leads to reduced genomic damage from PARPi-induced nonhomologous end joining and at least partial restoration of homologous repair and survival for BRCA1-deficient cells. However, clinical data supporting this mechanism are lacking [80].

Further specific treatment for BRCA1 cancer risk includes targeting estrogen production and signaling and prophylactic surgery to eliminate or reduce the number of potential tumorigenic cells. Bilateral salpingo-oophorectomy has been shown to significantly reduce the risk of breast cancer incidence by about 50% and breast cancer mortality by 90% [45, 81]. The use of tamoxifen, independent of estrogen receptor status, reduced the risk of contralateral breast cancer in BRCA1 mutation carriers with HR 0.38 (95% CI, 0.27–0.55) in a pooled prospective/retrospective cohort [82]. Specific therapy to prevent formation of ROS or toxic estrogen metabolites without endocrine ablation or blockade present a possible target that would modify cancer risk without the possible side effects and complications of surgery and iatrogenic menopause, including loss of fertility. Alternatively, strategies that exploit increased oxidative stress in tumor cells may provide strategy for targeted therapy. In normal cells, ROS are produced at low concentrations and can be effectively neutralized by the antioxidant system of the cells. In contrast, cancer cells produce elevated levels of ROS due to increased metabolic activity, resulting in a state of chronic oxidative stress. As noted above, BRCA1-mutant cells have a dysregulated response and increased sensitivity to oxidative stress as well as a decreased ability to repair DNA lesions resulting from ROS. As such, induction of ROS-mediated damage in cancer cells by proper pharmacological agents that either promote ROS generation beyond the cellular antioxidative capacity or disable the cellular antioxidant system have been considered as a “radical” therapeutic strategy to preferentially kill cancer cells [83]. Elesclomol, a small molecule that increases ROS production in mitochondria and induces apoptosis, has shown *in vitro* potential for treating breast cancer cells with defective DNA repair [84, 85].

The PI3K-AKT pathway is another target for therapy. PI3K is involved in both oxidative stress and escape mechanisms of DNA repair from PARP inhibitors. Combination treatment with PI3K and PARP inhibitors showed significant efficacy in inhibiting tumor cell growth *in vitro* and reducing tumor volume in mouse models [86]. PI3-AKT also functions downstream of Notch, a critical cancer stem cell regulator associated with basal-like breast cancer, in suppression of apoptosis [87, 88]. Although targeting the Notch pathway alone is not sufficient to reduce proliferation or cause cell death, combination with inhibition of the EGFR

pathway or AKT pathway results in enhanced cell death [88]. These results suggest that combination therapies targeting signaling pathways implicated in basal-like breast cancer or BRCA1-regulated cell function would provide new avenues for combating BRCA1-related breast cancer.

4. Conclusion

A great volume of knowledge regarding the molecular functions of BRCA1 and BRCA2 has developed in the last 20 years since the cloning of the genes. Despite some challenging issues in understanding BRCA1-mutant breast cancer development, there is great potential for advances in tying elucidated molecular pathways in cell and animal models to the clinical and epidemiological presentation of BRCA1/2-related breast cancer (**Table 1**). Such translational advances may be exploited not only to advance the treatment of breast cancer but also to diminish the risk described by Pierre Paul Broca—inaccessible to treat, impossible to escape—so long ago.

Challenges in BRCA1-associated breast cancer	Opportunities in BRCA1-associated breast cancer
1. Mechanisms for the tissue specificity of the BRCA1-related cancer risk	1. Development of targeted therapy based on hyperactive signaling pathways in BRCA1-associated breast cancer
2. Mechanisms for the unique BRCA1-related breast cancer phenotype	2. Improvement of synthetic lethal approaches in the treatment of BRCA1-associated breast cancer
3. Differential effects of BRCA1 mutations in different mammary epithelial cell and breast cancer cell populations	3. Prevention strategies based on BRCA1-associated breast cancer biology
4. Individualized breast cancer risk prediction for BRCA1 mutation carriers	

Table 1. Challenges and opportunities in BRCA1-related breast cancer research and treatment.

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