Breast cancer arising in a BRCA-mutated background: therapeutic implications from an animal model and drug development

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To date, the presence of a hereditary background has not influenced the selection of drug treatment in breast cancer. However, increasingly, negative hormone receptors and Her2 (often referred to as ‘triple negative’) or a medullary carcinoma histology has been reported in BRCA mutation carriers. Accordingly, such patients are often considered for adjuvant protocols based on chemotherapy (and not based on endocrine manipulations or trastuzumab). Mouse models introducing a conditional BRCA-null expression in the breast have recently provided powerful support for cisplatin-based treatment and have implications for the design of adjuvant studies in these patients.

Key words: BRCA, triple negative, breast cancer, PARP1 inhibitor, cisplatin

introduction

Breast cancers display marked heterogeneity in their clinical presentation and outcome. This heterogeneity, in part, has been highlighted by past traditional methods that have been used to classify breast cancer into different subgroups: including immunohistochemical staining of estrogen, progesterone, and her2neu receptors and the assessment of the morphologic characteristics of the tumor. These methods have aided in determining the prognosis and the development of treatment paradigms tailored to various subclasses of tumor. Gene expression profiling of breast tumors has further enhanced our ability to prognosticate and possibly treat the subtypes of breast cancer [1–4].

Gene expression profiling has subdivided breast cancer into five different groupings depending on patterns segregating into different clusters. The luminal A and B subtypes are characterized by estrogen receptor positivity, similarity in appearance to the luminal epithelial cells that line the inner layer of the mammary duct, and high levels of gene expression and positive immunohistochemical staining for luminal cytokeratins 8/18. Another subtype characterized by gene profiling is a group of breast cancers that overexpress her2neu. A ‘normal breast’ subtype is defined by heightened expression of basal epithelial genes and adipose cells and reduced expression of luminal epithelial genes. Finally, the basal subtype of breast cancer is distinguished from the other groups of breast cancer by demonstrating gene expression profiles similar to basal cells that line the outer basal layer of the mammary duct. They are characterized by negativity for estrogen and progesterone receptors and for her2neu (thus, ‘triple negative’) [1, 5–8], by displaying morphologic characteristics similar to the basal myoepithelial cells and by staining positive for Her1, cytokeratins 5/6, and/or cytokeratins 14 [2–9]. Immunohistochemical profiling beyond triple negative enhances the prognostic significance of the findings versus classifications based just on hormone receptor and her2neu negativity [9].

The histologic hallmarks of basal-like breast cancer include a high mitotic rate and grade, focal areas of necrosis, pushing margins suggestive of invasion, and lymphocyte infiltration within the stroma [10]. Basal-like breast cancer has also been found to contain frequent TP53 mutations [11].

Given these pathologic features, it is not surprising that basal-like tumors have been associated with a poor prognosis, displaying a greater tendency toward early and visceral recurrences, shorter breast cancer-specific survival and overall survival when compared with the other subtypes of breast cancer [8, 11–15]. In one population-based study, basal-like breast cancer was more prevalent in premenopausal African-American women and it has been shown to occur at an earlier age than other types of breast cancer [14].

Patients with the basal-like subtype of breast cancer who are treated with surgery and adjuvant anthracycline-based therapy experience shorter metastasis-free and shorter breast cancer-specific survival [16]. On the other hand, multiple clinical trials have demonstrated that triple-negative tumors are more sensitive to neo-adjuvant therapy. In fact, these patients are more likely to achieve a pathologic complete response than patients with luminal tumors [17–20]. Those who achieve
a complete pathologic response to chemotherapy have a better prognosis, regardless of histologic type. However, patients with triple-negative breast cancer who have residual disease after neo-adjuvant therapy are more likely to relapse early and experience shorter survival than patients with other types of breast cancer [17, 19]. As would be anticipated, these patients do not benefit from hormonal therapy or her2neu-targeted therapy. Clearly, better therapies tailored to this subtype of breast cancer are needed.

**BRCA1 and the development of basal-like breast cancer**

BRCA1 is responsible for several cellular functions, with particular emphasis to those related to sensing DNA damage. DNA double-strand breaks (DSBs) can be repaired by one of three mechanisms: nonhomologous end joining (NHEJ), homologous recombination (HR), and single-strand annealing (SSA). During the G1 phase of the cell cycle, NHEJ takes precedence over HR and DSBs are repaired by resealing the two ends of damaged DNA, culminating in a depletion of varying amounts of DNA. SSA results in repair of DSBs by the joining of similar sequences of DNA that surround the DSBs. Consequently, such repair mechanism may result in deletion of the bypassed DNA or translocation of the DNA onto a different chromosome containing a similar DNA sequence to the damaged one. NHEJ and SSA pathways are prone to errors while HR repairing DNA during the S and G2 phases of the cell cycle is ‘error free’: DSBs are copied from the identical sister chromatid. BRCA1 mediates HR via the formation of RAD51 nuclear foci. RAD51 protein forms complexes with BRCA2 and proteins involved in the Fanconi anemia pathway to promote strand invasion and pairing of homologous DNA sequences. HR is thus responsible for repair of stalled replication forks and DNA DSBs. The absence of a functional BRCA1 or BRCA2 gene consequently renders the cell incapable of repairing DNA DSBs by HR and the cellular machinery becomes dependent upon more error-prone methods of DNA DSBs repair—NHEJ and SSA [21]. Accordingly, loss of a functional BRCA1 gene, via a mutation, epigenetic silencing, or enhanced transcription of a negative regulator of BRCA1, promotes chromosomal instability and often results in carcinogenesis. Why breast cancers are the most prevalent manifestation of BRCA dysfunction is unknown.

Gene expression profiling has revealed that tumors from patients who are BRCA1 mutated segregate within the basal subgroup of breast cancers [4]. Tumors from BRCA1 mutation carriers are likely to stain positive for basal cytokeratins 5/6 and 14 and to exhibit negative estrogen receptor staining. These breast carcinomas also commonly stain for epidermal growth factor receptor [22]. BRCA1-associated tumors are commonly high grade and p53 mutated. They also have a tendency to occur in younger women and carry a poor prognosis [23].

Tumors arising in a BRCA1 mutation background most often display a basal-like genotype and similar morphologies and immunohistochemical staining patterns. It is also possible that impaired functioning or reduction of expression level of BRCA1 gives rise to sporadic basal-like breast cancers. Since the BRCA1 gene is located on 17q12-21, Wei et al. assessed whether epigenetic silencing of BRCA1 via promoter methylation and or loss of chromosome 17 could affect BRCA1 expression levels in breast tumors [24]. They found that there were fewer copies of BRCA1 and CEP17 in breast tumors that contained methylated BRCA1 promoters. These molecular findings were more common in young women, with high-grade histology and with negative estrogen and progesterone receptors.

Other mechanisms may be implicated in the silencing of the BRCA1 gene in basal-like breast cancer. Turner et al. [25] examined 37 basal-like breast cancers and 37 age- and histologic grade-matched controls for differences in BRCA1 expression levels. They did not find any differences in BRCA1 promoter methylation, but the basal-like breast cancers had significantly lower levels of BRCA1 messenger RNA and significantly higher levels of ID4 expression than the controls. ID4 is a negative regulator of BRCA1 and so its heightened expression in this cohort of basal-like breast cancer could account for the downregulation of BRCA1.

All this evidence suggests that BRCA1 dysfunction may play a role in the development of triple-negative, basal-like cancers. Recently, this defect in DNA DSB repair associated with BRCA functions has also been used to develop targeted treatment for this aggressive subtype.

**targeting BRCA-defective cells: PARP inhibitors**

Poly (ADP-ribose) polymerase-1 (PARP1) is one of a pair of enzymes involved in base excision repair, which is the principal mechanism for the repair of DNA single-strand breaks (SSBs). Inhibition of PARP1 may lead to decreased repair and increased production of SSBs in DNA throughout the cell cycle. In S-phase, an accumulation of DNA SSBs induces the formation of DNA replication fork arrests, giving rise to DNA DSBs requiring repair. Inhibition of PARP1 in a cell incapable of HR forces the cell to repair damaged DNA with more error-prone pathways like NHEJ and SSA resulting in chromosomal instability, cell cycle arrest, and apoptosis [26].

To assess the effects of inhibition of PARP1, Farmer et al. transfected a plasmid expressing a short interfering RNA-targeting mouse PARP1 into wild-type embryonic stem (ES) and ES cells lacking a wild-type BRCA1 and BRCA2. PARP inhibition diminished clonal growth of the BRCA1- and BRCA2-deficient cells when compared with the wild-type ES cells. Reduction in survival was also observed in BRCA1- and BRCA2-deficient ES cells in comparison to wild-type cells when exposed to the PARP1 inhibitors, KU0058684 and KU0058948. After exposure to the PARP1 inhibitor, KU0058684, ES cells lacking wild-type alleles were found to contain many chromosomal aberrations and to be arrested in the G2 or M phase of the cell cycle. There was also a considerable increase in apoptosis, suggesting that reduced cell survival was due to cell cycle arrest followed by apoptosis [26].

RAD51 subnuclear foci form to execute strand invasion and HR in response to DNA damage. RAD51 foci form in wild-type cell lines and cell lines with a functional BRCA1 and BRCA2 gene indicating that PARP inhibition increases the number of
DNA lesions that need to be repaired via HR. PARP inhibition does not induce the formation of RAD51 foci in cells lacking BRCA1 and BRCA2 [26, 27]. PARP inhibition is also able to inhibit the formation of BRCA2-deficient tumors that are implanted into athymic mice [26]. Taken together, these data demonstrate that tumors lacking functional BRCA1 or BRCA2 are sensitive to PARP1 inhibition.

Malfunction in the HR pathway may engender sensitivity to PARP inhibition [28]. Cell lines lacking several HR-related genes were treated with increasing concentrations of the PARP inhibitor KU0058948. Plasmids containing siRNAs directed against RAD51 and other genes integral to the functioning of RAD51, DSS1, and RAPA1 were transfected into cell lines and exposed to the PARP1 inhibitor. Cell viability decreased after drug treatment [28]. Further support for the importance that HR plays in cells exposed to PARP inhibition was evident in the experiments conducted by Bryant et al. [27]. Cells defective in XRCC2 and XRCC3 were sensitive to PARP inhibition. Reconstitution of these genes rendered the cells insensitive to PARP inhibition.

Thus, the sensitivity of BRCA1- and BRCA2-mutated cells to PARP inhibition appears to be mediated by the inability to repair DNA DSBs by the error-free mechanism of HR. In fact, PARP inhibition reduces the viability of cells depleted of the machinery necessary to maintain a functional HR pathway. PARP inhibitors may effectively target breast cancers that demonstrate a BRCA-like phenotype, namely basal-like triple-negative breast cancers. Moreover, one would expect that enhanced susceptibility of these cells to lethal effects when agents causing SSBs are combined with PARP inhibitors.

targeting BRCA-defective cells: cisplatin

Cisplatin treatment induces the formation of inter- and intrastrand cross-linked DNA adducts. BRCA1 wild-type cells have been shown to display RAD51 foci after being treated with cisplatin, whereas BRCA1-mutated cell lines display few, if any, RAD51 foci. This implies that cisplatin-induced DNA damage is repaired by HR and inability to repair renders BRCA1 mutant cells much more sensitive to cisplatin than BRCA1 wild-type cells [29].

BRCA1 function, therefore, may be a major determinant of sensitivity to cisplatin and perhaps to other chemotherapeutic agents that damage DNA. Accordingly, cell lines carrying a BRCA1 mutation are more sensitive to cisplatin treatment and more resistant to treatment with paclitaxel. Conversely, cell lines with functional BRCA1 are more resistant to treatment with cisplatin and more sensitive to treatment with paclitaxel. Transfection of functional BRCA1 into BRCA1 mutant cell lines reverses their chemosensitivity profile with the cells becoming more sensitive to paclitaxel and less sensitive to cisplatin. Reconstitution of BRCA1 results in increased apoptosis in response to treatment with paclitaxel and reduced cell death in response to treatment with cisplatin [30].

Given that BRCA1-deficient cells have an impaired ability to repair DNA damage via HR, cisplatin may effectively target breast cancers that demonstrate a BRCA-like phenotype basal-like triple-negative breast cancers. BRCA1 expression modulates sensitivity to different therapeutic regimens, suggesting that BRCA1-mutated and basal-like breast cancers that harbor dysfunctional BRCA1 may benefit from a more targeted approach to therapy than the empirically, historically derived regimens used so far.

developing targeted therapies directed against basal-like breast cancers

mouse models of BRCA1-mutated tumorigenesis

Conventional BRCA1 knockouts that are bred to homozygosity do not survive. Heterozygous BRCA1 female mice do not readily develop tumors. A BRCA1-null mouse model was, therefore, needed to examine the role that BRCA1 plays in breast cancer tumorigenesis, to study the mechanisms that contribute to the growth of these BRCA1-mutated tumors and to assess treatment interventions. For the latter, however, it would have been difficult to utilize tumor suppressor gene knockouts in mice simulating human tumors because the targeted mutation would be present in every cell of the animal.

A clue in developing an appropriate mouse model was that tumors arising in women with BRCA-1-mutated tumors often harbored TP53 mutations. Jonkers and his group set out to construct a mouse model that contained loss of BRCA1 and p53 [31]. Since P53 knockout mice typically develop lymphomas and sarcomas rather than epithelial tumors, they generated a conditional mammary tumor model with tissue-specific inactivation of p53. They first crossed p53 knockout mice with K14cre mice that had cre recombinase expression restricted to several types of epithelial tissue, including mammary tissue. Mice with conditional knockout of BRCA1 were then crossed with K14cre mice and the K14cre/p53 knockout mice to develop mice with epithelial-specific loss of BRCA1 and mice with epithelial loss of BRCA1 and p53. To study the effects of epithelial BRCA1 and p53 on mammary tumor development, mice with complete knockout of p53/BRCA1, mice with loss of one BRCA1 allele and complete knockout of p53, and mice with complete knockout of BRCA1 and loss of one p53 allele were generated [31].

The majority of tumors developing in the conditional mouse model with knockout of both p53 and BRCA1 resembled invasive ductal carcinoma with high-grade features. The tumors were an intermediate to high grade with a high mitotic count and nuclear grade. The tumors were estrogen receptor negative and basal cytokeratin 5 positive. They contained significantly more chromosomal aberrations than tumors from the mouse with only knockout of p53. The mammary tumors were deemed similar to the tumors that arise in women who carry mutations for BRCA1 [31]. This mouse model of carcinogenesis was, therefore, queried for testing the effectiveness of various therapies designed to target BRCA1-associated or basal-like breast cancers.

further implications for drug resistance

Metastatic tumors often become resistant to anticancer therapy and patients are left with few, if any, viable treatment options. Discerning mechanisms of resistance and how to overcome...
them can broaden therapeutic options available to patients [32]. The BRCA1/p53 knockout mouse may prove useful for in vivo testing of drug efficacy and resistance. The mammary tumors that developed in the Jonkers mouse model were treated with docetaxel, doxorubicin, and cisplatin. The tumors never developed resistance to cisplatin, but did develop resistance to doxorubicin. Gene expression profiling was used to analyze doxorubicin- and docetaxel-resistant tumor as well as untreated tumors to discover any gene expression patterns that correlated with drug resistance. Hierarchical clustering revealed that 45 genes were significantly upregulated in the doxorubicin-resistant tumors. The only genes that could possibly explain resistance to doxorubicin were Mdr1a and Mdr1b which encode a P-glycoprotein, known to confer drug resistance by transferring drugs out of cells; moreover, there was documentation of increased transport of doxorubicin out of the doxorubicin-resistant tumors [32].

By contrast, tumors that arise in the conditional BRCA1/p53 knockout are initially sensitive to cisplatin, but they eventually recur. Treatment of clinically recurrent mouse mammary tumor with cisplatin is characterized by initial response with eventual rapid progression. These data suggest that there may be a small group of cells within the tumor that are resistant to cisplatin and these cells tend to be selected preferentially with more cisplatin treatment. Tumor cells resistant to cisplatin treatment exhibit enhanced clonogenic survival capability indicating that these might be progenitor or cancer stem cells that give rise to a progeny of sensitive cells but eventually are selected with continued cisplatin exposure [33].

A hallmark of BRCA-mutated cancers that has emerged is their sensitivity to cisplatin. This relationship has been further strengthened by evidence that changes in the BRCA mutational status may induce cisplatin resistance in these cancers. Recently, it has been shown that secondary mutations that develop in the BRCA2 gene in BRCA2-associated ovarian and pancreatic cancer cell lines can restore function to BRCA2. Several of the cell lines with restoration of BRCA2 function exhibit RAD51 foci formation after exposure to ionizing radiation. They are also capable of repairing DNA DSBs with HR. Subsequently, these cell lines are no longer sensitive to cisplatin or PARP inhibitors. Examination of human BRCA2-associated ovarian carcinoma that is resistant to cisplatin has revealed loss of the BRCA2 mutant allele [34]. Experimentally, rescue of BRCA2 function restores the cell’s capacity for HR and causes tumors to become resistant to platinum [35].

The mechanism of platinum resistance that develops in BRCA1-associated breast tumors is not yet clearly understood clinically. Secondary BRCA1 mutations that arise in ovarian cancer restoring its function may confer resistance to cisplatin. Nine recurrent ovarian cancer tissue specimens from BRCA1 mutation carriers were analyzed to determine mutational status and how this affected sensitivity to cisplatin. Six of the cancers had developed resistance to cisplatin and three of the cancers were still responding to cisplatin. Four of six resistant tumors had developed secondary BRCA1 mutations and two of these tumors expressed BRCA1 protein on immunohistochemical staining. Rescue of BRCA1 function in these tumors may have restored the functioning of the HR pathway, causing the tumors to become resistant to cisplatin [36].

emerging approaches to the treatment of triple-negative breast cancer

Preclinical models of BRCA1- and p53-deficient cell lines have demonstrated sensitivity to topoisomerase I and topoisomerase II inhibitors and to platinum compounds [37]. Although breast and ovarian BRCA1-mutated breast cancers may eventually become resistant to platinum therapy, there has been a resurgence of interest in the use of platinum to treat triple-negative breast cancer. Given the degree of sensitivity that high-grade basal-like triple-negative breast cancer exhibit toward multiple chemotherapeutic agents and the increased likelihood that patients with triple-negative breast cancer may achieve a complete pathologic response to neo-adjuvant chemotherapy [17–20], platinums are being incorporated into neo-adjuvant chemotherapy protocols. In response to treatment with neo-adjuvant epirubicin, cisplatin, and infusional fluorouracil, 12 patients in a cohort of 30 patients experienced a complete pathologic response [38]. Phase III clinical trials are investigating platinums in the neo-adjuvant setting and in the treatment of metastatic triple-negative breast cancers [39].

Molecular profiling of basal-like breast cancer has identified genes besides BRCA that may modulate sensitivity to cisplatin. One of these genes is p63. Inhibition of p63 promotes p73-induced apoptosis in triple-negative breast cancers [40]. The p73 and/or p63 expression profile has been implicated as a biomarker that could further indicate cisplatin sensitivity in the neo-adjuvant and metastatic settings [41, 42].

Gene expression analysis has identified molecular markers that may serve as other potential therapeutic targets in basal-like breast cancer. Many of these breast cancers stain positive for EGFR, making it a candidate for targeted therapy [43–45]. Basal-like breast cancer cell lines are more responsive to EGFR inhibitors than luminal breast cancer cell lines. Carboplatin is synergistic with cetuximab in the treatment of these basal-like breast cancer cell lines [43]. This doublet may exhibit significant antitumor activity and may benefit from testing in clinical trials. Other potential proteins that should be targeted in the treatment of triple-negative breast cancer include src and abl kinases. Basal-like breast tumors exhibit a genetic profile that is sensitive to dasatanib, a multitargeted kinase inhibitor of src and abl [46]. Dasatanib reduces the growth of basal-type breast cell lines [47] providing support for the inclusion of dasatanib in clinical trials.

conclusions

Sporadic basal-like breast cancers often display striking similarities to BRCA-mutated breast cancers. There are drugs currently available, namely the platinum agents, and drugs that are undergoing widespread clinical testing, such as the PARP inhibitors that have been shown to effectively target BRCA1-mutated breast cancers. These drugs, as well as inhibitors directed to certain molecular markers like the EGFR and src, have been incorporated into clinical trials designed to treat triple-negative breast cancers. Combinations of targeted and chemotherapeutic agents seem worth exploring, particularly since targeted agents may potentiate the therapeutic effects of
chemotherapy with lesser contribution to its toxicity. Clinicians need to be aware of the refinements in the definition of triple negative vis-à-vis basal-like breast cancer, the pathogenetic link with BRCA protein function, and other emerging biological concepts in order to evolve the next generation of clinical trials in this unique and important subset of breast cancers.

**references**


