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BRCA1 deficient Mouse Models to Study Pathogenesis and Therapy of Triple Negative Breast Cancer

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Abstract

Genetically engineered mice along with allograft and xenograft models can be used to effectively model triple negative breast cancer both for studies of pathophysiology as well as preclinical prevention and therapeutic drug studies. In this review eight distinct genetically engineered mouse models of BRCA1 deficiency are discussed in relationship to the generation of triple negative mammary cancer. Allograft models derived from some of these genetically engineered mice are considered and xenograft models derived from breast cancers that developed from *BRCA1* mutation are presented. Examples of the use of genetically engineered, allograft and xenografts models for preventive and therapeutic studies are presented.

Keywords

BRCA1; mouse models; triple negative breast cancer

INTRODUCTION

One of the clinical settings in which triple negative breast cancer (TNBC) occurs is when patients carry a mutation in the Breast Cancer 1 (*BRCA1*) gene [1–4]. In the laboratory there are a number of preclinical mouse models of BRCA1 deficiency that can be used to study the pathogenesis and therapy of TNBC. This review provides detailed comparative information on the pathophysiology and mammary cancer pathology of eight different genetically engineered mouse models of *Brca1* mutation as well as consideration of current allograft and xenograft models in relationship to the study of TNBC.

In the published literature to date, five distinct genetic approaches have been used to develop genetically engineered conditional mouse models of BRCA1 deficiency and perspectives on some of them have been discussed previously [5,6]. One approach deletes only exon 11 of the full-length *Brca1* gene leaving expression of the short *Brca1* transcript (*Brca1*^{Δ11}) [7–14].

Four other approaches delete different exon groups (exon 2 (*Brca1*^{Δ2}) [15], exons 5–6 (*Brca1*^{Δ5–6}) [16], exons 5–13 (*Brca1*^{Δ5–13}) [17] or exons 22–24 (*Brca1*^{Δ22–24}) [18]) that are reported to result in functionally null *Brca1* alleles. Loss of full-length BRCA1 alone increases the prevalence of mammary cancer development but is associated with long latency in the exon 2 deletion model [15] and a low frequency of cancer development in the exon 11 deletion model [7]. Combining BRCA1 loss with loss of p53 function significantly increases cancer incidence [8,9]. Loss of normal p53 function is reported to frequently occur in basal-type human breast cancer that develop due to *BRCA1* mutation [19] and can be found in the genetically engineered mouse models with disrupted BRCA1 [15]. All of the models discussed above are termed conditional mouse models because loss of BRCA1 is conditionally targeted to mammary epithelial cells using Cre-lox technology [20]. Conditional gene targeting is used because germ-line BRCA1 deficiency results in death during embryogenesis [21–23]. When Cre-lox technology is used the DNA encoding the areas of the *Brca1* gene to be deleted are flanked by lox sites, which are DNA elements recognized by the enzyme Cre recombinase that mediates a genetic recombination event resulting in deletion of specific areas of the *Brca1* gene. Expression of the Cre recombinase is spatially regulated by the use of enhancer/promoter sequences that target Cre expression to mammary epithelial cells including the Mouse Mammary Tumor Virus Long Terminal Repeat (MMTV-LTR), Whey Acidic Protein (WAP), K14 and β-Lactoglobulin (BLG) [24–26]. The MMTV- and K14- Cre transgenes are active in mammary epithelial cells of non-pregnant mice but the WAP- and BLG- Cre transgenes require pregnancy for efficient gene deletion in mammary epithelial cells. Cre lines with sufficient but low consistent expression are preferentially used due to the possibility of Cre-induced toxicity when it is expressed at high levels [27]. These genetically engineered preclinical mouse models of BRCA1 deficiency are reasonable phenotypic copies of the human disease. As detailed below, some already have been characterized as developing TNBCs sharing the same aberrant gene expression patterns and/or histology found in women [28]. Since this phenotype mirrors what is observed frequently in *BRCA1* mutation carriers, the models are useful for preclinical tests of prevention strategies [12] and cancer therapeutics [29–32].

One model of germ-line *Brca1* haploinsufficiency with coincident germ-line p53 haploinsufficiency (*Brca1*^{+/-}/*p53*^{+/-}) has been reported that develops basal TNBC following delivery of 5 Gray (Gy) of ionizing radiation at 4–6 weeks of age [28,33].

Two knock-in *Brca1* mutant mouse models were generated to study specifically the interaction between BRCA1 and CHK2 checkpoint homolog (*S. pombe*) (CHK2) (*Brca1*^{S971A/S971A} mice) [34] and BRCA1 and Ataxia-telangiectasia (ATM)-mutated (*Brca1*^{S1152A/S1152A} mice) [35]. These mouse models develop mammary cancers following 4 cycles of 3 Gy irradiation delivered between 4 and 8 weeks of age; however, the cancers are not yet sufficiently characterized to determine if any represent the basal or TNBC phenotype.

Alternatives to the use of the genetically engineered mice include allograft and xenograft mouse models of BRCA1 deficiency. The allograft models utilize mammary epithelial cancer cells from one of the genetically engineered mouse models that are then implanted into new murine hosts. The xenograft models employ human breast cancer cell lines that carry *BRCA1* mutations implanted into immunologically deficient mice. The advantage of these approaches for therapeutic studies is that a number of host mice can be implanted simultaneously and followed in real-time to test the effect of different cancer therapeutic interventions [9,29,36,37].

Overview of BRCA1 Function in Mammary Epithelial Cells

BRCA1 is a multi-functional protein. It is involved in DNA damage repair, regulation of cell cycle checkpoints and centrosome duplication [38], interacts with multiple cellular proteins including the nuclear hormone receptors Estrogen Receptor alpha (ER α) and Progesterone Receptor (PR) [39–44], CHK2 [16,34], ATM-mutated [35], demonstrates ubiquitin 3 ligase activity in combination with BRCA1 associated RING domain 1 (BARD1) [45], and appears to have a role in regulating the growth of mammary epithelial stem cell populations [46]. Mouse models have shown that loss of BRCA1 function causes genetic instability [47], increasing the risk for cancer development, especially when another critical genetic lesion occurs, like a mutation in p53 [9,15]. Loss of BRCA1 disrupts the normal regulation of estrogen and progesterone signaling pathways in the mammary gland and mice lacking full-length BRCA1 in their mammary epithelial cells show abnormal growth responses to both estrogen and progesterone [11,13,48]. ER α over-expression in combination with BRCA1 deficiency and p53 haploinsufficiency increases incidence of cancer development with approximately half of the cancers developing characterized as ER α and PR negative [13]. Ovariectomy [10] and administration of the progesterone and glucocorticoid receptor antagonist mifepristone [14] reduce cancer prevalence in mouse models although, interestingly, tamoxifen acts as an agonist in the setting of BRCA1 deficiency in mice resulting in an increased cancer development [12]. Significantly the cancers that develop are all ER α -negative. Ovariectomy is recognized as an important intervention that can reduce the risk of breast cancer development in women who carry a *BRCA1* mutation [49]. In women *BRCA1* mutation is associated with an expanded luminal progenitor mammary cell population whose growth was shown to both show a reduced requirement for and respond more vigorously to a B27 supplement (http://tools.invitrogen.com/content/sfs/productnotes/F_051222_B-27WOvitaminA-TS-TL-MKT-HL.pdf) containing a variety of growth factors including corticosterone and progesterone that are known to influence normal and BRCA1 deficient mammary epithelial cell growth and survival [14,48,50,51].

GENETICALLY ENGINEERED MOUSE MODELS OF BRCA1 DEFICIENCY AND DEVELOPMENT OF TRIPLE NEGATIVE BREAST CANCER

The *Brca1*^{f11} mouse models

The initially published *Brca1*^{f11} model carried one floxed exon 11 allele (*Brca1*^{f11}) and one allele with a complete exon 11 deletion (*Brca1* ^{Δ 11}) [7]. When exon 11 is deleted only the full length BRCA1 protein is lost and expression of the short form of the BRCA1 protein is retained. Expression of the full-length form of the BRCA1 protein is required for normal development [52]. Even though the short form of the BRCA1 protein is expressed in the *Brca1*^{f11/ Δ 11} mice, the short form is unable to rescue embryonic lethality. This illustrates the functional differences between the full-length and short forms of the BRCA1 protein and the necessity for full-length BRCA1 for normal embryogenesis. The initial report on the *Brca1*^{f11} mouse model concentrated on analysis of pregnancy-associated mammary gland development and carcinogenesis following conditional exon 11 deletion mediated by the WAP-Cre transgene, but also included a subset of mice in which the floxed exon 11 was deleted by a MMTV-Cre transgene. The mice lactated normally however the authors did find that loss of BRCA1 during pregnancy mediated by the WAP-Cre transgene was associated with an approximately 20% reduction in mammary fat pad filling. An increased rate of apoptosis was reported 4 hours after parturition in a subset (30 and 50%, respectively) of *Brca1*^{f11/ Δ 11/WAP-Cre} and *Brca1*^{f11/ Δ 11/MMTV-Cre} mice suggesting that loss of BRCA1 may influence mammary epithelial survival during this active transition time from pregnancy to established lactation [53]. In a genetic variation of the original model when both *Brca1* alleles carry a floxed exon 11 allele (*Brca1*^{f11/f11/MMTV-Cre}), mammary gland development in nulliparous mice approximates wild-type mice unless they are

exposed to an excess of either estrogen or progesterone, which stimulates an abnormal and exaggerated growth response [13,48]. When loss of full-length BRCA1 is combined with either germ-line p53 haploinsufficiency (*Brca1*^{Δ1/Δ1/p53+/-/MMTV-Cre}) or conditional loss of p53 in mammary epithelial cells through Cre-Lox mediated disruption of p53 exons 5 and 6 (*Brca1*^{Δ1/Δ1/p53^{Δ5&6}/p53^{Δ5&6}/MMTV-Cre}), increased mammary epithelial cell proliferation associated with an increased number of ductal branch points is reported [11,14]. Taken together these studies indicate that both the expression level of p53 and hormonal exposure can influence the developmental phenotype of BRCA1 loss of function in the mammary gland. Significantly, these studies focusing on p53 and conditional *Brca1* exon 11 deletions in mammary epithelial cells parallel studies on germ-line *Brca1* deletion where survival of BRCA1 deficient embryos can be rescued by germ-line p53 deficiency [52,54, 55]. In the original report, mammary cancers were reported to develop in 2 of 13 *Brca1*^{Δ1/Δ11/WAP-Cre} and 3 of 10 *Brca1*^{Δ1/Δ11/MMTV-Cre} mice between ages 10–13 months. Aberrant p53 transcripts were noted in 2 of the cancers. Subsequent deliberate introduction of loss of function of one p53 allele resulted in 8 of 11 *Brca1*^{Δ1/Δ11/p53+/-/MMTV-Cre} female mice developing mammary cancers between ages 6 and 8 months. Succeeding reports using a slight genetic variation in that two floxed exon 11 alleles were combined with the MMTV-Cre transgene to effect mammary epithelial cell targeted BRCA1 loss (*Brca1*^{Δ1/Δ1/p53+/-/MMTV-Cre}) showed a similar high percentage of mice developing mammary cancer [12,13]. Approximately 50% of the mammary cancers that develop in the *Brca1*^{Δ1/Δ1/p53+/-/MMTV-Cre} mice demonstrate a TNBC or basal-like phenotype with a gene expression pattern paralleling that found in human TNBC and basal-type cancers [28]. The remainders of the cancers are usually ERα and PR negative and show diverse phenotypes including spindloid. ErbB2 is normally expressed in mammary epithelial cells and its expression has been detected in mammary cancers developing in this model but it is not clear if it is over-expressed at the level found in human Her2/ErbB2 positive cancers [9,56]. Unlike the WAP-Cre driven model, the *Brca1*^{Δ1/Δ1/p53+/-/MMTV-Cre} does not need to become pregnant for efficient development of mammary cancers [13].

The *Brca1*^{f2} mouse model

The *Brca1*^{f2} mouse model contains a conditional floxed exon 2 allele [15]. Exon 2 of the *Brca1* gene contains most of the RING domain and is a genetic model of the mutations in the RING domain of BRCA1 that are reported in some women carrying *BRCA1* mutations [15,45]. BRCA1 interacts with BARD1 and the BRCA1/BARD1 heterodimer has ubiquitin 3 ligase activity that is disrupted by mutations in the RING domain of either BRCA1 or BARD1. While only exon 2 was targeted for deletion in this model, function of BRCA1 is completely lost when exon 2 is deleted and the model functions as a complete BRCA1 null. Mammary cancer development was studied in these mice following conditional exon 2 deletion using the WAP-Cre transgene. The *Brca1*^{f2/f2/WAP-Cre} mice underwent at least one pregnancy-lactation cycle to activate Cre-mediated deletion. Thirty-one of 33 *Brca1*^{f2/f2/WAP-Cre} mice developed mammary cancers with a long latency (T₅₀ = 17 months). The majority of cancers were classified as Dunn Type B adenocarcinomas because they showed a medullary basal-type growth pattern [15], similar to some basal-type TNBCs found in women carrying *BRCA1* gene mutations. Expression of Cytokeratin (CK) 14, one of the markers for TNBC and basal-type mammary cancers, was reported in nearly all the cancers. Thirty out of 35 cancers were ERα negative, all were PR negative, and none showed HER2/ErbB2 amplification. Based on these histological and immunohistochemical analyses, approximately 88% of the cancers were determined to represent a basal or TNBC type. A sub-group of the cancers were analyzed for aberrant p53 expression. Seventy-eight percent showed abnormal nuclear accumulation of p53 and two that were examined demonstrated a R270H mutation corresponding to a known human mutation hot spot (R273). Significantly the *Bard1* gene conditionally mutant mice (*Bard1*^{f2/f2/WAP-Cre})

studied in the same report demonstrated a 90% incidence of medullary-basal-TNBC-type mammary cancers associated with a significant prevalence of p53 mutation. Given the very high prevalence of basal TNBCs in these models, both could be very useful for targeted studies of prevention or therapeutic regimens for basal TNBCs. However, the long latency of cancer formation in this model (17 months) is a relative contraindication to their use as it adds cost and age-related complications. Further characterization of the cancers in these models by gene expression analyses to assess the exact similarities and differences with human TNBC would be a useful next step in assessing their value for translational work. Their findings indicating that BARD1 is also a tumor suppressor for mammary epithelial cells is consistent with identification of *BARD1* gene mutations in familial breast cancer families [57].

The *Brca1*^{f5-6} mouse model [16]

The *Brca1*^{f5-6} mouse model was initially developed to study impact of BRCA1 loss of function in T lymphocytes [58] and later extended to a study that tested if CHK2 deficiency could rescue cell demise due to loss of BRCA1 function similar to what p53 deficiency does [16]. Conditional deletion of *Brca1* exons 5–6 results in a short 509 basepair RNA transcript with several termination codons that theoretically would yield a truncated BRCA1 protein consisting only of the first 49 NH₂-terminal amino acids (aa) from the full-length 1812 aa protein and would be predicted to function as a complete BRCA1 null [58]. Truncating mutations are a relatively common type of BRCA1 mutation found in familial breast cancer families [59]. CHK2 acts in a DNA damage response pathway that results in p53 activation. To study the impact of CHK2 loss on *Brca1* gene mutation related mammary carcinogenesis, *Brca1* floxed exons 5 and 6 alleles were conditionally deleted using a WAP-Cre transgene (*Brca1*^{f5-6/f5-6}/WAP-Cre). These mice were bred to CHK2 loss of function mice (*Chk2*^{-/-}) to generate *Brca1*^{f5-6/f5-6}/*Chk2*^{-/-}/WAP-Cre mice, and bred to p53 loss of function mice (*p53*^{-/-}) to generate *Brca1*^{f5-6/f5-6}/*p53*^{-/-}/WAP-Cre and *Brca1*^{f5-6/f5-6}/*p53*^{-/-}/WAP-Cre mice. Breeding females were used to study mammary carcinogenesis. Only 12% of *Brca1*^{f5-6/f5-6}/WAP-Cre mice develop mammary cancers with a long latency (mean age 19 months). In contrast, 50% of *Brca1*^{f5-6/f5-6}/*Chk2*^{-/-}/WAP-Cre mice develop mammary cancers in a slightly shorter timeframe (mean age 14 months) and 80% of *Brca1*^{f5-6/f5-6}/*p53*^{-/-}/WAP-Cre mice develop mammary cancers by a mean age just under 13 months. Mammary carcinogenesis could not be studied in the *Brca1*^{f5-6/f5-6}/*p53*^{-/-}/WAP-Cre mice due to their death from tumors in other sites secondary to p53 loss before the age at which mammary cancers appear. Cancers are described as acinar adenocarcinomas with some cancers showing areas of squamous differentiation. No further characterization was reported so it is not known whether or not the histology of any of them represent human-like TNBCs.

The *Brca1*^{f5-13} mouse model

Liu and colleagues generated a conditional mouse model with tissue-specific deletions of *Brca1* and *p53* genes resulting in somatic loss of both BRCA1 and p53 in mammary epithelial cells [17]. Deletion of floxed exons 5–13 of the *Brca1* gene results in a shift in the reading frame and the introduction of multiple termination codons in exon 14. The deletion appears to behave as a BRCA1 null as no viable mice carrying homozygous germ-line deletions of exons 5–13 were found suggesting embryonic lethality. This floxed *Brca1* allele was then combined with a *p53* allele in which exons 1–10 are surrounded by lox sites for conditional deletion resulting in null alleles for both the *Brca1* and *p53* genes [26]. Somatic conditional deletion of the *Brca1* and *p53* alleles was executed through the use of a K14-Cre transgene that is reported to mediate deletion in 5–30% of mammary epithelial cells including both luminal and basal myoepithelial cell populations [26]. Like the MMTV-Cre transgene, the K14-Cre transgene is active in other tissues including the salivary gland and

skin [25,26]. *Brca1*^{f5-13/f5-13/K14-Cre} mice showed normal mammary gland development and lactation and none developed mammary cancers even when aged up to 26 months. In contrast, 80% of *Brca1*^{f5-13/f5-13/p53^{fl-10}/K14-Cre} mice developed mammary cancer ($T_{50} = 7$ months). Ninety-one percent of these cancers were described as ER α negative high grade adenocarcinomas. Gene expression profiling demonstrated that these cancers could be grouped together on the basis of an expression pattern associated with the human basal TNBC-type phenotype (including expression of CK5 and p63) that can be clustered with breast cancers from human *BRCA1* gene mutation carriers. In this model the majority of mammary cancers that develop are reported to demonstrate a basal-type related gene expression pattern in contrast to the *Brca1*^{fl1/ Δ 11/p53+/-/MMTV-Cre} in which only approximately 50% demonstrate a basal TNBC type gene expression pattern [28]. However, the proportion of basal TNBCs versus other pathologies in women who carry *BRCA1* gene mutations is reported at 51% [60], more closely approximating the percentage reported in the *Brca1*^{fl1/ Δ 11/p53+/-/MMTV-Cre} mouse model.

The *Brca1*^{f22-24} mouse model

Mammary epithelial cell targeted conditional deletion of *Brca1* exons 22–24 that harbor the second terminal BRCA1 C-termini (BRCT) domain using a β -Lactoglobulin (BLG)-Cre transgene also results in the development of basal-triple-negative type mammary cancers when combined with *p53* haploinsufficiency (*Brca1*^{f22-24/p53+/-/BLG-Cre} mice) [18]. This *Brca1* conditional gene deletion was designed to mimic a truncated human BRCA1 protein mutation but instead results in a null mutation due to complete loss of protein expression. In the study reviewed here [18], mice underwent two pregnancy and lactation cycles to induce recombination and gene deletion. Like other conditional *Brca1* gene deletion mouse models *p53* haploinsufficiency increased the incidence and shortened the latency of mammary cancer development. Twelve percent of *Brca1*^{f22-24/BLG-Cre} mice developed mammary cancers by 15 months of age whereas 64% of *Brca1*^{f22-24/p53+/-/BLG-Cre} mice developed mammary cancer by 8 months of age. Like the MMTV-Cre and K14-Cre transgenes, the BLG-Cre transgene is active in the salivary gland. However, while MMTV-Cre driven conditional *Brca1*^{fl1/ Δ 11} deletion does not result in salivary carcinogenesis, salivary cancers were found at low frequency in these mice. The mammary cancers that developed in the *Brca1*^{f22-24/p53+/-/BLG-Cre} mice were largely ER α negative (26 out of 32), PR negative (all) and did not demonstrate ErbB2 expression. Eleven were classified as ductal carcinomas of no special type (ER α + and -). Significantly, the majority of the cancers contained either spindle differentiation or squamous metaplasia (29 out of 33). Twelve cancers were defined as spindle cell metaplastic breast cancers. Basal cell marker CK14 was expressed in nearly all the cancers including the ER α positive cancers. P63 was expressed in 7 spindle cell cancers and 5 cancers with squamous metaplasia. In comparison to other conditional *Brca1* gene deletion models, this model appears to develop a higher percentage of metaplastic (spindle type) mammary cancers. Spindle cell cancers make up only 30% of mammary cancers found in *Brca1*^{fl1/ Δ 11/p53+/-/MMTV-Cre} mice and are not categorized as basal or TNBC in this model [28]. Specific spindle cell histology is not described in the *Brca1*^{f5-13/f5-13/p53^{fl-10}/K14-Cre} [17] and *Brca1*^{f2/ Δ 2/WAP-Cre} [15] models although frequent squamous differentiation is reported in the *Brca1*^{f5-6/f5-6/p53+/-/WAP-Cre} mice [16].

The *Brca1*^{+/-p53+/-} Irradiation (IR) mouse model

The *Brca1*^{+/-p53+/-} IR mouse model was developed as part of an experiment to test the impact of 5 Gy of ionizing radiation exposure at 4–6 weeks of age on cancer development in the setting of *Brca1* and *p53* gene insufficiency [33]. These mice carry non-conditional germ-line deletions of *Brca1* and/or *p53*. In the absence of irradiation only 10% of *Brca1*^{+/-p53-/-} mice and none of *Brca1*^{+/-p53+/-} mice develop mammary cancers, although there is more frequent development of cancer in other (non-mammary) tissues. However, up

to 20% of the *Brca1*^{+/-p53+/-} mice develop mammary cancers following irradiation. Gene expression analyses have shown that at least some of them demonstrate expression patterns paralleling those found in human basal and TNBC [28].

The *Brca1*^{S971A/S971A} IR mouse model

A second model of irradiation induced mammary cancer formation in the setting of *Brca1* gene mutation is the *Brca1*^{S971A/S971A} IR mouse model. The *Brca1*^{S971A/S971A} model was originally generated to investigate the role of CHK2 mediated phosphorylation of BRCA1 function *in vivo*. Gene targeting was used to make an A971 substitution for S971 interrupting CHK2 induced BRCA1 phosphorylation [34]. *Brca1*^{S971A/S971A} mice do not form spontaneous mammary cancers but 15% developed mammary cancers following 4 weekly 3 Gy irradiation doses starting at 4–8 weeks of age. The cancers were not further characterized so it is not yet known if they represent the basal or TNBC phenotype.

The *Brca1*^{S1152A/S1152A} IR mouse model

A BRCA1 mutant mouse model substituting S1152 of mouse BRCA1 with A1152 by gene targeting interrupting ATM induced BRCA1 phosphorylation was generated to address the role of BRCA1 phosphorylation by ATM *in vivo* [35]. *Brca1*^{S1152A/S1152A} mice do not form spontaneous mammary gland cancers, however, they do develop mammary gland abnormalities such as atypical lobular hyperplasia and lobular carcinoma *in situ* by 18 months of age. Mammary cancers appeared in 18% of the mice following 4 weekly 3 Gy irradiation doses starting at 8 weeks of age. Both ERα positive and negative mammary adenocarcinomas were identified but were not further characterized.

ADJUVANT AND TARGETED THERAPIES IN GENETICALLY ENGINEERED MOUSE MODELS OF BRCA1 DEFICIENCY

BRCA1-deficient human breast cancers with TNBC molecular profiles reap little benefit from tailored therapy regimens that target ERα or HER2. However, hypersensitivity to DNA breaks is thought to be a targetable weakness in BRCA1-deficient cancers because BRCA1-dysfunction is known to compromise homology-directed DNA repair [61]. Drugs that induce DNA double-strand breaks thereby increasing genomic instability in the BRCA1 mutant cells, represent a promising direction for otherwise un-targetable TNBC. The enzyme poly (ADP-ribose) polymerase (PARP) is essential in the repair of single-stranded breaks (SSB) in DNA via the base excision repair pathway and aides in maintenance of genome stability [62]. Inactivation of SSB repair by PARP inhibitors may confer synthetic lethality to cells with defective homology-directed DSB repair [63]. In a 2008 study, Rottenberg and colleagues used the *Brca1*^{f5-13/f5-13/p53fl-10/K14-Cre} mouse model [17] to assess the therapeutic potential of the PARP1 inhibitor AZD2281 (olaparib) [29]. The BRCA1-deficient cancers arising in this model showed a prolonged response to the clinical PARP inhibitor without signs of toxicity resulting in increased survival of the mice. However, longer-term treatment with olaparib resulted in drug resistance as a consequence of significant up-regulation of the P-glycoprotein drug efflux pump. The same group then studied the combination of platinum therapy with olaparib since inhibition of PARP has also been reported to enhance the effects of DNA-damaging anticancer drugs such as platinum in BRCA1-deficient cells. The combination of cisplatin and carboplatin with olaparib significantly prolonged recurrence-free survival and overall survival when compared with platinum monotherapy, demonstrating that PARP inhibition does enhance the effects of DNA-damaging agents. However, similarly to their previous study, most cancers eventually relapsed following this therapy and the mice also showed increased toxicity with these combination regimens [29]. A subsequent investigation from the same group used the *Brca1*^{f5-13/f5-13/p53fl-10/K14-Cre} mouse model to test whether or not a Topoisomerase I

poison (topotecan) would enhance the anticancer effects of the PARP inhibitor olaparib [31]. Their results showed that topotecan alone or in combination with olaparib could be another therapeutic option for TNBC but, like their previous studies, they also found that the mammary cancers eventually acquired resistance and relapsed. Two mechanisms of resistance to Topoisomerase I inhibitors were found in their model, increased expression levels of the drug efflux transporter ABCG2 and reduced protein expression levels of the drug target Topoisomerase I.

CHEMORESISTANCE STUDIES IN GENETICALLY ENGINEERED MOUSE MODELS OF BRCA1 DEFICIENCY

Successful chemotherapy of BRCA1-deficient cancers can be hampered by the development of multidrug resistance. Although *BRCA1* gene mutation associated breast cancers often have a good initial response to DNA cross-linking agents such as platinum analogues and to PARP inhibitors, many cancers become resistant to chemotherapeutic drugs over time resulting in refractory disease [63,64]. Rottenberg and colleagues used the *Brca1*^{f5-13/f5-13/p53^{f1-10}/K14-Cre} mouse model [17] to study the response to doxorubicin, docetaxel and cisplatin and analyze mechanisms of acquired resistance [30,32]. They observed a heterogeneous initial response, similar to what is seen in human patients. However, eventually all cancers became resistant to doxorubicin and docetaxel with up-regulation of ATP-binding cassette (ABC) drug transporters including the drug efflux transporter ATP-binding cassette B1/P-glycoprotein (P-gp) as a mechanism responsible for resistance to doxorubicin. They found that the resistant phenotype could be completely reversed by the third-generation P-gp inhibitor tariquidar [32]. Notably in this study, acquired resistance to platinum compounds was not specifically identified even after multiple treatments. However, despite the lack of evident resistance, the tumors could not be completely eradicated, and cells invariably re-grew from the small tumor remnants remaining after treatment.

BRCA1 DEFICIENT ALLOGRAFT MOUSE MODELS

Genetically engineered mouse models of breast cancer were generated to gain insight into the pathogenesis of BRCA1 mutant cancers. As previously discussed, these models have proven to be essential tools for understanding specific genetic events involved in tumorigenesis and for testing whether molecularly targeted drugs affect an expected target. However, genetically engineered mouse models are sometimes criticized for the unpredictable appearance of tumors, time involved, and complicated breeding schemes. An alternative for this is the generation of mutant cells from mouse tumors and transplanting them as allografts. Deng and others generated three cell lines (W0069, W525 and W780) from primary mammary cancers of *Brca1*^{Δ11/Δ11/p53^{+/-}/WAP-Cre} mice to further characterize and identify other genetic alterations associated with *Brca1* gene mutation related mammary carcinogenesis [9]. These tumor cells displayed genetic alterations of p53 at both DNA and protein levels. The authors characterized these cells as ERα negative, with overexpression of ErbB2/HER2, c-Myc, cyclin D1, Cdc2 and p27 by Western and immunohistochemical analyses. TNBCs lack over-expression of ErbB2/HER2 so these three lines would not be truly representative of TNBC and additional lines may need to be generated for allograft studies of TNBC. However, the generation of these lines suggests that it may be possible to use the same technology to make additional lines, some of which could better represent TNBC. All three of these lines are tamoxifen resistant, but respond to doxorubicin and radiation [9]. Tumor allograft studies using cell line W780 revealed that a high dose of the PARP-1 inhibitor AG14361 results in the inhibition of mammary tumor growth but failed to achieve complete remissions. A discussion of the advantages and disadvantages of tumor allograft studies in predicting the sensitivity and specificity of PARP-1 inhibitors is included

in the report [36]. The same cell line (W780) was used in allograft experiments to study the effect of genistein on mammary tumor growth [37]. The data indicates that genistein suppresses the growth of BRCA1 mutant tumors through cell cycle arrest and mitotic catastrophe. Because W780 cells are p53 deficient, the cell cycle arrest and apoptosis caused by genistein treatment are considered p53 independent.

The use of immunocompromised mice for preclinical testing raises concerns as it makes it difficult to predict the role of the immune system in response to therapies. To avoid this complication, Jonkers and others orthotopically transplanted mammary cancer cells from *Brca1^{f5-13/f5-13/p53^{fl-10}/K14-Cre}* mice, a transgenic line with a high prevalence of TNBC when studied *in situ*, to wild-type mice to study the effect of the PARP inhibitor AZD2281 (olaparib) alone and in combination with platinum drugs [29]. Treatment with olaparib inhibited tumor growth without signs of toxicity but long-term treatment resulted in the development of drug resistance. The combination of olaparib with cisplatin or carboplatin increased overall survival of the mice, suggesting that this agent potentiates the effect of platinum drugs.

Besides their use as preclinical cancer models to evaluate drug efficacy and response, cancer allografts serve as experimental tools to study cancer pathogenesis. To test if over-expression of the protein deacetylase NAD-dependent deacetylase sirtuin-1 (SIRT1) inhibits tumor formation, W0069 cells were transfected with SIRT1, sorted and transplanted into nude mice [65]. The study revealed that transfection of SIRT1 significantly inhibited the ability of W0069 cells to form tumors *in vivo*. The same study showed that pretreatment with resveratrol significantly delayed tumor initiation, and treatment after tumor appearance significantly reduced tumor formation through resveratrol induced apoptosis associated with reduced survivin expression.

BRCA1 MUTANT XENOGRAPH MODELS

The advantage of having BRCA1 mutant human breast cancer cell lines representative of TNBC is that the impact of pathogenic human *BRCA1* gene mutations can be evaluated in the context of a human genetic background. HCC1937 was the first cell line identified as a BRCA1 mutant human breast cancer cell line [66]. The common founder mutation in this cell line consists of the insertion of a cytosine residue at position 5382 of *BRCA1*, resulting in translation of a truncated protein. A study of 41 human breast cancer cell lines subsequently identified three new human BRCA1 mutant cell lines; MDA-MB-436, SUM149PT and SUM1315MO2 [67]. In the MDA-MB-436, the 5396 + 1G>A mutation results in an inframe deletion of 28 amino acids and a truncated BRCA1 protein. In the SUM1315MO2 cell line, the authors identified the common pathogenic AG dinucleotide deletion at position 185 that is prevalent in the Ashkenazi Jewish population. This study also reported a novel BRCA1 mutation in the SUM149PT cell line, consisting of a deletion of a thymine residue at position 2288 resulting in a truncated BRCA1 protein. All four BRCA1 mutant cell lines represent TNBC, but only HCC1937 and MDA-MB-436 harbor p53 protein mutations.

In an effort to establish and characterize a new BRCA1 mutant human cell line, Johannsson and others transplanted tumor fragments from a breast cancer lymph node metastasis carrying a germ-line *BRCA1* gene mutation into immunodeficient mice [68]. The tumor xenograft (designated L56Br-X1) led to the establishment of the L56Br-C1 BRCA1 mutant human cell line. The presence of the common founder germ-line mutation 1806C>T leads to a truncated BRCA1 protein. Immunohistochemical studies confirmed this xenograft to be a TNBC, p53 mutant, and epidermal growth factor receptor (EGFR) and CK8 negative.

Although studies have shown very similar histopathological properties in BRCA1 mutant breast cancers from humans and transgenic mice, human xenograft tumors are a fundamental tool for the study of drug treatments *in vivo*. Experiments using mouse xenografts have been the gold standard in cancer drug development due to the high degree of predictability and rapidity of tumor formation and simplicity in following tumor growth. One of the main limitations in the use of mouse xenografts is that many human cell lines have been cultured on plastic for years. Some argue that cells passaged for so many generations on plastic are not representative of the original tumor in its native state and lack some of the cellular complexity of *in vivo* tumors.

HCC1937 xenografts have been successfully used to study the effect of cisplatin on tumor inhibition, demonstrating the high sensitivity of BRCA1 mutant tumors to platinum-based compounds and the impact of these drugs on the cell cycle [69]. The MDA-MB-436 cell line has been used extensively in xenograft studies, but not all of the investigations concentrated on linking the studies to BRCA1 mutation. However, one preclinical study using the BRCA1 mutant MDA-MB-436 xenograft model was used to demonstrate the efficacy and tolerability of the PARP inhibitor MK-4827 [70]. Pharmacological studies of the heat shock protein 90 (Hsp90) inhibitor PU-H71 using the MDA-MB-436 xenograft model demonstrated a complete response and tumor regression without toxicity [71]. This study showed that TNBCs can rely on the chaperoning function of Hsp90 for proliferation, survival and metastatic behavior and demonstrated that TNBCs, like HER2+ positive tumors, are sensitive to Hsp90 inhibition. A preclinical study on the orally active PARP inhibitor ABT-888 using the MX-1 breast carcinoma xenograft model revealed the ability of this compound to potentiate the efficacy of platinum agents [72]. The study not only showed that the compound ABT-888 in combination with cisplatin or carboplatin resulted in more pronounced effects on tumor regression, but also identified a novel BRCA1 variant, a 33636delGAAA mutation in the *BRCA1* gene that results in translation of a truncated BRCA1 protein. The MX-1 cell line also contains two single-nucleotide polymorphisms in the *BRCA2* gene.

CONCLUSIONS

Genetically engineered mice along with allograft and xenograft models can be used to effectively model TNBC for studies of pathophysiology as well as to test preclinical prevention and therapeutic drug studies. To date, several genetically engineered mouse models have been generated that reproduce effects of BRCA1 deficiency, recapitulate the TNBC phenotype, and can be used to study both cancer prevention and therapy (Table 1). BRCA1 mutant human cell lines of TNBC that can be grown as xenografts model the genetic complexity of invasive TNBC and provide important insights into therapy of this disease (Table 2). This review highlights the applicability of genetically engineered mouse, allograft and xenograft models of BRCA1 deficiency to aid in predicting response and resistance to both single and combination therapeutic regimens including either more general chemotherapeutics or targeted therapies. In the future, mouse models carrying BRCA1 mutations that mimic the common founder mutations in humans may be useful adjuncts to improve translation of the knowledge gained from preclinical studies into the clinic. Additional murine BRCA1 deficient TNBC cell lines that carry different secondary genetic mutations and are able to grow as allografts would facilitate comparative studies of drug response in different genetic backgrounds. Development of more human BRCA1 deficient TNBC cell lines that reflect the genetic diversity of BRCA1 deficient TNBC and function in xenografts studies are a third important goal.

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Table 1

Mouse models of BRCA1 deficiency and phenotype of mammary cancers

Model	Mammary cancers						Agents studied	References
	Brca1 exon deletion or mutation	ERα	PR	HER2	Triple negative-basal gene expression pattern	Histology		
<i>Brca1</i> ^{f11/Δ11} /WAP-Cre	11	NR	NR	NR	NR	Adenocarcinoma	No	[7]
<i>Brca1</i> ^{f11/Δ11} /MMTV-Cre	11	NR	NR	NR	NR	Adenocarcinoma	No	[7]
<i>Brca1</i> ^{f11/Δ11} /p53 ^{+/+} /MMTV-Cre	11	NR	NR	NR	NR	Adenocarcinoma	No	[7]
<i>Brca1</i> ^{f11/Δ11} /p53 ^{+/+} /MMTV-Cre	11	–	–	–	yes	Basal. Adenocarcinoma, Spindloid	Tamoxifen (prevention)	[12,13,28]
<i>Brca1</i> ^{f11/Δ11} /p53 ^{Δ6} /p53 ^{Δ6} /MMTV-Cre	11	NR	NR	NR	NR	NR	Mifepristone (prevention)	[14]
<i>Brca1</i> ^{f12/Δ2} /WAP-Cre	2	–	–	–	NR	Adenocarcinoma (Tubular, solid, squamous)	No	[15]
<i>Brca1</i> ^{f5-6/Δ5-6} /p53 ^{+/+} /WAP-Cre	5–6	NR	NR	NR	NR	Adenocarcinoma	No	[16]
<i>Brca1</i> ^{f5-6/Δ5-6} /Ch2 ^{-/-} /WAP-Cre	5–6	NR	NR	NR	NR	Adenocarcinoma, Adenosquamous	No	[16]
<i>Brca1</i> ^{f5-13/Δ5-13} /p53 ^{fl-10} /K14-Cre	5–13	–	NR	NR	yes	Adenocarcinoma	Doxorubicin, Docetaxel, Cisplatin, Carboplatin, Olaparib, Topotecan, Tarquidar	[17,29,30,31]
<i>Brca1</i> ^{f22-24/Δ22-24} /p53 ^{+/+} /BLG-Cre	22–24	–	–	–	NR	Adenocarcinoma, Spindle, squamous	No	[18]
<i>Brca1</i> ^{f1/-} p53 ^{+/+} IR	11	NR	NR	NR	yes	Carcinoma	No	[28,33]
<i>Brca1</i> ^{f1/-} p53 ^{-/-} IR	11	NR	NR	NR	NR	Carcinoma	No	[33]
<i>Brca1</i> ^{f971A.971A} IR	Mutation	NR	NR	NR	NR	Adenocarcinoma, squamous	No	[34]
<i>Brca1</i> ^{fS152A.S152A} IR	Mutation	+	NR	NR	NR	Adenocarcinoma	No	[35]

NR – not reported.

IR: Irradiation.

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Table 2

BRCA1 mutated human cell lines used in xenograft studies

Cell line	BRCA1 mutation	ERα	PR	HER2	p53	Agents studied	Reference
HCC1937	5382insC	-	-	-	mut	Cisplatin	[66,69]
MDA-MB4-36	5396 + 1G>A	-	-	-	mut	MK-4827 (PARP inhibitor) PU-H71 (Hsp90 inhibitor)	[67,70,71]
SUM149PT	2288delT	-	-	-	wt	No	[67]
SUM1315MO2	185delAG	-	-	-	wt	No	[67]
L56Br-C1	1806C>T	-	-	-	mut	No	[68]
MX-1	33636delGAAA	-	-	-	wt	ABT-888 (PARP inhibitor)	[72]

mut – mutated.
wt – wild type.