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Focus On

Validation of transgenic models of breast cancer: ductal carcinoma in situ (DCIS) and Brca1-mutation-related breast cancer

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Abstract Available mouse models of ductal carcinoma *in situ* (DCIS) and BRCA1-mutation-related breast cancer are reviewed. The best validated mouse models of human DCIS are the conditional estrogen receptor α in mammary tissue (CERM) model initiated by deregulated estrogen receptor α and the serial explant mouse model initiated by p53 deficiency. At present the most useful and best validated mouse model of BRCA1-mutation-related breast cancer uses the cre-lox system to make a conditional Brca1 deletion targeted to mammary epithelial cells. The major shortcoming of the non-conditional Brca1 models is the high incidence of non-mammary tumor development. The use of mammary gland transplants or explants from these mice into nude hosts is one approach that could be used to circumvent this deficiency. Development and validation of a Brca1-mutation-related mouse model of basal cell breast cancer is an important next step.

Keywords: Brca1; Breast cancer; DCIS; ERα; Mouse models

Introduction

In developed countries breast cancer is diagnosed in as many as 1 of 8 women at sometime during their lifetimes. As breast cancer can be managed successfully when it is diagnosed and treated at pre-invasive stages, there is enormous clinical interest in finding better ways to identify women who are at high risk for invasive cancer. Understanding the pathophysiology of human breast cancer is a key factor that can be used to develop improved means of identifying and treating women at high risk in order to prevent the development of invasive breast cancer. Genetically manipulated mouse models are one approach to study breast cancer pathophysiology. Specific genetic changes can be engineered into the mice with development of the disease occurring within the context of normal physiological function and without exogenous chemical carcinogens or radiation exposure.

A non-invasive neoplastic condition that is known to predispose women to the development of invasive breast cancer is Ductal Carcinoma In Situ (DCIS) [1]. The molecular cause(s) of DCIS remain unidentified, but to date investigators have correlated the appearance of DCIS with increased estrogen receptor alpha (ERα) expression [2,3] and telomere shortening [4]. One or both events may be a critical early event in neoplastic progression, the first leading to alterations in cell proliferation and the second predisposing to development of genetic instability. Seventy five percent of all cases of human DCIS are ERα positive [5,6]. ERα is expressed in 100% of non-comedo DCIS and over 90% of cells in those lesions express ERα. The remaining 25% of DCIS that do not express the receptor are predominantly high-grade comedo lesions.

Genetic diseases in which germline mutations predispose women to the development of DCIS and

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invasive breast cancer are BRCA1 and BRCA2 mutation [7–10], Cowden's syndrome [11,12] and Li-Fraumeni syndrome [13]. Loss of Brca1 function has been associated with both genetic instability and enhanced estrogen signaling [14–19]. BRCA1-associated breast cancers are frequently ERα, ErbB2 and cyclin D1 negative, are defined as high grade and demonstrate p53 expression [14,20–22]. An over-representation of BRCA1-mutation-related adenocarcinomas has been described within the category of basal epithelial cell mammary cancers suggesting that loss of BRCA1 function potentiates development of this specific type of breast cancer [23,24].

Mouse models of DCIS

To date three models have been identified that develop human-like DCIS histopathology (Table 1): a Conditional Estrogen Receptor α in Mammary tissue (CERM) model: mouse mammary tumor virus (MMTV)-rtTA/tet-op-ERα mice [25], a WAP-TAg model [26], and a serial mammary tissue explant mouse model with p53 deficiency in the Balb/C strain background [27]. These models present four different types of DCIS that are found in humans: cribiform, clinging, solid and comedo [28,29].

In the CERM model increasing steady-state levels of nuclear-localized ERα in mammary epithelial cells combined with an inability to downregulate ERα expression in response to estrogen exposure is correlated with the development of DCIS. This mechanism is consistent with one of the proposed etiology of human DCIS; that is, in women, increased levels of nuclear localized ERα in mammary epithelial cells of ductal hyperplasia and DCIS is correlated with an increased risk of developing ERα positive invasive breast cancer [2]. This is the only mouse model of human-like DCIS in which lesion development is not dependent upon expression of an oncogene or deletion of a tumor suppressor gene. In this model, over-expression of ERα induces increased rates of mammary epithelial cell proliferation and DCIS lesions develop as early as 2 months of age and hyperplastic nodules appear by 12 months of age. The DCIS lesions in this model express ERα and progesterone receptor (PR). Gene and protein expression profiles were used to validate CERM mice as a model of ERα and PR positive DCIS. Lesion development in the CERM model is hormone dependent as the lesions regress in response to anti-estrogen treatment and ovarian function is required for lesion development. The murine DCIS lesions demonstrate expression of nuclear localized cyclin D1 and p27, similar to ERα positive DCIS lesions in humans [30–32]. To date all studies of the CERM model have been carried out in the C57Bl/6 strain background.

The WAP-TAg model develops cribiform-, roman arch-like-, clinging- and comedo-type DCIS [26]. Lesion development is dependent upon SV40 Tag-induced inactivation of tumor suppressor genes including pRb and p53 and cell cycle control pathways [33]. p53 mutations [34–36] and deregulation of p16-cyclin D-Cdk4(6)-pRB-E2F and p14/ARF pathways have been described in human DCIS lesions [37–39]. However, in this model, lesion development is dependent upon pregnancy-induced expression of

Table 1. Transgenic mouse models of DCIS.

Model/mouse strain	Histopathology	Initiating event/ model type	Relationship to human DCIS	Gene expression in the DCIS	Reference
CERM transgenic mice/C57B1/6	Ductal hyperplasia, lobular hyperplasia, cribiform, clinging and solid DCIS	Over-expression and deregulation of ERα in mammary epithelial cells/ conditional tetracycline responsive gene targeting to mammary epithelial cells/ disease in nulliparous mice	Increased and deregulated ERα expression is found in the majority of human DCIS	ERα/PR/p27 positive, increase in nuclear-localized cyclin D1	[25]
WAP-TAg transgenic mice/BALB/c	Cribiform-, roman arch-like-, clinging-, and comedo-type DCIS	SV40 TAg expression in mammary epithelial cells/ targeting to mammary epithelial cells/pregnancy-induced lesion development in 5 of 6 lines	Loss of p53 function and deregulation of p16-pRb pathway are found in some human DCIS	No information available	[26]
p53 null and heterozygous mammary explants in nude mice/ BALB/c explants	Cribiform- and comedo-type DCIS	Loss of functional p53 in mammary epithelial cells/serial transplantation of p53 null and heterozygote mammary tissue into nude mice	Loss of functional p53 is found in 20–50% of human DCIS	Information available only on outgrowth lines from DCIS	[27]

the TAg oncogene in 5 of 6 lines, whereas in humans pregnancy is described as a protective factor [40]. In addition, because the WAP promoter is active in the alveolar epithelium in this model, lesion development is thought to arise from alveolar cells rather than ductal cells. In contrast, in human disease there is more concern about the lesions arising from the ductal epithelium in non-pregnant women. There is no published information available on gene and protein expression profiles in the WAP-Tag model. The studies described above using the WAP-TAg model were carried out in the BALB/c strain background.

The p53-deficient model consists of a series of serially transplanted premalignant outgrowth lines derived from p53 null and heterozygous mice in the Balb/C strain background. DCIS arises after 5 to 8 serial transplantations. The initiating event in this model is inactivation of p53 function. Loss of functional p53 is found in some patients at risk for breast cancer (Li-Fraumeni syndrome) and in 20–50% cases of human DCIS [34–36]. Loss of genetic stability has been described in human DCIS lesions [37,41]. Genetic instability can result in aneuploidy. In this model, investigators characterized different outgrowth lines with and without aneuploidy. Both diploid and aneuploid outgrowths were found to go on to develop DCIS. The presence or absence of aneuploidy specifically in the DCIS lesions that developed were not examined. Telomerase activity was higher in outgrowth lines but was not directly evaluated in the DCIS lesions. ERα immunohistochemistry has been performed on the outgrowth lines but not specifically in the DCIS lesions. Nine of 11 outgrowth lines show ERα expression levels equal or greater than normal mammary gland without p53 mutation. Two of the 11 outgrowth lines show ERα expression levels lower than normal levels.

There are two genetically manipulated mouse models that develop non-invasive Mammary Intraepithelial Neoplasia (MIN) [42] lesions that are biologically but not histopathologically similar to human DCIS: the C3(1)/SV40 Tag on an FVB/N background [43,44] and the polyoma middle T on an FVB background [45,46]. The MIN lesions that develop in the C3(1)/SV40 Tag transgenic mouse model lose ERα expression as they progress to less differentiated lesions indicating that this may be a more valid model for ERα negative MIN as compared to ERα positive disease. Cancer development in this model can include amplification of the *Ki-ras* gene; however, amplification of this gene is not specifically associated with DCIS or invasive breast cancer in humans.

An alternative to the use of genetically manipulated mouse models to study the pathophysiology of DCIS is a xenograft model derived from human mammary epithelial cells that are implanted in nude mice. The DCIS lesions that develop histopathologically resemble human disease [47].

Mouse models of Brca1-mutation-related breast cancer

Brca1-deficient homozygous knockout mouse models result in early embryonic lethality due to developmental abnormalities and cellular proliferation defects [48–51]. To date, three different approaches have been used to generate Brca1-mutation-related mouse models (Table 2). All of these models depend upon interruption of exon 11 to disrupt Brca1 function. The protein domain encoded by exon 11 is required for many of the cellular functions ascribed to Brca1 [52]. There are mouse strain differences in the impact of *Brca1* disruption on embryonic development.

A cre-lox-based approach is used to direct a targeted deletion of Brca1 exon 11 to mammary epithelial cells and thereby avoid embryonic toxicity (*Brca1*$^{\text{conditional (co/co)}}$/MMTV-Cre/*p53*$^{+/-}$ mice) [52,53]. These mice continue to express the normal splice variant of Brca1 that lacks exon 11 in the targeted cells. Similar to human BRCA1-mutation-related breast cancers, a significant percentage of mammary adenocarcinomas developed in these mice are classified as high-grade undifferentiated adenocarcinomas, demonstrate loss of p53 function and both the cancers and precancerous hyperplastic lesions are ERα negative [53,54]. Unlike human BRCA1-mutation-related cancers, ErbB2 and cyclin D1 over-expression are described in a significant proportion of these cancers, and c-myc over-expression also can be found [53,54]. Mammary cancer incidence in this model is very low when both p53 alleles are intact but is increased significantly by p53 haploinsufficiency [52]. In the *Brca1*$^{co/co}$/MMTV-Cre/*p53*$^{+/-}$ mice, hyperplastic nodules and foci composed of highly proliferative mammary epithelial cells appear at least 2–6 months before development of adenocarcinomas and the prevalence of these abnormalities increases with age [54]. While tumor development is generally solitary, all glands can demonstrate varying numbers of hyperplastic nodules and foci. Metastatic disease to the liver and lung are found in some although not all mice with mammary adenocarcinomas. The majority of palpable adenocarcinomas appear between 6 and 18 months of age. Significantly, mammary cancers do not develop in mice that have only one *Brca1* allele disrupted, only in mice with both alleles disrupted. This stands in contrast to human disease, which is believed to initiate in mammary epithelial cells with *BRCA1* haploinsufficiency.

An alternative approach is to generate a mouse model more similar to human disease in which mammary cancer development is initiated by *Brca1*

Table 2. Transgenic mouse models of *BRCA1*-mutation-related breast cancer.

Model/mouse strain	Histopathology	Initiating event/ model type	Relationship to initiation of human *BRCA1*-mutation-related disease	Gene expression in mammary cancers	Reference
Brca1$^{co/co}$/ MMTV-Cre/*p53*$^{+/-}$/ C57B1/6	Mammary hyperplasia, hyperplastic nodules, undifferentiated mammary adenocarcinomas	Loss of full-length Brca1 through exon 11 deletion with *p53* haploinsufficiency/ conditional cre-lox targeting to mammary epithelial cells/ disease in nulliparous mice	Human disease is initiated by a spectrum of heterozygous *Brca1* mutations. *p53* mutation found in ~50%	ERα/PR negative, ErbB2, cyclin D1 and c-myc over-expression in a subset	[53,54]
Brca1$^{+/-}$/ p53$^{-/-}$ and *Brca1*$^{+/-}$/ p53$^{+/-}$ with irradiation at age 4–6 weeks/strain not specified	Papillary, tubular, intraductal, anaplastic, unclassifiable mammary cancers	Targeted mutation to exon 11 in one Brca1 allele with either loss of p53 or *p53* haploinsufficiency/ germline mutation	As above	Loss of wild-type Brca1 expression in a subset	[55]
Brca1$^{tr/tr}$ and *Brca1*$^{tr/tr}$/ p53$^{+/-}$/ 129/Sv or MF1	Infrequent preneoplasia and DCIS, solid, papillary, cribiform, tubular, acinar, mucinous, adeno-acanthomatous, sarcomatous mammary cancers	Loss of full-length Brca1 through introduction of a STOP codon into exon 11 with and without *p53* haploinsufficiency/ germline mutation	As above	Most ERα/PR negative, many p53, p21 and cyclin D1 positive, ErbB2 in a subset	[57]

haploinsufficiency. However because cancer development does not occur in the mammary gland secondary to loss of one *Brca1* allele, disruption of one *Brca1* allele is combined with loss of either one (heterozygote) or two (null) *p53* alleles and exposure to 5 Gray (Gy) of ionizing radiation at 4–6 weeks of age [55]. The utility of these models is compromised by the high incidence of lymphomas and other non-mammary cancers that develop in these mice. In the *p53* null mice, 10% (4/41) of mice with *Brca1* haploinsufficiency (*Brca1*$^{+/-}$/*p53*$^{-/-}$) develop mammary cancers (reported as papillary, tubular or unclassifiable), but 90% (36/41) develop lymphomas or other non-mammary solid tumors. If the mice are *p53* heterozygotes then irradiation is required to induce mammary cancer development. In the absence of radiation there are no mammary cancers, but 25% (5/21) of irradiated *Brca1*$^{+/-}$/*p53*$^{+/-}$ mice develop mammary cancers. Similar to the *Brca1*$^{+/-}$/*p53*$^{-/-}$ mice the usefulness of this model is compromised by the fact that 95% (23/24) of the mice develop lymphomas or other non-mammary solid tumors. Loss of wild-type Brca1 expression is described in a subset of the mammary cancers just as loss of heterozygosity (LOH) at the *BRCA1* locus found in human disease [56]. Expression patterns of ERα, PR, ErbB2 or other markers in the mammary cancers that develop in these models have not yet been reported.

A third approach is to modify the *Brca1* locus so that a truncated mutant protein that maintains embryonic development is expressed but the C-terminal half of Brca1 required for its tumor suppressor effects is lost (*Brca*^{tr}/*Brca*^{tr} mice) [57]. It is important to note that this truncated protein maintains embryonic development only in specific mouse strains (129/Sv or MF1). Mammary cancers develop in 16% (12/76) of the mice but 85% (76/89) develop lymphomas or other non-mammary solid tumors compromising the utility of the model. The percentage of mice developing mammary cancers is not significantly increased by deletion of one *p53* allele (2/7) and no mammary cancers develop in p53 null mice (0/8). One hundred percent of both *p53* heterozygote and null *Brca*^{tr}/*Brca*^{tr} mice develop lymphomas or other non-mammary solid tumors. Both mammary preneoplasia and cancer are described in the *Brca*^{tr}/*Brca*^{tr} mice but extensive proliferative mammary disease is uncommon. Mammary cancers demonstrate solid, papillary, cribriform, tubular, acinar, mucinous,

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adenoacanthomatous, and sarcomatous pathology. The majority of the mammary cancers are ERα and PR negative, frequently p53, p21 and cyclin D1 positive with 1/3 demonstrating ErbB2 expression.

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