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Review

Transgenic mouse models for the prevention of breast cancer

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Abstract

Breast cancer prevention research has made remarkable progress in the past decade. Much of this progress has come from clinical trials. However, in the future to test the many promising agents that are now available, pre-clinical models of breast cancer are needed. Such models are now available. Useful models include rat and mouse models, particularly, the genetically engineered mice (GEM). Many transgenic mouse models have been generated by manipulating growth factors and their receptors, cell cycle regulators, signal transduction pathways, cellular differentiation, oncogenes and tumor suppressor genes. The transgenes are induced to express in the mouse mammary glands under the control of various transgenic promoters, which have respective characteristics in expression pattern and other biological attributes. These models are providing invaluable insight on the molecular mechanisms of breast tumorigenesis. In this review, we discuss the relative relevance of the most commonly used transgenic mouse models for breast cancer prevention studies, and provide examples of how these transgenic models can be used to conduct cancer prevention research. Due to the multi-factor, multi-step nature of breast cancer, many factors should be incorporated into a valid prevention study. However, many barriers to progress must be overcome, including access to and availability of new cancer preventive drugs, and difficulties in conducting studies of combinations of preventive agents.

Keywords: Transgenic models; Genetically engineered mice; Breast cancer; Pre-clinical application; Molecular prevention

Contents

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1. Introduction

Each year, there are about 200,000 newly diagnosed cases of breast cancer in the United States, and around 40,000 patients die of this disease [1]. The incidence of breast cancer is now steady and is showing a slight declining trend, suggesting the progressive effect of the application of mammographic screening, adjuvant chemotherapy and intensive therapy for the existing cancer. Recent breast cancer prevention clinical trials demonstrated that the selective estrogen receptor (ER) modulators (SERMS) tamoxifen and raloxifene reduced breast cancer incidence in high-risk women by approximately 50% [2,3]. These studies proved that it is possible to reduce breast cancer risk by blocking the effect of estrogen on the human breast. However, the SERMS cannot prevent all breast cancers. Notably, there was no decrease in the incidence of ER-negative breast cancer, which accounts for 30–40% of all breast cancers. It is therefore imperative to develop effective therapeutic and preventive agents for both ER-positive and ER-negative breast cancers.

Breast cancer is considered as a multi-stage, multi-factor process that involves genetic and non-genetic factors. Progress in understanding the molecular mechanism of breast carcinogenesis comes from in vitro and in vivo studies done over the last two decades. One of the most important advances in understanding breast carcinoma was the development of animal models of breast cancer. Of the many models that have been developed, transgenic or genetically engineered mice (GEMs) are among the most useful. The first reported transgenic mouse model of breast cancer was the mouse mammary tumor virus (MMTV)-myc model in which overexpression of the myc transcription factor in the mammary gland resulted in spontaneous mammary adenocarcinoma [4]. Since then, more than 100 transgenic models (mainly murine) have been generated for the study of mammary gland biology and breast cancer therapy and prevention [5,6]. Several extensive reviews have described the generation, characteristics and applications of transgenic mouse models in breast cancer research [7–11]. In this review, we will focus on those transgenic mouse models suitable for breast cancer prevention studies. We will also cover major strategies and recent progress in testing chemopreventive agents. Newly developed applications for mouse transgenic models will also be discussed.

2. Information resources for breast cancer prevention research

Electronic access to the established network of the mouse models will greatly facilitate and enhance the dissemination of new ideas and results, establish collaboration in the mammary gland research community, and explore new insights for the prevention studies. The

Table 1
Electronic resources for mammary biology and transgenic mice research

Websites and URL links	Main functions	Available resources
http://emice.nci.nih.gov	NCI sponsored mouse model network	Model information, mice repository
http://mammary.nih.gov	NIH sponsored mammary gland biology site	Models, tools, genome, etc.
http://mammary.nih.gov/Annapolis-guidelines/	NIH sponsored mammary gland biology site	Guidelines for transgenic mice research, pathology workout, etc.
http://histology.nih.gov	NIH sponsored mammary gland histology database	Data viewing, comparison, submission, classification
http://romulus.cit.nih.gov/m1/lgp/weblinks/index.html	Provide useful links to mammary gland biology	Various topics and information
http://www.jax.org	Mouse model, availability, genome	Strain information, model database, training programs
http://www.criver.com/products/research_models/	Animal model information, availability	Strain information, various animal models

National Cancer Institute (NCI) established the Mouse Models of Human Cancer Consortium (MMHCC) in 1999 to serve this purpose [5]. Useful links to these websites are listed in Table 1. From the MMHCC site (http://emice.nci.nih.gov), researchers can access Mouse Models and Resources submenus to obtain valuable information regarding mouse models, the available mouse repositories and databases pertaining to mouse genetics, models, various mouse resources and websites, genes and pathway information. NIH has also created a web site listing information important for breast cancer researchers (http://mammary.nih.gov). From here, researchers may link to almost everything related to mammary gland biology, such as experimental models, tools and technologies, methodology and histology atlases, mouse genome and reviews. In addition, to facilitate transgenic mice research, NIH researchers established guidelines to define mammary histology, proliferative lesions, tumor classification and pathology of mammary tumors in mice [6]. This information can be accessed at http://mammary.nih.gov/Annapolis-guidelines/. In addition to this website, a CD-ROM is available for the mammary biology research community [5]. Other useful links include a histology database sponsored by NIH (http://histology.nih.gov), from which researchers may interactively learn, compare and submit relevant histological data from breast cancer studies. Additional useful information about transgenic models in breast cancer prevention research can be found in the websites of commercial vendors. For example, the Jackson Laboratory (http://www.jax.org) provides transgenic animal strain information, availability, model database, mouse genome informatics and a sub-classification of models for cancer research.

3. Transgenic mouse models of mammary tumorigenesis

Numerous transgenes have been used to generate mouse models to mimic human breast cancer. Most mouse transgenic models for prevention studies are generated through gain of function or knockout of critical components in oncogenic pathways. The most commonly used models for breast cancer studies cover a wide range of various targets such as growth factors, receptors, cell cycle regulators, signal transduction pathways, cellular differentiation, oncogenes and tumor suppressor genes (Table 2). The relevance of these models to breast cancer prevention studies is also indicated for reference. The Oncogene journal had a special issue discussing some important models in the January issue of 2000.

4. Promoters to drive the expression of transgenes

We have compiled a list of the promoters commonly used to generate mouse mammary tumor models (Table 3).

1. MMTV promoter: mammary gland-specific expression of the transgenes is desired to avoid inducing tumors in other organs. Many mouse models have

Table 2
Transgenic models for breast tumorigenesis and prevention studies

Target category	Transgene	Multi-transgene manipulation	Promoters	Species	Phenotype	Relevance to prevention studies	Reference
Growth factors	*TGFα*	*p52, myc*	MMTV, WAP, MT	Mouse rat	Abnormal mammary gland development, hyperplasia, adenoma, adenocarcinoma	High	[12–17]
	TGFβ		MMTV, WAP	Mouse	Impaired lobular development, inability to produce milk, early senescence	Median	[18–20]
	Heregulin	myc	MMTV	Mouse	Hyperplasia, adenocarcinoma	High	[21,22]
	IGF-I		WAP	Mouse	Abnormal involution, hyperplasia	High	[23,24]
	IGF-II		BLG	Mouse	Adenocarcinoma	High	[25]
	HGF		MT, WAP	Mouse	Abnormal mammary gland development, adenocarcinoma, adenosquamous carcinoma, lung metastasis	Median	[26,27]
	FGF3 (Int 2)	Wnt-1	MMTV	Mouse	Hyperplasia, adenocarcinoma	High	[28]
	FGF7 (KGF)		MMTV	Mouse	Hyperplasia, adenocarcinoma	High	[29]
	FGF8	Wnt-1	MMTV	Mouse	Hyperplasia, adenocarcinoma	High	[30]
	c-src		MMTV	Mouse	Hyperplasia, occasional neoplasia	Median	[31]
Receptors for growth factors	ErbB-2 (Neu)	p53	MMTV, WAP	Mouse rat	Hyperplasia, adenocarcinoma, lung metastasis	High	[32–35]
	ERα	SV40T	MMTV, Tet-op	Mouse	Hyperplasia, adenocarcinoma	High	[36]
	RET-1		MMTV	Mouse	Hyperplasia, adenocarcinoma	High	[37]
	Tpr-MET		MMTV	Mouse	Hyperplasia, adenocarcinoma	High	[38]
Signal tranduction pathways	ras	p21, myc,	MMTV, WAP	Mouse	Hyperplasia, adenocarcinoma, accelerated tumorigenesis and metastasis	Median	[6,39–43]
	PTEN		MMTV	Mouse	Fibroadenoma, adenocarcinoma, metastasis to lung and lymph node	High	[44,45]
	CK2		MMTV	Mouse	Hyperplasia, adenocarcinoma	Median, long latency	[46]
	Cox-2		MMTV	Mouse	Hyperplasia, dysplasia, adenocarcinoma, metastasis to lymph node	High	[47]
	c-Rel		MMTV	Mouse	Hyperplasia, adenocarcinoma, adenosquamous carcinoma, spindle cell tumor	High	[48]
	PPARγ	PyV-mT		Mouse	Accelerated mammary tumorigenesis	High	[49]
Viral oncogenes	PyV-mT	Shc/Grb2 PPARγ	MMTV	Mouse	Hyperplasia, adenocarcinoma, lung metastasis	High	[49–52]
	SV40T	Bcl-2, p53, bax, maspin, K-ras	C3(1),WAP	Mouse rat	Hyperplasia, adenocarcinoma, lung metastasis, osteosarcoma, soft tissue sarcoma	High	[53–63]

Cell cycle regulators	p53	Wnt-1, Brca1, $p53^{172\text{-}H}$	Null, WAP, MMTV, Cre/loxP	Mouse	Rare mammary tumor in $p53^{-/-}$ mice, increased tumor incidence in $p53^{-/-}$/Wnt-1, -Brca1 mice	High	[35,64–68]
	p53	p53 somatic mutation	Cre/loxP	Mouse	ER-positive and ER-negative tumors, high metastasis	High	[69]
	Myc	Bcl-2	MMTV, WAP	Mouse	Hyperplasia, adenocarcinoma, accelerated tumorigenesis	High	[4,70,71]
	Cyclin D1		MMTV	Mouse	Hyperplasia, adenocarcinoma	High	[72]
	Cyclin D3		MMTV	Mouse	Squamous metaplasia	Low	[73]
	CDK4		Knock-in	Mouse	Adenocarcinoma, adenosquamous carcinoma	Median	[74]
	MNT		MMTV	Mouse	Adenocarcinoma	High	[75]
	Cyclin E		BLG	Mouse	Hyperplasia, adenocarcinoma	Median	[76]
	HCCR-2		Constitutive	Mouse	Mammary tumors and metastasis	Median	[77]
Differentiation	Wnt-1	FGF3, p53, pRB, ER	MMTV	Mouse	Hyperplasia, adenocarcinoma	High	[65,66,78]
	Wnt10b		MMTV	Mouse	Hyperplasia, adenocarcinoma	High	[79]
	β-Catenin		MMTV	Mouse	Hyperplasia, adenocarcinoma	High	[80]
	NOTCH4 (INT3)	TGFβ, pRB	WAP, MMTV	Mouse	Displasia, adenocarcinoma, lung metastasis	High	[81,82]
Miscellaneous targets	Stromelysin		WAP	Mouse	Hyperplasia, adenocarcinoma, carcinosarcoma, metastasis to lung and kidney	Median	[83]
	MTS-1		MMTV	Mouse	Adenocarcinoma, lung metastasis	High	[84]
	MMP-1 (membrane type)		MMTV	Mouse	Hyperplasia, dysplasia, adenocarcinoma	High	[85]
	CDC37	Myc, cyclin D1	MMTV	Mouse	Hyperplasia, adenocarcinoma, adenosquamous carcinoma	Median	[86]
	Prolactin		Neu-related lipocalin (NRL)	Mouse	Hyperplasia, adenocarcinoma, adenosquamous carcinoma	High	[48]
	CSF-1/c-fms colony-stimulating factor and its receptor		MMTV	Mouse	Hyperplasia, dysplasia, increased tumorigenesis	Median	[87]
	Aromatase	DMBA treatment	Constitutive	Mouse	Hyperplasia, dysplasia, increased tumorigenesis with DMBA treatment	High	[88–91]

Table 3
Promoters commonly used in transgenic mouse models

Promoter name	Expression pattern in mammary gland	Dependence on hormone/pregnancy	Advantages/drawbacks	Suitability for prevention study	Reference
Mouse mammary tumor virus longternimal repeat (MMTV-LTR)	Ductal, alveolar cells	Expressed in all developmental stages, high expression in pregnancy	Patchy, mosaic expression	++++	[92,93]
Whey acidic protein (WAP)	Preferentially alveolar cells	Mainly during lobularalveolar differentiation	Hormone/pregnancy dependent	++	[8]
C3(1) promoter from rat PSBP	Ductal, alveolar cells	Independent of estrogen and/or pregnancy	Unaffected by hormone	++++	[54,95]
Bovine β-lactoglobulin (BLG)	Specific to MG	Activated in late pregnancy and lactation	Hormone/pregnancy dependent	++	[101]
Metallothionein (MT)	Most mammary cells	No	Inducing transgene expression with heavy metal treatment	++	[15]
Tet-on, tet-off inducible systems	Ductal, alveolar and myoepithelial cells	Dependent on the collaborating promoter	May induce at any time, need at least two transgenes	++++	[70,98,99]
Neu-related lipocalin (NRL)	Most mammary cells	Independent of hormone stimulation	Unaffected by hormone	+++	[48]
Cre/loxP system	Dependent on the promoter in transgene	Dependent on the collaborating promoters	May induce to delete genes at any time	++++	[100]
K14	Most mammary cells	Independent of hormone	High expression in skin	+	[102]

used the MMTV long terminal repeat to drive transgene expression in the mammary gland. The MMTV promoter drives transgene expression in ductal and alveolar cells in all developmental stages of the mammary gland. Importantly, the MMTV promoter is regulated hormonally and is dramatically enhanced during pregnancy [92]. This promoter has been very useful for studies of mammary gland development and tumorigenesis. However, one potential drawback of this promoter is that the transgene is expressed in a non-uniform, mosaic pattern [93]. In addition, there is some embryonic expression of genes regulated by this promoter, which can lead to developmental problems [94].

2. Whey acidic protein (WAP) promoter: WAP promoter drives expression of transgenes preferentially in alveolar cells during lobuloalveolar differentiation. The WAP promoter is also regulated by hormones in pregnancy [8].
3. C3(1) promoter: the 5′ flanking region of the C(3)1 component of the rat prostate steroid binding protein has been used to express SV40 Large T antigen in the mammary gland of female mice that resulted in mammary tumors [54]. The C(3)1 promoter-driven transgene is activated in mammary ductal and alveolar cells, and is independent of estrogen and/or pregnancy regulation [95]. In addition, this promoter also drives expression in the prostate gland in male mice resulting in prostate cancer.
4. Bovine β-lactoglobulin (BLG) promoter: this promoter is activated in late pregnancy and during lactation. It has been used to drive the expression of growth factors and cell cycle regulators in mammary alveolar cells. Since pregnancy reduces the risk of breast cancer in humans [96], this promoter may not be the best choice for mammary cancer prevention studies.
5. Metallothionein (MT) promoter: the MT promoter is not a mammary gland-specific promoter. However, it can be used to induce transgene expression in most cell types, particularly when induced by heavy metals. Thus, treatment with heavy metals

such as zinc will induce increased expression of the transgene and has been shown to cause mammary tumors in transgenic models [97].

6. Inducible expression systems: inducible expression of transgenes has been used for mammary gland studies for almost a decade [98]. The MMTV promoter was used to express the tet-responsive transactivator, tTA. The tTA protein can activate the expression of a second transgene that is controlled by a tet-off operator in the absence of tetracycline. However, because the tet-off system only directs the expression of transgenes in a small fraction of mammary epithelial cells, its application in mammary biology is somewhat limited. More recently, Chodosh developed a reverse tetracycline-dependent transcriptional activator (rtTA) system with MMTV promoter to achieve mammary specific, tightly regulated homogeneous transgene expression in the presence of tetracycline or its derivative doxycycline. Using this system, the c-myc transgene was specifically induced in mammary epithelial cells [70,99]. This system, although cumbersome because of the requirement of at least two transgenes, is highly mammary gland-specific and inducible, and has great potential for future cancer prevention studies.
7. Conditional knockout: the recently developed Cre-loxP system is another inducible and regulatable mammary gland-specific expression system. In this approach, the Cre gene is under the control of MMTV or WAP promoters. Activation of the Cre gene causes conditional deletion of specific target genes. Deletion of Brca1 gene by this system resulted in abnormal ductal development and activated apoptosis [100]. This system, like the rtTA system described above, offers great promise for future studies of mammary gland biology and tumorigenesis.

To date, almost all promoters used in transgenic models for mammary tumor development induce transgene expression in the mammary gland. However, there is no specific promoter for mammary stroma. Considering that stromal structure has an important impact on mammary gland development and tumorigenesis, the identification and characterization of a stroma-specific promoter would be a major advance in mammary gland biology.

5. Commonly used mouse models for breast cancer prevention studies

A number of mouse models are particularly relevant for prevention studies of breast cancer.

5.1. TGFα models

The transcription factor TGFα plays an important role in mammary development and is overexpressed in 30–70% of breast cancer cases as reviewed by Rudland [103]. TGFα expression has been driven under MMTV-LTR, WAP and MT promoters [12,13,15,104]. The WAP-TGFα model has been shown to have diffuse mammary epithelial hyperplasia in pregnancy, multifocal hyperplastic alveolar nodules at latency of 2–6 months and mammary tumors at 6–12 months [15,105]. Yet, the TGFα-induced mammary tumors are focal and relatively fewer in number [15], indicating that additional tumorigenic mechanisms are needed to promote tumor development.

5.2. ErbB-2 (wild-type and activated forms) models

ErbB-2 (*HER2*, *Neu*) is one of the most intensively studied genes in breast cancer biology. The gene is amplified in 15–20% of human breast cancers, and is overexpressed in approximately 30% of breast cancers [106,107]. ErbB-2 is an indicator for clinical prognosis, metastasis and tamoxifen resistance [108–110]. ErbB-2 had been engineered to express under MMTV and WAP promoters. Wild-type and mutated ErbB-2 transgenic mice develop mammary tumors in several strains around 7 months of latency [32,33,111–114]. Multiple transgenic models have confirmed that the early ErbB-2 model carries a valine to glutamic acid substitution in the transmembrane domain that confers constitutive activation of the receptor in the absence of ligand [8]. More relevant to human breast pathology, a late wild-type ErbB-2 model develops mammary tumors that carry sporadic mutations in the transgene in the tumor, but not in the adjacent normal mammary tissue [114]. The mammary tumors in ErbB-2 transgenic mice are ER-negative and their pathologic appearance resembles lobular and alveolar phenotypes found in about 5% of human breast cancers [114].

5.3. Wnt-1 model

Wnt-1 was originally found to be activated after MMTV infection, and the resulting mice had a high incidence of mammary tumors. Wnt-1 is a glycoprotein that signals through the β-catenin pathway. Its expression is seen throughout mammary gland development, but not in the adult gland. Deregulation of the downstream effectors in the Wnt-1 signaling pathway is involved in the tumorigenesis of several tumor types including breast cancer [115]. MMTV-Wnt-1 expression causes ductal hyperplasia in late gestation and in prepubertal mice [78]. The Wnt-1 mice develop adenocarcinoma at 6–12 months of age [78,116]. These tumors demonstrated a moderately differentiated ER-negative phenotype, and are heterogeneous in ER-positive and/or ER-negative status. The MMTV-Wnt-1 mice have been crossed with MMTV-Fgf3, Sky$^{-/-}$, p53$^{-/-}$, ERα$^{-/-}$ and TGFβ [117]. There is a synergistic effect between Wnt-1 and Fgf3 [116], as these bigenic animals showed shortened latency to develop mammary tumors. The p53 KO mice bred with Wnt-1 mice develop mammary tumors significantly faster than the p53$^{+/-}$ counterpart [66]. Metastasis in Wnt-1 mice occurs to lymph node and lung, even after the primary tumors are removed. Therefore, Wnt-1 model is more relevant to human breast cancer in two aspects: stroma signaling is important in breast tumorigenesis since human mammary gland has a significant proportion of stromal structure; and metastatic route is similar to that of human breast cancer. In addition, Wnt-1 tumors may be heterogeneous in ER status, rendering it a unique model for breast cancer prevention studies.

5.4. Ras models

Ras mutation is infrequent in breast cancer [118]. However, wild-type ras is significantly activated in breast cancers overexpressing EGFR and/or ErbB-2 [119]. Ras driven by WAP and MMTV is sufficient to induce hyperplasia, adenocarcinoma, accelerated tumorigenesis and metastatic mammary tumors [41–43,120]. The MMTV-h-ras transgenic mice develop mammary gland tumors from 5 weeks to 6 months of age [39].

5.5. c-myc model

c-myc is a transcription factor that dimerizes with Max and regulates target gene promoters. A defined role for c-myc has been shown in cell cycle regulation and apoptosis. c-myc regulates normal mammary development and hormone-related proliferation, and also controls involution and remodeling [121]. Further, *c-myc* is deregulated in many human breast cancers. *c-myc* gene is amplified in approximately 15–20% of all human breast cancers and is overexpressed in up to 70% of breast cancers [122]. Several c-myc transgenic models have been developed in which the *c-myc* gene is expressed using MMTV or WAP promoters. The mice for each of these models develop mammary tumors at a high rate [4,15,123]. MMTV-c-myc mice develop spontaneous mammary adenocarcinomas within 4–8 months [4]. WAP-c-myc mice develop adenocarcinomas or solid carcinomas in 80% of female transgenic mice after multiparity, at latency of 5–10 months [15,123]. These c-myc-induced mammary tumors are ER-negative tumors. It is important to note that c-myc overexpression does not transform all mammary epithelial cells, as suggested by the long latency. This suggests that additional events are required for c-myc-induced transformation of mammary cells. In this regard, the c-myc model reflects the attributes of human breast carcinogenesis, and hence is a potentially ideal mouse model for cancer preventive intervention.

5.6. SV40 T-antigen models

Simian virus 40 large and small T-antigens (SV40 Tag) induce mammary tumors by inactivating the p53 and Rb tumor suppressor genes. When SV40 Tag is expressed using the promoter C3(1) from prostate steroid binding protein, the transgene induces mammary carcinomas in 100% of female mice and prostate tumors in all male mice [53–55]. The C3(1) model has several unique characteristics for breast cancer prevention studies. The model mimics a well-defined time course for progressive mammary lesions and tumorigenesis: atypical ductal epithelia at 8 weeks, mammary intraepithelial neoplasia (similar to human DCIS lesion) at 12 weeks and invasive carcinoma by 16 weeks of age [53]. Most interestingly, the C3(1) mice all develop tumors in virgin animals without the need of additional

hormonal stimulation from pregnancy, a superior attribute over several other transgenic models. Another valuable feature of this model is that the C3(1) promoter itself is not stimulated by estrogen or pregnancy, and the tumors are ER-negative and estrogen-independent. Therefore, C3(1) model is especially useful for studying ER-negative mammary tumorigenesis. The SV40T has also been expressed using the WAP promoter [56,57,59]. In this model, all female mice develop mammary tumors by 8–9 months of age. Histologic appearance of the tumor varies from well- to poorly-differentiated phenotypes. Pregnancy enhances the tumor development due to the WAP promoter, and the first tumors appear at 6 months of age after one pregnancy. Similar to C3(1) model, the tumorigenesis in this model is characterized by three distinct stages: initial proliferation, hyperplasia and adenocarcinoma [58]. An interesting attribute of this model is the high level of proliferation, apoptosis and fibrosis in the tumor. This model is potentially useful to explore the early events during mammary tumorigenesis, particularly with respect to cellular proliferation and cell death.

5.7. p53 models

Alterations of the p53 tumor suppressor gene are frequently detected in human breast cancer, with up to 40–50% of all human breast cancers having p53 mutations [124]. Several animal models have been developed that either overexpress a mutant p53 gene in mammary tissue or have the endogenous p53 gene deleted or disrupted in mammary gland cells [35,65,125,126]. Mammary tumors are infrequently observed in $p53^{null}$ mice because the mice first develop lymphoma and die of these tumors before development of mammary gland tumors. Medina developed a transplantable BALB/c-$p53^{null}$ mammary epithelium and demonstrated that lack of p53 function is sufficient to cause mouse mammary tumorigenesis, though hormone stimulation is an effective enhancer for the $p53^{null}$-induced tumorigenesis [64]. A WAP-$p53^{172R\text{-}H}$ transgenic mouse model was developed in which $p53^{172R\text{-}H}$ functions as a dominant-negative mutant [35,126]. The WAP-$p53^{172R\text{-}H}$ mice develop tumors in shorter latency after DMBA treatment. The mice developed by crossing MMTV-ErbB-2 with WAP-des-IGF-1 have shown significantly reduced latency [126]. An important characteristic of this model is that it develops mammary tumors similar to human high-grade breast adenocarcinoma in the presence of carcinogens and oncogenes. Thus, WAP-$p53^{172R\text{-}H}$ accelerates carcinogen- and oncogene-mediated tumorigenesis, and is useful for cancer preventive intervention.

5.8. Cyclin D1 model

Cyclin D1 is amplified in about 20% of human breast cancers [127], while the cyclin D1 protein is overexpressed in more than 50% of human breast cancers [128–130]. In addition, loss of cyclin D1 interferes with mammary tumorigenesis. Sicinski and coworkers crossed cyclin $D1^{-/-}$ KO mice to four different mammary oncomice and found that cyclin D1 mediated the MMTV-c-neu and MMTV-v-Ha-ras induced mammary tumors, but not MMTV-c-myc and MMTV-Wnt-1 induced mammary tumors, suggesting that cyclin D1 is essential for the Neu-Ras pathway and the tumors dependent on cyclin D1 [131]. Cyclin D1 overexpressing breast cancers have been modeled by Wang et al. who developed an MMTV-cyclin D1 transgenic model. These mice have enhanced proliferation of mammary epithelial cells, and develop mammary carcinomas at a mean age of 18 months [72]. Therefore, the cyclin D1 transgenic mouse models a significant proportion of human breast cancers, and thus may be useful to study mammary carcinogenesis.

6. Considerations in choosing models for breast cancer prevention studies

As mentioned earlier, breast cancer is a complex disease caused by dysregulation of many different oncogenes, tumor suppressor genes and growth factor pathways. The currently available models are valuable tools for the elucidation of the mechanisms of mammary tumorigenesis. However, it is important to recognize that no one model can represent all the different forms of human breast cancer. There are unique requirements for the models if one wishes to conduct prevention studies. Hence, when choosing an appropriate model for cancer prevention studies from the large repository, one should consider the following as general guidelines:

1. General relevance to human breast cancer: a number of models such as ErbB-2, myc or $p53^{-/-}$ and Brca1 or Brca2 models are highly relevant to human breast cancer, in terms of comparing gene expression profiles in mRNA and protein levels, and pathological presentations. Other models may be less relevant to human breast cancer (e.g., ras models).
2. Effect of chemopreventive agents on transgene expression: transgenic models are excellent choices for testing the efficacy of chemopreventive agents. However, because chemopreventive agents may have an effect on expression of the transforming transgene, it is imperative that studies using transgenic mouse models rule out this possibility. To be a useful study, the researcher must first demonstrate that the chemopreventive agent does not reduce the expression of the transgene. It is important to note that carcinogen-induced models and gene knockout models do not have this problem.
3. ER status of the tumors: human breast cancers can be categorized as ER-positive or ER-negative tumors. Yet, most mouse models produce ER-negative mammary tumors. For example, ErbB-2 model produces ER-negative tumors. While this model is useful to study ER-negative tumorigenesis, other models will need to be used to study ER-positive tumorigenesis. Useful models to study ER-positive breast cancer include the DMBA-induced rat model, estrogen-induced ACI rat model and the $p53^{null}$ mouse model. Each of these develops mammary tumors that are ER-positive. So the choice of which animal model to use will depend on whether one is attempting to prevent ER-positive, or ER-negative breast cancer, or both.
4. Other factors: each individual transgenic model has its own unique characteristics in transgene expression (which is dependent on the promoter chosen) and kinetics of tumorigenesis. Therefore, the time of tumor development, tumor multiplicity, pathological presentation, critical molecular pathways, biomarkers and metastatic potentials vary among models. When designing a prevention study, the kinetics of tumorigenesis, as well as the tendency to develop pre-invasive, invasive and metastatic cancers, need to be integrated into the planning of the experiments.

7. Examples of chemopreventive drug trials using transgenic mouse models

7.1. SERMS

While classic SERMs such as tamoxifen and raloxifene are now being compared in the NSABP STAR trial to compare their efficacy and safety profiles, other hormone-regulating agents are also being tested in animal models. The human clinical trials show that SERMs are only able to prevent ER-positive tumor formation. However, in pre-clinical studies using MMTV-ErbB-2 mice, the mammary tumor incidence was reduced significantly in mice given tamoxifen at an earlier age (8–18 weeks of age) [132,133]. In addition, a combination of tamoxifen and angiostatin cDNA delivery achieved greater suppression of tumor growth than tamoxifen or angiostatin alone [134]. A further combination of tamoxifen, angiostatin and TIMP-2 achieved 90% reduction of tumor incidence in the MMTV-ErbB-2 model [135]. These results suggest that in some cases, anti-estrogen SERMs can suppress the development of ER-negative cancers. The underlying mechanism is unknown at this time.

7.2. Aromatase inhibitors

Aromatase is a key enzyme in synthesizing endogenous estrogen in peripheral tissue. The transgenic model overexpressing aromatase demonstrates increased tissue estrogenic activity and induction of hyperplastic and dysplastic lesions in mammary glands with or without circulating estrogen [90,91]. These preneoplastic changes appear to be further stimulated by the carcinogen DMBA, leading to an increased incidence of mammary tumors in mice. Low dose letrozole, an aromatase inhibitor, inhibits expression of ER, PR and cell cycle regulators, and reduces mammary cell hyperplasia and the index of proliferation marker, PCNA [88–91]. These studies have provided a vivid example of how to use a transgenic mouse model to elucidate important tumorigenic mechanism.

7.3. Retinoids

Retinoids are Vitamin A analogs that mediate the transcriptional regulation with their receptors RAR and RXR. Studies in our laboratory have demonstrated that RXR-selective retinoids are much less toxic than

RAR-selective retinoids. LGD1069 (Bexarotene, Targretin), an RXR-selective retinoid, prevents ER-negative mammary tumors in C3(1) SV40T and MMTV-ErbB-2 transgenic mouse models [136,137]. Another newer RXR-selective retinoid, LG100268, has been reported recently by Suh and colleagues to reduce tumor incidence in the NMU rat model by promoting TGFβ-dependent apoptosis [138,139]. The most striking finding in these studies is that when LG 100268 was used in combination with a third generation SERM, arzoxifene, only very low dosages of both arzoxifene and LG100268 were needed to cause significant reduction of tumor burden [138]. Similar results were obtained using the MMTV-ErbB-2 model.

7.4. Tyrosine kinase inhibitors (TKIs)

EGFR (HER1, ErbB-1) or other members of its receptor family (HER2, 3, 4) are overexpressed in a portion of human breast cancers and are highly expressed in ER-negative tumors [140,141]. TKIs can effectively block the tumorigenic potentials that arise from the EGF signaling pathway. ZD1839 (IRESSA) is the prototype of this class of drugs [142]. Recent work in our laboratory has demonstrated that this drug prevents ER-negative tumor formation in MMTV-ErbB-2 mice. The median time to tumor formation was approximately 230 days in vehicle-treated mice and more than 310 days in mice treated with ZD1839 at 100 mg/kg ($P < 0.001$). This effect was achieved by reducing proliferation and increasing expression of the cell cycle regulator p27 [143].

7.5. Cox-2 inhibitors

One of the most promising new class of chemopreventive agents is the Cox-2 inhibitors. Cox-2 is one of the rate-limiting enzymes in converting free arachidonic acid to PGG2. The two downstream products PGE1 and PGE2 enhance mitogenesis in mammary cells stimulated with EGF [144]. Cox-2 is overexpressed in 56% of breast cancers including DCIS as well as infiltrating ductal and lobular carcinoma [145,146]. Mammary glands from transgenic MMTV-Cox-2 mouse model demonstrate hyperplasia, dysplasia and development of metastatic tumors [47]. The specific Cox-2 inhibitor, celecoxib, is currently being tested for its ability to prevent cancer in humans. When given at 500 ppm, celecoxib significantly suppresses tumor incidence and PGE2 levels in the MMTV-ErbB-2 model [147]. This drug is also under evaluation in our laboratory using other transgenic models.

8. Barriers to progress

Tremendous progress has been made in the last 20 years in the field of breast cancer prevention. The most important advance came from the observation from clinical trials that anti-estrogen SERMs prevent the development of breast cancer. Although SERMs are effective in reducing the incidence of ER-positive breast cancer, there are no effective strategies to prevent ER-negative breast cancer. Identification of novel targets and development of effective cancer preventive agents will be necessary to prevent all breast cancers. While many investigators are attempting to develop effective strategies to prevent breast cancer, there remain several major barriers that slow the progress. These include:

1. The Dupont Patents

   On 12 April 1988, Drs. Philip Leder and Timothy Stewart patented the “OncoMouse”. This is the first time that a living animal was given patent protection by the US Patent and Trademark Office. This mouse was created by inserting a cancer-causing gene into its genomic DNA. Thus, the mouse is cancer-prone, and is suitable for cancer biology and cancer prevention studies. The patent was extremely broad in terms that it covers *all* genetically engineered non-human mammals [148]. DuPont subsequently purchased the rights for this patent and other related patents for $6 million dollars. The OncoMouse portfolio now contains three patents, and DuPont sells licenses to use transgenic mice in biomedical research. DuPont has attempted to patent the OncoMouse in Europe, Canada and Japan. The European Patent Office has recently restricted the patent to “transgenic mice” only [149]. On 5 December 2002, the Supreme Court of Canada ruled that the OncoMouse cannot be patented in Canada, making Canada the only Western country to deny a patent of a cancer-prone mouse. Because these patents [148,150,151] could slow the progress of cancer research, NIH reached an agreement with DuPont that NIH staff and grantees can use transgenic mice if for non-profit use. Although this agreement does facil-

itate NIH-funded research, the DuPont OncoMouse patents certainly impede collaborative studies with industry. Thus, these patents do slow the pace of progress in cancer prevention research [152–154].

2. Access and availability of new agents and drugs

   Prevention studies frequently involve the testing of new agents or drugs in pre-clinical models. These new agents are developed in academic laboratories, in federal laboratories and by pharmaceutical companies. Certainly, the largest number of novel agents is held by pharmaceutical companies. To make rapid progress, these agents will need to be tested rapidly and effectively. Thus, collaboration between pharmaceutical companies and translational researchers is essential. Unfortunately, legal concerns over intellectual property and licensing issues often impede or delay these collaborative activities. To make rapid progress and identify the most effective agents for the prevention of breast cancer, these barriers must be overcome.

3. Combination of preventive agents and drugs

   Considering that cancer is a genetic disease composed of multiple stages and factors, the combined use of several preventive agents to block multiple oncogenic pathways is likely to be necessary to prevent cancer. Recent data from Sporn and coworkers has demonstrated that the combination of arzoxifene and an RXR-selective retinoid has achieved convincing results in preventing breast cancer [138]. However, the testing of this combination in humans has to date been hampered by difficulties in testing a combination of drugs from two different pharmaceutical companies. If the drugs to be used in combination are developed by different pharmaceutical companies, the combined use of these agents is often not permitted because of concerns over intellectual property, licensing and the potential for direct comparison of the different agents. To overcome these difficulties, a better system to foster the cooperation among pharmaceutical companies, academic prevention researchers and government agencies is clearly needed at this time.

9. Future directions

In the past decade, the generation of transgenic models for breast cancer research has provided the mammary biology research community with new tools to understand breast tumorigenesis mechanisms. Using these models, several important classes of chemopreventive agents have already been shown to have impressive preventive effects (SERMs, RXR retinoids, TKIs and Cox-2 inhibitors). In addition, recent studies have shown synergism between drugs with different targets (e.g., arzoxifene and LG100268 [138]). In the future, the study of a combinatorial approach using multiple chemopreventive agents will need to be undertaken to effectively prevent breast cancer.

New transgenic models will also be needed to better represent human breast cancer. The majority of available models represent either ER-positive breast cancers or ER-negative breast cancers. Clearly, models that mimic human breast cancer etiology in developing *both* ER-positive and ER-negative breast cancers are needed. Several recent transgenic models fulfill this need [64,69,155], and more models will be developed in the future. The long-term goal of animal model research is to develop and use animal models to identify effective strategies for the treatment and prevention of human cancer.

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