Genetically engineered ERα-positive breast cancer mouse models

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Abstract

The majority of human breast cancers are estrogen receptor-positive (ER+), but this has proven challenging to model in genetically engineered mice. This review summarizes information on 21 mouse models that develop ER+ mammary cancer. Where available, information on cancer pathology and gene expression profiles is referenced to assist in understanding which histological subtype of ER+ human cancer each model might represent. *ESR1*, *CCDN1*, prolactin, *TGFα*, *AIB1*, *ESPL1*, and *WNT1* overexpression, *PIK3CA* gain of function, as well as loss of *P53* (*Trp53*) or *STAT1* are associated with ER+ mammary cancer. Treatment with the PPARγ agonist efatutazone in a mouse with *Brca1* and *p53* deficiency and 7,12-dimethylbenz(a)anthracene exposure in combination with an activated myristoylated form of AKT1 also induce ER+ mammary cancer. A spontaneous mutant in nude mice that develops metastatic ER+ mammary cancer is included. Age of cancer development ranges from 3 to 26 months and the percentage of cancers that are ER+ vary from 21 to 100%. Not all models are characterized as to their estrogen dependency and/or response to anti-hormonal therapy. Strain backgrounds include C57Bl/6, FVB, BALB/c, 129S6/SvEv, CB6F1, and NIH nude. Most models have only been studied on one strain background. In summary, while a range of models are available for studies of pathogenesis and therapy of ER+ breast cancers, many could benefit from further characterization, and opportunity for development of new models remains.

Key Words
- mammary gland
- estrogen receptor
- breast
- carcinoma
- pathogenesis

Endocrine-Related Cancer (2014) **21**, R195–R208

Introduction

Breast cancer is a heterogeneous disease consisting of four clinically relevant categories based on the expression patterns of estrogen receptor α (ERα; *ESR1*) and V-Erb-B2 avian erythroblastic leukemia viral oncogene homolog 2 (*HER2*) (Guiu *et al*. 2012). Molecular studies divide breast cancers-expressing ERα into luminal A and luminal B subtypes that are distinguished by different expression patterns of proliferation-related genes. Together they are referred to as ER+ breast cancers. But within this classification exists different morphological/histological subtypes (Habashy *et al*. 2012). The majority of human invasive ER+ breast cancers are classified as invasive ductal, smaller percentages are defined as invasive lobular, while other histological subtypes including tubular, papillary, invasive cribriform, mucinous, adenocystic, adenosquamous, spindloid, and adenomyoepithelioma appear less commonly. Ductal carcinoma *in situ* (DCIS) is a non-invasive cancer. ER+ breast cancer may or may not express progesterone receptor (*PGR*) and/or *HER2*+. Triple-negative breast cancer does not express ER, PGR, or enriched HER2 and includes basal-like breast cancer. ER+ luminal subtypes represent ~70% of all invasive

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DOI: 10.1530/ERC-13-0512

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breast cancers diagnosed in the USA each year (Yanagawa *et al*. 2012).

ER+ breast cancer is defined if 1–10% of the cancer cell nuclei stain for ERα by immunohistochemistry (Harvey *et al*. 1999, Hammond *et al*. 2010). Women diagnosed with ER+ breast cancer are candidates for anti-hormonal therapy including tamoxifen, raloxifene, fulvestrant, and the aromatase inhibitors letrozole, exemestane, vorozole, formestane, and fadrozole (Ariazi *et al*. 2006, Larionov & Miller 2009, Geisler *et al*. 2012).

Classically, ERα is activated by binding to estrogens, resulting in nuclear translocation with binding to estrogen response elements and expression of estrogen target genes as a major mechanism of action. Membrane/cytoplasmic and G-protein-coupled activities are also described (Renoir *et al*. 2013). ERβ, which is encoded by a different gene, is generally characterized as being anti-proliferative in breast cancer (Fox *et al*. 2008). A wide range of molecular activities are known to influence the expression levels and activity of ERα, with many of these pathways having the capacity to contribute to breast cancer development (Manavathi *et al*. 2013). In both women (Allred *et al*. 2004) and mice (Frech *et al*. 2005), deregulated estrogen signaling can result in increased proliferation of the mammary ductal epithelium, leading to cancer progression.

Creation of immortalized ER+ breast cancer cell lines was an important step for the experimental study of ER+ breast cancer pathogenesis and therapy *in vitro* (Holliday & Speirs 2011, Wong & Chen 2012). Application of these cell lines to xenograft models has enabled a wide variety of *in vivo* studies examining response to therapy including anti-hormonal approaches (Brodie *et al*. 2005). Norway rats exposed to chemical carcinogens develop ER+ mammary cancer and have been used in different types of *in vivo* experiments exploring pathogenesis and treatment (Shull 2007).

Genetic engineering of mouse models to produce ER+ mammary cancer represents an alternative choice for *in vivo* study of ER+ breast cancer pathogenesis and treatment. Mice are more readily genetically manipulated than rats, there are many genetically engineered mouse models to breed into ER+ breast cancer models for further study, and mice are, in general, less expensive to maintain than rats. Mouse models of ER+ breast cancer have been reviewed previously (Mohibi *et al*. 2011, Kirma & Tekmal 2012). In this study, we update the discussion with more recently developed models as well as include new complementary information on those that have been described before. To date, five major types of models have been published (Table 1). The first type develops ER+ mammary cancer from direct overexpression of ERα in mammary epithelial cells (Table 1A). The second type exhibits ER+ mammary cancer as a result of genetic aberrations of other molecules within the estrogen-signaling pathway (Table 1B). The third type develops ER+ mammary cancer as a result of pharmacologic treatment in combination with genetic aberrations of other molecules within the estrogen-signaling pathway (Table 2). The fourth type results from exposure to a chemical carcinogen in combination with genetic aberrations of other molecules within the estrogen-signaling pathway (Table 3). The fifth type is derived from brother–sister matings of nude mice (Table 4).

At the present time, it is clear that there in no one mouse model that develops all of the histopathological types or molecular subtypes of ER+ human breast cancer (Malhotra *et al*. 2010). Human breast cancers are classified as non-invasive or invasive, and ER+ cancers are found in both categories. Invasive (also called infiltrating) ductal carcinoma is the most common type of human breast cancer and the majority of these are ER+. Histopathological types of human breast cancer that are even more commonly ER+ include invasive lobular, papillary, and tubular. Not all of the published studies that report the development of genetically engineered mice provide sufficient detail to be able to definitively assign the histopathology developing in the mouse to the corresponding human histopathology. Similarly, not all models have been yet adequately molecularly analyzed to be able to accurately assign molecular subtype. However, where this information is available, it is reported in the text as part of the description of the model.

The transcriptomes of mouse and human mammary epithelial cells demonstrate significant similarities in gene and pathway activation (Lim *et al*. 2010). However, there are differences in ER expression patterns. Luminal progenitor cells in humans are reported to express higher levels of ER than that in mice (Visvader 2009). In both normal human and mouse mammary gland, ER is expressed in a portion of the non-proliferating luminal mammary epithelial cells, whereas premalignant and malignant lesions demonstrate proliferating ER+ mammary cells (Anderson *et al*. 1998, Anderson & Clarke 2004). However, while human mammary stroma does not demonstrate expression of ER, ER is expressed in the mouse mammary stroma where it is able to act in a paracrine fashion on the mammary epithelium (Parmar & Cunha 2004).

The genetically engineered mouse models presented here utilize the mouse mammary tumor virus (*Mmtv*) long-terminal repeat and the rat neu-related lipocalin (*Nrl*)

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Table 1 Genetically engineered mouse models that develop ER+ breast cancer

Published nomenclature	Genetic nomenclature	Background strain	Age range of mice demonstrating cancer development (months)	Percentage of mice with mammary cancers within this age range (%)	Percentage of mammary cancers designated ER+ (%)	Parity required for tumor development	References
(A) Models developing ER+ mammary cancer from direct overexpression of ERα							
tTA/TAg/*ERα*	*Tet-op–Esr1*$^{MMTV–tTA/tet-op–SV40-TAg}$	C57Bl/6	10–12	37	100	No	Tilli *et al.* (2003)
CERM (conditional estrogen receptor α in mammary tissue)	*Tet-op–Esr1*$^{MMTV–rtTA}$	C57Bl/6	10–12	3–5	50	No	Miermont *et al.* (2012) Miermont *et al.* (2010) Frech *et al.* (2005)
AIB1Δ3/CERM	*Tet-op–Esr1*$^{MMTV–tTA/tet-op-AIB1Δ3}$	C57Bl/6	19–26	7	50	No	Nakles *et al.* (2011)
Brca1$^{f/f;}$ MMTV-Cre/p53+/−/CERM	*Tet-op–Esr1*$^{MMTV–rtTA/Brca1 f11/f11/MMTV-Cre/p53+/−}$	C57Bl/6	9–16	100	50	No	Herschkowitz *et al.* (2007)
(B) Models developing ER+ mammary cancer as a result of genetic alterations of molecules impacting estrogen signaling							
MMTV-cyclin D1	*MMTV-Ccdn1*	C57Bl/6	12	5	100	No	Miermont *et al.* (2012) Wang *et al.* (1994)
MMTV-cyclin D1	*MMTV-Ccdn1*	FVB	20–23	47.5	37.5	Yes	Lin *et al.* (2008)
MMTV-D1T286A	*MMTV-Ccdn1*^{T286A}	FVB	16–20	51	50	Yes	Lin *et al.* (2008)
NRL–PRL, line 1655–8	*Lcn2–Prl*$^{1655–8}$	FVB	12–21	80	50	No	Rose-Hellekant *et al.* (2003)
MMTV-Wnt1	*MMTV-Wnt1*	FVB	3–7	80	86	No	Zhang *et al.* (2005)
P53(R270H/+)WAPCre	*p53*$^{R270H/+/WAP-Cre}$	129Sv/C57BL/6	7–8	87	67	Yes	Wijnhoven *et al.* (2005)
P53$^{fp/fp}$*WAPCre*^{c}	*p53*$^{fp/fp WAPCrec}$	CB6F1× C57BL/6	8–12.5	92	40	No	Lin *et al.* (2004)
p53 null	*p53*−/−	BALB/c	11–12	24–55	21	No	Medina *et al.* (2002)
Stat1$^{-/-}$	129S6/SvEvTac-*Stat1*^{tm1Rds}	129S6/SvEv	18–26	62	90	No	Chan *et al.* (2012)
TGFα×*p53*+/−	*Nrl–TGFα*$^{p53+/−}$	FVB/N	9–21	100	'Most'	No	Rose-Hellekant *et al.* (2007)
MMTV–AIB1	*MMTV–AIB1*	FVB/N	12–25	76	40	No	Torres-Arzayus *et al.* (2004)
MMTV–Espl1	*MMTV–Espl1*	C57Bl/6	10–11	80	100	Yes	Mukherjee *et al.* (2013)
MMTV–Espl1, *p53*+/−	*MMTV–Espl1*$^{p53+/−}$	C57Bl/6	10–11	100	45	Yes	Mukherjee *et al.* (2013)
Pik3ca^{H1047R}	R26-Pik3ca$^{H1047R/MMTV-CreNLST}$	FVB	5–16	69	96	Yes	Adams *et al.* (2011)

Table 2 Model developing ER+ mammary cancer as a result of pharmacologic treatment in combination with genetic alterations of molecules impacting estrogen signaling

GEM model published nomenclature	Genetic nomenclature	Background strain	Age range of mice demonstrating cancer development (months)	Percentage of mice with mammary cancers within this age range (%)	Percentage of mammary cancers designated ER+ (%)	Pharmacological inducer	Parity required for tumor development	References
Brca1$^{f11/f11/p53+/-/MMTV\text{-}Cre}$	*Brca1*$^{f11/f11/p53+/-/MMTV\text{-}Cre}$	C57Bl/6	10–12	100	23	Efatutazone administered at age 4 months	No	Nakles *et al.* (2013)

promoter to drive coding sequences of cancer-inducing proteins. The *Mmtv* and whey acidic protein (*Wap*) promoters are used to drive the expression of the *Cre* recombinase. The *Mmtv* and *Wap* promoter sequences contain hormone-responsive elements, while the Nrl promoter is hormonally non-responsive. Molecular studies *in vitro* demonstrate that while the *Mmtv* promoter is responsive to glucocorticoid receptor, androgen receptor, and PGR, it is not an ER-responsive promoter (Prefontaine *et al.* 1999). *In vivo*, expression of *Erbb2* from an *Mmtv* promoter is not significantly increased by coincident expression of aromatase, which increases estrogen production (Tekmal *et al.* 2007). The *Wap-Cre* transgene is active only during lactation, while *Mmtv-Cre* is active throughout mammary development (Wagner *et al.* 1997). Studies in which ovariectomy is performed to demonstrate estrogen responsiveness of MMTV-driven cancers should take into account that ovariectomy will result in loss of both estrogen and progesterone, rendering the possibility that tumor regression could be confounded by a decrease in transgene expression levels due to the loss of progesterone. Appropriate controls to perform are to directly assess transgene expression in the presence and absence of ovariectomy. Similarly, if exogenous hormones including estrogen and progesterone are used, their impact on transgene expression levels should be characterized. The same transgene in different integration sites can exhibit different regulatory behavior, so one cannot generalize about the hormone dependency of a specific transgene from one line to another (Wagner *et al.* 2001). Finally, some hormonally unresponsive promoters within the transgenes can nevertheless demonstrate a dependency for the expression on hormonally regulated developmental stages such as the C3(1)/T(AG) transgene, whose expression is turned on with puberty but does not demonstrate differences in expression in response to isolated alterations in estrogen or ER levels (Yoshidome *et al.* 2000). Finally, hormonally responsive promoters can lose their dependence upon hormonal signals as reported for the *Wap-rtTACre* transgene (Lin *et al.* 2004).

ER1 overexpression mouse models

The addition of murine *Esr1* expression to a mouse model in which expression of simian virus 40 T antigen (TAg) is directed to epithelial tissues using a conditional tetracycline

Table 3 Model developing ER+ mammary cancer as a result of carcinogen exposure in combination with genetic alterations of molecules impacting estrogen signaling

Published nomenclature	Genetic nomenclature	Background strain	Age range of mice demonstrating cancer development (months)	Percentage of mice with mammary cancers within this age range (%)	Percentage of mammary cancers designated ER+ (%)	Chemical inducer	Parity required for tumor development	References
MMTV-myr*Akt1*	*MMTV–AKT1*^{myr}	C57Bl/6	6–12	40	100	7,12-Dimethyl-benz(a)anthracene (DMBA) treatment for 5 weeks beginning at 9 weeks of age	No	Blanco-Aparicio *et al.* (2007)

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Table 4 Model developing ER+ mammary cancer as a result of brother–sister matings of nude mice

Published nomenclature	Background strain	Age range of mice demonstrating cancer development (months)	Percentage of mice with mammary cancers within this age range (%)	Percentage of mammary cancers designated ER+ (%)	Parity required for tumor development	References
Spontaneous mammary tumor model	NIH Nude	3.5–12	62	100	Yes	Kumar *et al.* (2007)

responsive gene expression system that induces the appearance of ER+ mammary adenocarcinoma in 37% of the female of *tetracycline-operator (tet-op)–Esr1*$^{MMTV–tetracycline\ transactivator\ (tTA)/(tet\text{-}op\text{-}TAg)}$ mice by 12 months of age (Tilli *et al.* 2003; Table 1A). All of the mammary cancers that appear in this model are ER+. The cancers have been shown to bind estrogen and demonstrate estrogen dependence. In this model, expression of both *Esr1* and *simian virus 40 TAg* are targeted together to epithelial cells and temporally regulated using an *Mmtv–tTA* 'tet-off' transgene (Hennighausen *et al.* 1995, Furth 1997). A 'tet-off' regulated transgene is expressed in the absence of exposure of the mice to a tetracycline compound such as doxycycline. Administration of doxycycline to a transgenic mouse carrying the 'tet-off' system results in doxycycline binding to the tetracycline responsive transactivator protein, which changes its conformation and renders it unable to bind to the tetracycline responsive promoter. In contrast, in the 'tet-on' system a mutated tetracycline responsive transactivator protein is expressed, which binds a tetracycline responsive promoter only when it is bound to a tetracycline compound such as doxycycline. Both systems can be used equally as efficiently in transgenic mice to temporally direct transgene expression. Significantly, in the absence of coincident TAg expression, *Tet-op–Esr1*$^{MMTV–tTA}$ transgenic mice do not develop mammary gland cancer. The adenocarcinomas that develop in the *tet-op–Esr1*$^{MMTV–tTA/tet\text{-}op–SV40\text{-}TAg}$ mice histologically model human ER+ ductal adenocarcinoma.

In contrast, utilization of a different conditional transgene (*MMTV*–rtTA; Gunther *et al.* 2002) to target murine *Esr1* overexpression to mammary epithelial cells results in 3–5% of the *Tet-op–Esr1*$^{MMTV–rtTA}$ mice exhibiting mammary adenocarcinoma by 12 months of age (Miermont *et al.* 2010, 2012; Table 1A). Half of the invasive adenocarcinomas that appear are ERα+ and neither cancer prevalence nor percentage of ERα+ adenocarcinoma is altered by low-dose 7,12-dimethylbenz(a)anthracene (DMBA) exposure, loss of the signal transducers and activators of transcription factor 5a (*Stat5a*) gene, or coincident cyclin D1 overexpression. Prevalence of preneoplasia is higher than that of cancer with 30% of the mice reproducibly demonstrating ductal hyperplasia (DH) and 40% hyperplastic alveolar nodules (HANs) by age 12 of months (Diaz-Cruz *et al.* 2011, Nakles *et al.* 2011). In this model, expression of ERα is increased from 1.5- to 2-fold in mammary epithelial cells, resulting in the appearance of increased mammary ductal epithelial cell proliferation and the appearance of DH and DCIS by 4 months of age (Frech *et al.* 2005). Disease appearance is dependent upon the presence of cyclin D1 (Frech *et al.* 2008). Loss of one copy of *p53* (*Trp53*) significantly increases preneoplasia prevalence but not cancer (Diaz-Cruz & Furth 2010). Significantly, ERα+ invasive adenocarcinomas have developed on tamoxifen in this model and tamoxifen delivered at 10 months of age fails to induce a significant reduction in HAN prevalence, consistent with the presence of a significant degree of intrinsic tamoxifen resistance (Miermont *et al.* 2012). The impact of a 'tet-off' transgene (*tet-op–tTA*), whose expression is on an autoregulatory loop (Shockett *et al.* 1995), to target *tet-op–Esr1* expression to both epithelial and non-epithelial tissues has also been investigated (Tomic *et al.* 2007). Mammary DH also appears in these mice by 4 months of age (Tilli *et al.* 2003). The higher rates of preneoplasia and cancer development in *Tet-op–Esr1*$^{MMTV–rtTA}$ as compared with *Tet-op–Esr1*$^{MMTV–tTA}$ mice is correlated with a significantly higher percentage of mammary epithelial cells demonstrating targeted transgene expression (Hennighausen *et al.* 1995, Gunther *et al.* 2002). The *tet-op–Esr1*$^{MMTV–rtTA}$ mice histologically model human DCIS and ductal adenocarcinoma. Adenosquamouscarcinomas, modeling a less common human subtype, appear less frequently.

Amplified in breast cancer 1 (AIB1), also known as steroid receptor coactivator 3 or thyroid hormone receptor activator molecule 1, impacts the activity of both hormone-dependent and -independent pathways in breast cancer and has been proposed as a modulator of tamoxifen resistance (Lahusen *et al.* 2009, Xu *et al.* 2009). A splice variant lacking the N-terminal domain (AIB1Δ3/AIB1-Δ4)

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DOI: 10.1530/ERC-13-0512

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is a more potent inducer of estrogen-mediated gene transcription (Chien *et al.* 2011). Tri-transgenic *tet-op–Esr1*$^{MMTV–tTA/tet-op–AIB1}$ and *tet-op–Esr1*$^{MMTV–tTA/tet-op–AIB1\Delta3}$ mice were generated to compare the impact of AIB1 and AIB1Δ3 on ERα-mediated mammary carcinogenesis (Nakles *et al.* 2011; Table 1A). ER+ mammary adenocarcinomas modeling human invasive ductal carcinoma was developed in the *tet-op–Esr1*$^{MMTV–tTA/tet-op–AIB1\Delta3}$ mice, but the prevalence was not significantly different than that found in the *Tet-op–Esr1*$^{MMTV–rtTA}$ mice and cancers did not appear until 19–26 months of age, rendering no advantages of this genetic combination over the *Tet-op–Esr1*$^{MMTV–rtTA}$ mice for the study of ER+ mammary cancer.

Loss of the BReast CAncer 1, early-onset 1 (*BRCA1*) gene is a genetic risk factor for the development of breast cancer. A high percentage of women born with deleterious mutations in the *BRCA1* gene will develop breast cancer by age 70 (Chen & Parmigiani 2007). The predilection for cancer development in estrogen responsive tissues in women carrying *BRCA1* mutations may be related to the ability of *BRCA1* to downregulate the activity of ERα (Eisen *et al.* 2008). *Tet-op–Esr1*$^{MMTV–rtTA}$ mice were mated to mice with genetically engineered conditional deletion of exon 11 of the *Brca1* gene in the mammary epithelial cells to generate *Tet-op–Esr1*$^{MMTV–rtTA/\text{Brca1-floxed exon 11 }(f11)/f11/MMTV-Cre}$ for testing the impact of ERα overexpression on cancer development initiated by loss of *BRCA1* function (Jones *et al.* 2008). In the absence of *Esr1* overexpression, 53% of *Brca1*-deficient mice (Brca1$^{f11\,(f11)/f11/MMTV-Cre/p53+/-}$) develop triple-negative (ER−/PGR−/HER2<2+) adenocarcinomas with gene expression patterns paralleling those found in human breast cancers (Herschkowitz *et al.* 2007). In contrast, in the presence of *Esr1* overexpression, 100% of *Tet-op–Esr1*$^{MMTV–rtTA/\text{Brca1-floxed exon 11 }(f11)/f11/MMTV-Cre/p53+/-}$ mice develop mammary adenocarcinomas and 50% of these are ER+ (Table 1A). HAN prevalence is also 100% by 12 months of age and 50% of the hyperplasias are ER+ (Jones *et al.* 2008). The mammary cancers that develop in the *tet-op–Esr1*$^{MMTV–rtTA/Brca1\ \ f11/f11/MMTV-Cre/p53+/-}$ mice most commonly histologically model human-invasive ductal carcinoma.

Models that develop ERα+ cancer through alterations in the molecules interacting with the estrogen-signaling pathway

Cyclin D1 overexpression mouse models

Cyclin D1 plays an important role in the regulation of estrogen signaling in mammary tissue (Fu *et al.* 2004, Casimiro *et al.* 2013). Expression of cyclin D1 is positively correlated with ERα expression in breast cancer (van Diest *et al.* 1997, Bostrom *et al.* 2009). Cyclin D1 forms a complex with CDK4 or CDK6 to regulate the cell cycle at the G1/S phase and CDK4/6 inhibitors such as PD 0332991 are being studied in combination with anti-hormonal agents for the treatment of ERα+ cancer (Sutherland & Musgrove 2002, Lange & Yee 2011). Depending upon the length of time observed, specific transgenic line, and strain background studied, cyclin D1 overexpression targeted to mammary epithelial cells in *Mmtv-Ccdn1* transgenic mice results in the development of mammary cancer in 5–47.5% of mice (Wang *et al.* 1994, Lin *et al.* 2008, Miermont *et al.* 2012; Table 1B). Adenocarcinoma and adenosquamouscarcinoma histologic types appear corresponding to the same histologic subtypes in invasive human breast cancers. On an FVB background, 47.5% of *Mmtv-Ccdn1* mice develop mammary cancer between 20 and 23 months of age, 37.5% of these cancers are ER+, and isolated ER+ cancer cells placed in tissue culture are reported to demonstrate estrogen responsiveness (Lin *et al.* 2008). *Mmtv-Ccdn1*^{T286A} transgenic mice carry a genetically engineered cyclin D1 allele with an activating mutation. The mutant cyclin D1 encoded by the genetically engineered allele cannot be phosphorylated. This lack of phosphorylation interrupts cyclin D1 nuclear export, resulting in its retention in the nucleus and continuing activity (Lin *et al.* 2008). On an FVB background, 51% of these mice develop mammary cancer by 16–20 months of age, 50% are ER+, and the isolated cancer cells in tissue culture demonstrate estrogen responsiveness and tamoxifen growth inhibition (Lin *et al.* 2008). A high proportion of mammary adenocarcinomas arising in the *Mmtv-Ccdn1* mice demonstrate papillary histology, modeling human papillary breast cancer, while secretory glandular histology predominates in *Mmtv-Ccdn1*^{T286A} mice, an uncommon histological subtype in humans.

Prolactin overexpression mouse models

Prolactin (PRL) is a peptide hormone essential for normal mammary growth and development that, when overexpressed in mammary tissue, can induce mammary cancer development in mouse models. In women, the role of PRL in breast cancer development remains under investigation (Clevenger *et al.* 2003, Tworoger *et al.* 2013). PRL has the ability to activate ERα in the absence of ligand (O'Leary *et al.* 2013), and high levels of PRL have been associated with tamoxifen and aromatase inhibitor

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DOI: 10.1530/ERC-13-0512

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resistance (Dowsett *et al*. 1983, Bhatavdekar *et al*. 1994, Bami *et al*. 1998). Genetically engineered mice overexpressing rat *Prl* from the hormonally unresponsive rat *Nrl* promoter in the mammary epithelial cells was generated to study the potential role of local PRL overexpression in mammary cancer (Rose-Hellekant *et al*. 2003, Arendt *et al*. 2011; Table 1B). ER+ adenocarcinomas developed in one of the two FVB/N *Prl–Nrl* founder lines generated (line 1655–8; Table 1B). Line 1655–8 demonstrates elevated levels of serum PRL and 80% of the female mice from this line develop mammary adenocarcinomas between 12 and 21 months of age, and 50% of the cancers are ER+. Papillary is the predominant histology (44%) followed by glandular (22%) and solid (22%). Different percentages of ER+ cancer cells are described in the different cancer histologies: glandular (10%), solid (4%), papillary (21%), adenosquamous (32%), and carcinosarcoma (8%), modeling different histologic subtypes of some of the less commonly diagnosed human ER+ breast cancers. Significantly, aged (~22 months of age) FVB/N mice with pituitary prolactinomas also develop ER+ mammary cancers (Radaelli *et al*. 2009).

Wnt1 mouse models

Molecules involved in the Wnt1 signaling pathway (including cyclin D1, c-myc, and β-catenin) have been implicated in breast cancer (Li *et al*. 2000). Wnt1 signaling increases β-catenin levels, which transcriptionally activates cyclin D1 and c-myc. The impact of Wnt1 signaling on cyclin D1 ultimately affects the downstream estrogen–ER complex that regulates gene transcription and expression. The role of *Wnt1* in mammary cancer was initially investigated because it is one of the randomly selected sites of integration for the MMTV. Eighty percent of female *Mmtv-Wnt1* transgenic mice develop mammary cancer between 3 and 7 months of age with ~86% categorized as ER+ as defined by at least 5% of the mammary epithelial cancer cells demonstrating ERα expression (Zhang *et al*. 2005; Table 1B). Growth of the cancers, however, is not repressed by loss of estrogen signaling. Instead ER+ cells are lost, and selection of proliferating ER negative cells maintains cancer growth. Histology of the ER+ mammary cancers was not defined. While neither *Ras* mutation nor *Pten* insufficiency impact the percentage of Wnt1-induced mammary cancers demonstrating ER expression, *p53* haplo-insufficiency, *p53* insufficiency, and HER2/Neu overexpression lead to loss of ER positivity (Zhang *et al*. 2005, Fuchs-Young *et al*. 2011).

p53 mutation, *p53* deletion, and *p53* deletion transplant mouse models

Mutations in the *p53* gene are reported in 20–23% of ER+ breast cancer and are reported to negatively impact response to anti-hormonal therapy (Gasco *et al*. 2002, Uji *et al*. 2013, Yamamoto *et al*. 2014). A variety of cellular processes regulated by *p53*, including cell cycle control, apoptosis, senescence and response to DNA damage, can affect breast cancer development and therapy response (Lai *et al*. 2012, Walerych *et al*. 2012). *In vitro* *p53* has been shown to regulate ERα transcription by recruiting essential transcription factors to the ERα promoter in MCF-7 cells (Shirley *et al*. 2009). However, *In vivo*, *p53*-deficient mice have been shown to develop ER+ tumors, indicating that p53 is not required for ER expression.

Three different mouse models of functional *p53* loss develop ER+ mammary cancer (Table 1B). Expression of a *p53* R270H mutant allele targeted to mammary epithelium and activated during pregnancy using a *WAP-Cre* transgene (Wijnhoven *et al*. 2005) generates a mouse model in which mammary cancers appeared in 87% of the mice with a mean latency of 5 months following activation of Cre recombination during pregnancy. Sixty-seven percent of the cancers are reported to exhibit ER stained cells. Papillary and carcinosarcoma subtypes, modeling two less commonly seen human histologic subtypes, demonstrate epithelial cell staining, and a sarcoma subtype shows only positive mesenchymal cells. In another model, the *Wap-Cre* transgene is used to delete both copies of *p53* in mammary epithelium (Lin *et al*. 2004). Ninety-two percent of the parous mice develop mammary tumors with a median tumor latency of 9.5 months. Forty percent of the cancers are ER+ and include adenocarcinoma, myoepithelial adenocarcinoma, adenosquamous carcinoma, and spindle cell histologies. The adenocarcinomas would model human ductal carcinoma, while the others would model less frequently diagnosed subtypes. In contrast, if an *Mmtv-Cre* transgene is used to execute the *p53* deletion, none of the mammary cancers that develop are ER+, whether they are parous or virgin. A third p53-related model developed in BALB/c mice uses implants of mammary epithelium from 8-week-old female mice with germ line loss of *p53* that is placed into the cleared mammary fat pads of 3-week-old mice to generate a mouse model of human DCIS (Jerry *et al*. 2000, Medina *et al*. 2002, 2003). Between 24 and 55% of the implanted mice develop disease by 11 or 12 months following implantation, and 21% of the lesions are ER+.

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STAT1-deficient mouse model

STAT1 plays a role in the mediation of innate immunity, lying downstream of type 1 and 2 interferons, and is reported to both promote leukemogenesis and inhibit mammary carcinogenesis in mice (Koromilas & Sexl 2013). *STAT1* is expressed in human breast cancer epithelial cells with some, but not all, studies demonstrating a positive correlation with ERα and disease prognosis (Furth 2013). Ninety percent of the mammary carcinomas that develop in *Stat1*-deficient (129S6/SvEvTac-*Stat1*^{tm1Rds}) mice are ER+, show hormone-dependent growth, and demonstrate luminal-type cancer surface markers (Chan *et al*. 2012; Table 1B). Mammary cancers develop between 18 and 26 months of age. While only 62% of nulliparous mice develop mammary cancer, this increases to 91% in multiparous mice. The molecular signature of the mammary carcinomas developing in the mice resembles that of human luminal-type breast cancers. Histology is only specified as carcinoma and not further subtyped. Follow-up studies suggest that *Stat1* suppresses mammary cancer formation through regulation of *Jak2* activity by suppressor of cytokine signaling 1 (*Socs1*; Chan *et al*. 2014).

Transforming growth factor α overexpression mouse model

Transforming growth factor α (TGFα) is a member of the epidermal growth factor family that is overexpressed in some human breast cancers (Booth & Smith 2007). It promotes epithelial development and proliferation. Upon binding to its receptor, ERBB1, it creates either a homodimer or heterodimer with another member of the ERBB family of proteins (Roepstorff *et al*. 2008). Some of the dimers have been observed in ER− or ER+/PR− tumors spurring an interest in the role of TGFα in breast cancers (Holbro *et al*. 2003). TGFα expression can be found in 50–70% of human breast tumors and has been found to be downregulated by tamoxifen in ER+/PR+ breast cancers (Ciardiello *et al*. 1991, Noguchi *et al*. 1993). TGFα signaling stimulates cytoplasmic *Pi3k*, which triggers *Akt*. Genetically engineered FVB/N mice that overexpress TGFα in mammary epithelial cells due to an *Nrl–Tgfα* transgene will develop ER+ mammary cancers (Rose-Hellekant *et al*. 2007; Table 1B). It is reported that 'most' mammary cancers are ER+ but PGR− appear in virgin mice between ages 9 and 21 months and parous mice between ages 8 and 14 months of age. Cystic papillary histopathology is found in all the mice with the development of 'solid adenomatous' tumors in some mice, the first type may correspond to the papillary subtype in human. Ovariectomy reduces mammary tumor incidence from 100% in mice with ovaries to 67% in ovariectomized mice and increased the mean age at which tumors appeared by 4 months. Significantly mammary cancers that appear in *Nrl–Tgfα/p53*+/− mice are ER negative.

Aib1 overexpression mouse model

Mmtv–Aib1 mice on an FVB/N background were generated to test the impact of high levels of *Aib1* expression targeted to mammary epithelial cells (Torres-Arzayus *et al*. 2004; Table 1B). ER+ and ER− mammary tumors appear in 40 and 8%, respectively, of female mice with the majority appearing between 12 and 25 months of age. There was no significant difference in the time of onset or incidence between virgin and parous mice. Both mammary preneoplasia and tumors are reported to demonstrate high levels of the phosphorylated forms of insulin-like growth factor 1 receptor, the p70S6 kinase, and phospho-S6 ribosomal protein. Other tumors that appear in these mice include the frequent appearance of pituitary adenomas, uterine leiomyosarcomas, and lung adenocarcinomas and less frequently fibrosarcomas, skin papillomas and squamous cell carcinomas, ovarian teratomas, lymphomas, osteomacrophage sarcomas, and adrenocortical tumors of the kidney. ER+ tumor histopathology is reported as microacinar and comedo type, types corresponding to non-invasive DCIS-type breast cancers in women.

Separase overexpression mouse model

Espl1 encodes the gene for separase, a cysteine protease that hydrolyzes cohesin, mediating progression from metaphase to anaphase. It is overexpressed in some ER+ human breast cancers. Eighty percent of multiparous transgenic C57Bl/6 mice carrying an *Mmtv–Espl1* transgene develop mammary tumors by 10–11 months of age (Mukherjee *et al*. 2013; Table 1B). Tumors also develop in primiparous but not nulliparous mice. Nuclear and cytoplasmic staining for ER is described in all histological types of mammary cancers that developed in these mice: spindle-like, squamous, solid, and glandular, the first two subtypes representing less common histologic human breast cancer subtypes with further information required to know if the other histologies correspond to human ductal carcinomas. Significant intra- and inter-tumor heterogeneity demonstrating both luminal and basal features are present with more and less differentiated areas exhibiting different ER expression levels. An immune

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reaction with hyperproliferative stroma is present in 12-month-old mice. Introduction of a *p53/−* background into the mice did not significantly alter tumor penetrance or latency; however, lung metastases are found only in *Mmtv–Espl1/p53+/−* mice and the percentage of ER+ cells is reduced to ~45%. The *Mmtv–Espl1/p53+/−* model is described as being the representative of the more aggressive forms of human breast cancer that exhibit genomic instability, cell cycle defects, and metastases.

Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α gain-of-function mutation mice

Activating mutations in the phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α (*PIK3CA*) gene occur in between one-quarter and one-third of human breast cancers, with 40% of these mutations located to the kinase domain. Introduction of a *Pik3ca* gene sequences carrying the *H1047R*-activating mutation ($Pik3ca^{H1047R}$) into the *Rosa26* (*Gt(Rosa)26Sor*) locus with activation through expression of an *Mmtv-Cre* transgene results in 43% of the $Pik3ca^{H1047R/p53f/+/MMTV\text{-}CreLineA}$ mice developing mammary tumors by 10 months of age when the more strongly expressed $Mmtv\text{-}Cre^{LineA}$ transgene is used, and 69% of mice by 17 months of age when the more mammary cell targeted but heterogeneously expressed $Mmtv\text{-}Cre^{NLST}$ transgene is used (Table 1B; Adams *et al.* 2011). Lymphomas/thymomas as well as other tumors also develop in these mice at significant frequencies (84 and 43% respectively). Tumors appear in both virgin and parous mice. Loss of one *p53* allele increases the percentage of $Pik3ca^{H1047R/p53f/+/MMTV\text{-}CreNLST}$ mice, demonstrating mammary tumors to 80% by ~10 months of age. Ninety-six percent of the tumors developing in the $Pik3ca^{H1047R/p53f/+/MMTV\text{-}CreNLST}$ mice are reported as either adenomyoepithelioma or adenosquamouscarcinoma, and ER+ cells are noted to be found in each of these histological cancer types, although specific percentages are not reported. These represent some of the less commonly seen ER+ histological subtypes found in women. Spindle cell tumors also appear.

Pharmacological interventions can promote the development of ERα+ mammary cancer in genetically engineered mice

PPARγ is expressed in human breast cancers where, when activated, it can exert a differentiating, apoptotic, and/or growth inhibiting effect (Kotta-Loizou *et al.* 2012). Efatutazone was tested as a cancer preventative in *Brca1* mice because it is a high affinity PPARγ agonist that does not demonstrate activation of related receptors PPARα or PPARΔ and is currently in human clinical trials for cancer therapy. *Brca1* mice were used because they have intrinsic resistance to tamoxifen (Jones *et al.* 2008) and efatutazone represents an alternative preventative. The percentage of $Brca1^{f11\ (f11)/f11/MMTV\text{-}Cre/p53+/-}$ mice developing ERα+ cancers can be increased by exposure to the peroxisome proliferator-activated receptor γ agonist efatutazone (Nakles *et al.* 2013; Table 2). While expression of ERα is rare (0–1%) in cancers developing in untreated $Brca1^{f11/f11/MMTV\text{-}Cre/p53+/-}$ mice, it rises to 23% in cancers that develop on efatutazone treatment initiated at 4 months of age. ER+ histologic subtypes that appear on efatutazone in $Brca1^{f11/f11/MMTV\text{-}Cre/p53+/-}$ mice include papillary and DCIS, modeling the same histological subtypes found in women.

Carcinogen exposure can promote the development of ERα+ mammary cancer in genetically engineered mice

Nuclear localization of phosphorylated V-Akt murine thymoma viral oncogene homolog 1 (*AKT1*) is correlated with ER positivity in human breast cancers (Bostner *et al.* 2013). *AKT1* lies downstream of phosphoinositide-3-kinase (PI3K) signaling. This pathway can play numerous roles in carcinogenesis (Klarenbeek *et al.* 2013). To explore the role of AKT1 activation in breast cancer, a genetically engineered mouse model with the expression of an artificially constitutively activated form of *Akt1* targeted to mammary epithelium using the *Mmtv* promoter was generated (*Mmtv-myrAkt1* mice) (Blanco-Aparicio *et al.* 2007; Table 3). Localization of *Akt1* to the membrane through myristoylation generates a mouse model with constitutive AKT activation. Both lines of transgenic mice generated develop mammary cancer, but only after exposure to the chemical carcinogen, DMBA beginning at 9 weeks of age and continuing for 5 weeks. Roughly 40% of the mice exhibit either mammary adenocarcinoma (papillary or poorly differentiated) or adenosquamous carcinoma between 13 and 39 weeks after DMBA administration (between 6 and 12 months of age). All of the cancers are ER+. The poorly differentiated cancers may model the more commonly found invasive ductal carcinomas in women while the other two subtypes would model less commonly found morphologies. Given the cytoplasmic localization of *Akt1* in these transgenic mice, they do not directly model the nuclear *AKT1* localization

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reported in human ER+ breast cancer. However, WT mice exposed to DMBA demonstrate predominantly ER− mammary tumors (Yin *et al.* 2009). The appearance of ER+ mammary cancers in this model appears to be functionally related to expression of the activated *Akt1*.

Spontaneous ER+ mammary cancer nude mouse model

Although not deliberately genetically engineered, brother–sister matings of heterozygous NIH nude mice resulted in the development of a line of mice with high serum estrogen levels, in which 62% of females develop Erα-positive metastatic mammary cancers by a mean age of 7 months (Kumar *et al.* 2007; Table 4). Mammary adenocarcinomas appear only in breeding females. Loss of ovarian hormone stimulation through ovariectomy leads to tumor regression. Histologically, the tumors are described as having tubular features, one of the less common ER+ subtypes found in women, generally associated with a good prognosis.

Summary and conclusions

In this study, we describe a spectrum of genetically engineered mouse models that develop ER+ mammary cancer. Different strain backgrounds are represented. While there is significant variability in the percentage of cancer cells demonstrating ER positivity, reported levels fall within the criteria used to define ER+ breast cancer. Some, but not all, of the models have been tested to determine their response to anti-hormonal agents and/or investigated for hormone dependency for growth. An important step in the validation of genetically engineered mouse models of different breast cancer subtypes is to compare their transcriptional profiles with those found in human breast cancers (Pfefferle *et al.* 2013). ER+ breast cancer models that have been more rigorously investigated for parallels to human disease include Wnt1 overexpression and *BRCA1*, *STAT1*, and *p53*-deficient models. The long latency (>12 months of age) of many models renders them challenging and expensive to work with; however, application of mammary epithelial transplant techniques could make them more tractable for study. There does not appear to be one best mouse model of ER+ mammary cancer consistent with the fact that there is not one type of ER+ human breast cancer. Instead, like all experimental tools, the model system selected should be that most suitable for the experimental design and goals. For example, if there was a reason to directly control the timing of ERα expression or co-express ERα with another gene, then a conditional system would be most appropriate. If the goal is to determine factors that regulate the appearance or maintenance of ERα expression in mammary cancers, then one of the spontaneous ERα+ models may be more useful. More uniform and comprehensive information on hormone responsiveness, response to anti-hormonal agents, and gene expression patterns as compared with human ER+ breast cancers as well as characterization of the genetically engineered mice on different strain backgrounds would be useful.

Future directions

Opportunity for generation of new genetically engineered mouse models remains. There is a strong need for ER+ models that reliably develop metastatic disease. Further characterization of the model developed in nude mice (Kumar *et al.* 2007) is required before it can be effectively used for experiments that might address the pathophysiology of ER+ metastatic disease. Another approach to develop more sophisticated models reflective of individual ER+ breast cancer subtypes will be to combine transgenic *Esr1* expression with other genetic manipulations as was accomplished for both *TAg* expression and loss of *Brca1/p53*. For example combining *Tet-op–Esr1*$^{MMTV–rtTA}$ mice with a mouse model of *ErbB2/Her2* mutation (Ursini-Siegel *et al.* 2007) could generate a model for luminal B ER+ breast cancer. Although joining Esr1 overexpression with germ line *p53* haplo-insufficiency did not accelerate tumor development (Diaz-Cruz & Furth 2010), combining *p53*$^{R270H/+/WAP\text{-}Cre}$ with *Tet-op–Esr1*$^{MMTV–rtTA}$ mice might be more potent as mutant *p53*^{R270H} has more molecular impact than simple reduction of *p53* expression levels. Moreover, a model with mutant *p53* would be translationally relevant for breast cancers carrying somatic p53 mutation in contrast to the germ-line insufficiency model that more closely parallels Li–Fraumeni syndrome. Bringing *Esr1* overexpression into the *Nrl–Prl* line 1655–8 model could make a new laboratory tool for further study of the epidemiologically defined risk of elevated PRL on breast cancer (Tworoger *et al.* 2013). Loss of *Stat1* accelerates mammary cancer development in *Mmtv-Neu–IRES-Cre* mice (Klover *et al.* 2010). If loss of *Stat1* in *Tet-op–Esr1*$^{MMTV–rtTA}$ mice accelerated cancer development to under 12 months, this would be a more tractable model for further study of luminal type breast cancer. Targeted development of new models and refinement of existing models will need to build upon the new information

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emerging from deep sequencing and genetic characterization of ER+ breast cancer in humans.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

Funding

Supported by NCI, NIH 1RO1CA112176 (P A Furth), NCI, NIH 5P30CA051008 (P A Furth) and NCI, NIH T32 Ruth L Kirschstein National Research Service Award Institutional Training Grant 5T32CA009686 (S A Dabydeen).

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Received in final form 25 January 2014
Accepted 30 January 2014
Made available online as an Accepted Preprint
30 January 2014

Published by Bioscientifica Ltd.