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How to Choose a Mouse Model of Breast Cancer, a Genomic Perspective

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Abstract

Human breast cancer is a heterogeneous disease with numerous subtypes that have been defined through immunohistological, histological, and gene expression patterns. The diversity of breast cancer has made the study of its various underlying causes complex. To facilitate the examination of particular facets of breast cancer, mouse models have been generated, ranging from carcinogen induced models to genetically engineered mice. While mouse models have been generated to mimic the initiating event, including p53 loss, BRCA loss, or overexpression of HER2 / Neu / erbB2, other genomic events are often not well characterized. However, these secondary genetic events are often critical to the mouse tumor evolution, subtype, and outcome, just as they are in human breast cancer. As such, these other genomic events are a critical component of what models are chosen to study specific subtypes of human breast cancer. Here we review the genomic analyses that have been completed for various genetically engineered mouse models, how they compare to human breast cancer, and detail how this information can be used in choosing a mouse model for analysis.

Keywords Mouse models · Breast cancer · Genomics

Introduction

Classification of breast tumor pathology, and the grading of breast tumors dates back to the early twentieth century when the potential importance of histological heterogeneity was noted [1, 2]. With the recent advent of powerful research tools, including microarrays and sequencing technologies, heterogeneity at the molecular level has revealed the complexity of breast cancer [3]. At a clinical level, heterogeneity compounds the difficulty of treatment, especially for late stage tumors where metastatic lesions are often vastly different from their primary tumor counterparts at both a molecular and histological level. Given the complexity of breast cancer, mouse models were initially used to study some of the well known driving events, but have more recently been shown to mimic human breast cancer through significant molecular heterogeneity [4]. From a genomic perspective, this review will explore the necessary considerations for choosing the correct mouse model when studying particular aspects of the disease.

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Human Breast Cancer

On a broad histological level, human breast cancer is classified into in situ and invasive carcinoma [5]. Both of these classification schemes are further delineated and there are now more than 20 recognized histological subtypes of human breast cancer [6]. Focusing on the invasive disease, which typically yields a worse prognosis, invasive ductal carcinoma (IDC) accounts for approximately 74% of cases [7]. Invasive lobular carcinoma (ILC), accounts for 15% of invasive breast cancer cases, with other rare histologies, including papillary and lipid rich carcinoma, comprising the remaining 10%. [7, 8].

Contrary to their names, IDC and ILC do not always arise in the mammary ducts or lobules respectively [9]. ILC is marked by E-cadherin loss and often has mutations in *PTEN*, and *FOXA1* [10, 11]. Typically, ILC patients have a better prognosis, with estrogen receptor/progesterone receptor (ER/PR) positive, and HER2 negative staining. IDC, otherwise known as invasive carcinoma of no special type, is itself marked by varying histologies, and is classified as such through the lack of any specific differentiation [9].

Clinically, certain histologies, such as invasive cribriform and mucinous, offer a better prognosis than others [9]. While IDC is typically diagnosed at earlier stages than ILC, survival rates for IDC patients are lower when stage matched to their ILC counterparts [12].

Breast cancer subtypes have also been defined through gene expression data [3, 13]. This has since been refined to the PAM50 subtypes, which include luminal A, luminal B, human epidermal growth factor 2 (HER2)-enriched, basal-like, and claudin-low [14]. The luminal A and B subtypes are typically distinguished from the other three subtypes through a number of factors including lower expression of proliferation markers, higher expression of estrogen/progesterone receptors (ER/PR), and lower HER2 expression [15, 16]. Between the luminal subtypes, luminal B tumors tend to have higher staining for the proliferation marker Ki-67, and lower expression for PR. While both luminal subtypes typically have a better prognosis than the HER2-enriched, basal-like, and claudin low subtypes, Luminal B tumors tend to have a worse prognosis than their luminal A counterparts [17–19]. As their name suggests, HER2-enriched breast tumors tend to have high expression of the *ERBB2* gene while having low expression of basal-like genes such as *FOXC1* [15]. HER2-enriched tumors also display a high frequency of *TP53* mutations, with approximately 72% having a mutation. Clinically, PAM50 defined HER2-enriched tumors have been shown to have a better treatment response with the addition of Trastuzumab to standard chemotherapies [20]. Basal-like tumors have the worst prognosis of any of the intrinsic subtypes, with the majority of them being triple negative (ER-/PR-/HER2-) [21]. Basal-like tumors tend to have high expression of proliferation markers, and also have the 2nd highest mutation rate of the intrinsic subtypes, with *TP53* being mutated in 80% of triple-negative tumors [22]. Finally, claudin-low tumors tend to have high expression of epithelial to mesenchymal transition markers, and low expression of luminal markers [14]. A majority of the claudin-low tumors fall into the triple negative subset, and have a poor prognosis [14].

In addition to transcriptomic data for human breast cancer, The Cancer Genome Atlas (TCGA) has genomically cataloged more than 30 types of cancer from over 11,000 patients, providing unique insight on copy number alterations, somatic mutations, and how this correlates with mRNA expression. The large number of samples provided by TCGA allows researchers to explore previously hidden genomic alterations and add statistical robustness to their study. TCGA is a powerful tool for researchers looking for important genomic trends or individual genes involved in cancer, and is even more robust when combined with validation studies done in vitro or in vivo.

There is a long and rich history of using immortalized cancer cell lines to study human breast cancer. More recently, tumor microenvironment research, including on the stroma and immune system, has revealed the impact of these systems on both the formation of primary tumors and their progression to metastatic lesions. The in vitro study of cell lines, or injection of cell lines into immunocompromised mice represents a limitation to the study of cancer cell lines. In order to overcome some of the shortcomings of in vitro studies, patient derived xenografts (PDXs) have been generated by implanting human tumor cells or tissues into immunodeficient mice [23, 24]. While the primary advantage of PDX systems is the lack of accumulated mutations and genetic drift that occurs within in vitro systems cultured on plastic, recent work has illustrated shortcomings of PDX models, including genetic drift in late passaged tumors, as well as the necessity of using immunocompromised recipients [25–29].

Mouse Models

Carcinogen Based Models

A common method for modeling breast cancer is through mouse model systems. Currently there are numerous systems, each with advantages and disadvantages, used to generate different models. Modeling cancer in animals began with the application of coal tar on rabbits and mice, leading to the formation of tumors [30]. Since that point, a wide array of carcinogens employed in mice have been used to study cancer, including N-methyl-N-nitrosourea (MNU), 3-methy1cholanthrene (MCA), and perhaps the most widely used 7–12,Dimethylbenz[a]anthracene (DMBA) [31, 32]. Tumors in mice treated with carcinogens often express a variety of genomic alterations including mutations in *PTEN*, increased expression of *CCND1* and *MYC*, and the activation of important cellular pathways including NF-κB, Wnt, and PI3K/AKT [33, 34]. Histologically, these tumors vary greatly between models, with MPA treated mice often exhibiting type-B adenocarcinomas, and DMBA treated mice often having tumors of the adenomyoepethelial and myoepithelial histologies [31, 35].

Transplant Mouse Models

To further study facets of human cancers in a more biologically relevant setting, transplantable mouse models have been developed. These include the mouse intraductal (MIND) model in addition to the previously mentioned cell lined xenografts and patient derived xenograft models. In order to study the progression of human cancers from ductal carcinoma in situ (DCIS), the MIND model mimics human DCIS through the injection of human DCIS cells into the ducts of SCID-beige mice [36]. Indeed, this method allows for the subtypes of DCIS to be maintained in a mouse model [36, 37]. However, despite their clear strengths, these models are not readily amenable to modification or manipulation to allow quick and easily genetic testing of hypotheses.

Genetically Engineered Mouse Models

The complexity of human cancer may best be modeled through the various forms of genetically engineered mice, including transposon based, transgenic, knock-in, knock-out, and inducible mouse systems. One of their largest advantages these models possess is the acquisition of impactful mutations [38, 39], analogous to the development and progression of human breast cancer.

One method of generating mice with cancer in the mammary glands is through the use of transposable elements [40–42]. These systems are used for germline transmission, as well as generating somatic mutations for the study of cancer [43]. Use of these systems allowed mice to be characterized with mutations in key genes. As mentioned above, patients with ILC tend to have loss of E-Cadherin. Using the Sleeping Beauty (SB) transposable system, Kas et al. showed the importance of particular genes, including *Myh9*, and *Ppp1r12b*, contributing to tumor formation in mice with ablated E-Cadherin [44].

To study potential oncogenes, transgenic mice are developed to determine whether overexpression of that particular gene results in tumor formation. In these mice, tissue specific promoters direct oncogene expression to a particular organ or tissue. Promoters for the study of breast cancer in mice include the commonly used mouse mammary tumor virus (MMTV) and whey acidic protein (WAP), as well as others including keratins [45–47]. Overexpression of a number of important oncogenes with these promoters has illustrated the importance of key genes, including *C-MYC*, RAS, and *ERBB2* [45, 48, 49]. In addition to the simple overexpression systems, work from the Chodosh lab introduced numerous inducible systems where expression of key oncogenes could be turned on or off in the mammary gland through introduction of doxycycline to the water [38, 50–52]. These systems revealed that while tumors were initially dependent upon the initiating oncogene, they accumulated enough mutations that when expression of the primary driving gene was withdrawn, tumors that initially regressed eventually relapsed. Other studies have used a combination of the inducible and standard transgenic systems to demonstrate oncogene dominance, where only one oncogene in a two oncogene system is needed to maintain tumor viability [53, 54].

In addition to transgenic models with overexpression of various oncogenes, knock-in models have been generated to express oncogenes in their native genomic location. This has allowed for expression of oncogenes under the control of the Rosa26 promoter, resulting in lower levels of transgene expression [55]. Other groups have placed a lox-stop-lox cassette between the endogenous promoter and an oncogene. The advantage of this system is that normal temporal and spatial control of gene expression occurs [56], but depending on timing of the excision event, mice can adapt to oncogene expression [57]. Importantly, with the lox-stop-lox system, erbB2 knock-in mice developed amplification and overexpression of the oncogene, analogous to HER2 + ve breast cancer [56]. Numerous other knock-in models have been created to study breast cancer genes, including R273H, R248W, and R175H *Tp53* mutant mice, as well as H1047R *Pik3ca* mutant mice [58, 59].

Alongside overexpression of oncogenes, knock-out mice permit the study of tumor suppressor genes in vivo. *TP53*, the most mutated gene in breast cancer, as well as *BRCA1*, which has germline mutations in 5–10% of human breast cancer, have been studied extensively through the use of knockout models [60]. The combination of knockout models with transgenic models, where expression of Cre is linked to the transgene, have also allowed the study of specific facets of tumor development while lacking signaling pathways [61, 62].

In addition to standard transgenic and knock-in / knockout systems, engineered nuclease systems, including TALEN and CRISPR, are used to generate mouse models. These systems allow for the deletion, addition, and replacement of desired DNA sequences into numerous models, including mice. While TALEN systems are capable of editing genes anywhere in the genome, as opposed to CRISPR needing nearby PAM motifs, CRISPR has become a more widely used tool due to its simplicity and cost effectiveness. Studies utilizing the power of TALEN and CRISPR systems have investigated numerous genes important to breast cancer, including *BRCA1* and *CDH1* [63, 64]. These systems can be employed through manipulation of mouse embryonic cells, or through direct injection of the system components into wildtype mice, and mice containing the cas9 protein under control of the cre-lox system [65, 66]. Gene specificity is achieved in these systems through the use of guide RNAs. A further review of these systems can be found here [67]. With the recent advent of CRISPR systems easing the transgenic process, it will also be interesting to see whether there is a resurgence in the use of ER+ rat models. Another tool potentially capable of faithfully recapitulating human breast cancer progression is the replication-competent avian sarcoma-leukosis virus – tumor virus A receptor (RCAS-TVA) system reviewed here [68]. This system can be used for the delivery of oncoproteins and dominant negative tumor suppressors in a timely matter, but is often limited to small insertions into the virus.

With the heterogeneity of human breast cancer and the large number of mouse models available to study the disease, the central question becomes, which model is the best fit for a particular study? This is obviously dependent on the experimental question, but the characterization of the models and their relation to human breast cancer should be considered. This is true on a phenotypic, genomic, and gene expression level.

Mouse Phenotypes

On a phenotypic level, there is a large amount of variation between the various mouse models of breast cancer. In terms

of latency, models range from the rapid MMTV-PyMT in the FVB background, to the prolonged GR/J, with tumors appearing at 45 days, and 12 months respectively. Other notable models with strikingly different latency periods include MMTV-NeuNT transgenics relative to the conditional expression of NeuNT under the control of the endogenous promoter, where tumors appear at 89 days and 15 months respectively [69, 70]. Variation is also observed in the tumor growth rate in various strains. While MMTV-Neu mice grow to 2500mm^3 from first palpitation in approximately 45 days [71], other models such as MMTV-Myc mice with *Stat3* ablated, can take as long as 109 days to grow to 2500mm^3 from the first palpitation [72]. Fluctuations in tumor latency and growth rate are also context dependent, relying on differentially activated signaling pathways. This is exemplified with ablation of the E2F1 transcription factor in two different mouse models. Loss of E2F1 in the MMTV-Neu mouse model leads to increases in both tumor latency and growth rate, whereas in the MMTV-PyMT model, a decrease in latency and no alteration to growth rate was observed [71, 73]. These differences illustrate the importance of selecting particular models for a study.

Previous research has also shown histological differences between the primary tumors of various mouse models. A review of GEMMs by a panel of experts in 2000 found the majority of genetically engineered mouse tumors to have a set of histological forms unique from non-GEMM tumors such as carcinogen induced models [74]. Some GEM tumors, such as those from models expressing the *neu* and *src* transgenes, have also been found to have histologies similar to those of tumors from human patients [75]. Much like human breast cancer, a large amount of histological variation is seen within certain GEMMs. MMTV-Myc mice have been shown to harbor multiple tumor histologies including papillary, microacinar, and squamous tumors [39]. Similar pathologies were noted in the MMTV-Met mice [76]. In MMTV-PyMT mice, while approximately 40% of tumors have a microacinar histology, tumors also display a wide array of histological patterns including adenosquamous, glandular, and those of mixed histology [73]. More recently, certain GEMM tumor histological subtypes have been shown to correlate with particular transcriptional profiles within the model, much like the human disease. In fact, gene expression signatures have been generated that are capable of predicting histological patterns in mouse tumors [77].

The study of metastasis is also heavily reliant on mouse models. While the expression of some oncoproteins such as PyMT and Neu result in a heavy metastatic burden in mice, other transgenic models with potent oncogenes such as WAP-Ras and MMTV-Myc have lower metastatic rates, or fail to metastasize at all [46, 49, 69]. Strain background is also an important consideration in the ability of the primary tumor to metastasize, with expression of PyMT in FVB mice resulting in nearly all tumor bearing mice developing metastasis to the lung. However, the same transgenic line interbred to RF/J, C58/J, and other backgrounds dramatically reduced the metastatic burden [78]. Of GEMMs that metastasize, most result in metastases to the lungs. However, select models have the ability to metastasize to different organs. MT-Met mice have demonstrated metastasis to the heart and kidney as well as the lung, and tumors from p53$^{fp/fp}$ MMTV-Cre mice are able to metastasize to the liver [79, 80].

Gene Expression Data

The advent of microarray and sequencing technologies has made it possible to complete large scale gene analysis on large numbers of samples. In breast cancer, conserved gene expression patterns led to the definition of the intrinsic subtypes of breast cancer [3]. Since the initial work on human breast tumor expression data, numerous studies have applied microarrays to study GEMM mammary tumors. This has been done for individual models [39, 76, 81–87], as well as in a broader survey approach across models.

When examining individual models using array analysis, a surprising amount of molecular heterogeneity has been a recurring finding. Not surprisingly, this heterogeneity was present in tumors with long latency (MMTV-Myc), and correlated with histological subtypes. Predicting that tumors with a short latency would be less heterogeneous would appear to be a logical hypothesis, however, it is notable that tumors with extremely short latency, driven by PyMT, also have a surprising level of heterogeneity from tumor to tumor. Together these studies suggest that both models are dependent upon accumulation of other events for tumor formation and progression. Not all models have extensive heterogeneity, and models such as Wap-Myc, C3(1)Tag, and MMTV-Neu, have less heterogeneity based on gene expression profiles. Comparison of these individual models to human breast cancer has revealed that C3(1)-Tag and Wap-Myc models have expression patterns similar to basal-like human tumors, including high expression *CRYAB*, a known human basal-like tumor marker [88]. Expression signatures from other tumor types, such as luminal, do not correlate as well between mouse models and human tumors, although they still share some similar features, like positive staining for the K8/18 marker [88]. While the MMTV-Neu model fails to actually reflect human Her2+ breast cancer on a gene expression level, this may simply be due to the altered expression of other genes within the large HER2 amplicon. A mouse model with amplification of the endogenous erbB2 locus [56] should thus be assayed for similarities to human HER2 + ve breast cancer.

In addition to manuscripts that have profiled individual models, there have been several publications that compared various models. Herschkowitz et al examined 13 different models of breast cancer, identifying models with similarities to luminal

tumors, despite being ER-negative, and having heterogeneous expression patterns. They also identified other GEMMs resembling more basal like tumors. [88]. Hollern et al increased the number of samples analyzed (1156) as well as profiling numerous additional models to examine 26 major models with several additional variants (wild type *Myc*, T58A *Myc* etc.). This unsupervised approach demonstrated substantial heterogeneity in the majority of mouse models. Using both a gene expression and a signaling pathway approach, they also noted several similarities between the intrinsic subtypes of human breast cancer, and subsets of various mouse models. Importantly, it was noted that only a portion of tumors from an individual model reflected each of the intrinsic subtypes [4]. Further, Pfefferle et al. examined 356 samples from 27 models to identify 17 distinct mouse mammary tumor intrinsic subtypes, eight of which reflected subtypes in human breast cancer. However, this analysis used an intrinsic approach, a supervised method of clustering that may add bias to the study. Each of these three manuscripts provides an important examination of the diversity of mouse models of breast cancer and are an essential starting point when choosing a mouse model for analysis.

Genomic Copy Number Alterations

In tumor cells, regions of the genome are often deleted or repeated dozens of times, potentially serving to drive tumor formation or modify tumor progression. A prime example of copy number variation (CNV) in cancer is the amplification of human epidermal growth factor receptor type 2 (*HER2*), resulting in uncontrolled activation of downstream signaling cascades, including the MAPK pathway [89, 90]. While extensive CNV data from mouse tumor models has not been generated, use of an algorithm that predicts CNV from gene expression data has been generated and validated [91]. Applied to mouse models of breast cancer, the prediction of CNV noted variation across numerous mouse models of breast cancer. However, genes from some CNV regions, such as *Gsn*, are conserved among some models [91]. This same trend was seen within distinct mouse models, whereas some CNV events showed little conservation between mice in a given model, and other events were present in greater than 50% of mice in a given model [91]. More interestingly, integrated clustering of CNV events from mouse and human tumors showed conservation of some CNV events between the two species [91], demonstrating that mouse models can be an accurate depiction of human breast tumors in terms of copy number alterations.

Pathway Analysis

Research has shown that complex networks of proteins work together in regulatory pathways that control cellular function. These signaling pathways, including the MAPK/ERK and PI3K/AKT pathways, are often dysregulated in cancer [92, 93]. Expression data from the various genes that constitute these pathways and their downstream targets can predict activation or inactivation of particular pathways, making these pathway signatures an important tool for the study of breast cancer. To uncover pathway use, gene expression analysis has been coupled with bioinformatic tools like Gene Set Enrichment Analysis (GSEA), which has been widely applied to many models. Likewise, a Bayesian Regression Pathway signature system [94] has been applied to mouse models of breast cancer to predict cell signaling pathway activity [71–73]. Like differential gene expression data, pathway signatures often vary within GEMMs, the most prominent example of this perhaps being the *Myc* model [4]. In mice, pathway signatures have shown a correlation with histological subtypes, most notable being the microacinar histology associated with amplification events on chromosomes 11 and 15 [91]. Pathway signatures from mouse mammary tumors have also been found to correlate to human breast tumors. A set of highly expressed pathways found in tumors from Myc mice were also found to be highly expressed in Basal-like human tumors [95]. This trend has been seen in a number of pathway signature sets between mouse and human tumors.

Sequencing

Sequencing of human breast cancer samples has led to both the discovery of novel mutations important to breast cancer, such as *FOXP1* [96], as well as further characterization of genes already known to be important to cancer development including *HER2* and *PI3K* [97, 98]. In mouse models, sequencing studies in lung cancer have shown the mutational burden from GEMM tumors to be lower than that of human lung tumors. Tumors from *Kras*, and *Egfr* driven mice carry a mutational burden of ~.05 non-synonymous mutations per mega base, while human tumors harbor a mutational burden of ~4.1 non-synonymous mutations per mega base [99, 100]. While numerous publications have examined gene expression in mouse models of breast cancer, very few models have been examined at the sequence level. Recently, whole genome sequencing (WGS) from mouse mammary tumors (MMTV-Neu and MMTV-PyMT) has also led to the discovery of alterations in genes potentially important to human breast cancer, including *Col1a1* and *Phb* [101]. The potential impacts of these mutations on tumor behavior in such well characterized tumor models

underscores the need to complete WGS on mouse models of breast cancer [102].

Researchers are now beginning to appreciate the cellular and genetic heterogeneity of tumors not only between patients, but within single tumors [103]. Intratumoral and metastatic site heterogeneity present issues for tumor treatment, as targeted therapies may be effective for only part of the tumor. Single cell RNA sequencing (scRNA-seq) is beginning to confront these challenges through the understanding of the differences present within a primary tumor, and across the metastatic sites. Investigation of copy number alterations in single cell sequencing of two triple negative human breast tumors found four distinct populations of cells, with some shared CNV regions between the cell populations [104]. In mice, scRNA-seq has begun to show the distinct gene expression profiles of mammary epithelial cells at different developmental stages. In the mammary gland, a shift in gene expression from a basal-like transcriptional profile to a more luminal profile occurs around 5 weeks of age [105]. While more studies are needed using scRNA-seq, key insights into the single cell heterogeneity of cancer should continue to be uncovered as this technology continues to develop.

Other Considerations - Metabolomics and Proteomics

While cancer metabolomics is not a new area of study within the field, recent years have seen a surge in metabolic profiling of both human and mouse tumors. A 2018 study from Dai et al. focuses on the metabolic profiles for a number of mouse models, including PyMT, Wnt1, and Neu [106]. This study not only found metabolomic differences between tumor and normal breast tissue for each model, it also found that each oncogene had a unique metabolomic profile. Furthermore, the C3-TAg model was found to have metabolites of prognostic value, illustrating the importance of these studies.

Advances in mass spectrometry have also led to a rise in large scale proteomics analysis. These analyses in breast cancer mouse models have allowed both comparisons to the human disease, as well as enhanced the search for biomarkers capable of early cancer detection. Indeed, proteins found upregulated in the plasma of tumor bearing PyMT mice have been found to coincide with multiple human breast cancer cell lines, including MCF7 and BT474 [107]. In some cases, such as with the conditionally activated Neu mouse model, entire proteomic profiles have been made publically accessible in hopes of enhancing the search for novel cancer biomarkers [108].

Choosing a Model

Choosing the correct mouse model to investigate human breast cancer is an important experimental decision. As reviewed above, there are numerous categories stratifying the various models. Rather than simply using a model based on availability, investigators should carefully consider the choice of model. First, if the research question is one related to a particular signaling pathway, then this may dictate the choice of model. Numerous models have been profiled in comparison to each other in several reports [4, 95], and both GSEA and Bayesian pathway predictions have been reported for these models [4]. These data may be downloaded and signaling pathways searched to determine models with high or low activity for a pathway of interest. However, given the gene expression heterogeneity seen in various models [95, 109, 110], the number of tumors with the signaling pathway alterations in question should be considered when calculating the number of experimental subjects required.

If the primary consideration is a phenotype, such as metastatic progression, then the model choice will be constrained by that characteristic. While a majority of studies use the MMTV-PyMT strain for metastatic research, other strains that metastasize are available and are listed in Table 1. The short tumor latency and extensive metastasis are attractive characteristics for the PyMT transgenic mice, but if the gene expression profile and signaling pathways that are of interest do not match, then other strains are available with metastatic properties. Other characteristics, from tumor latency to promoter system, are also listed in Table 1.

For investigators simply looking to ask which mouse model most closely resembles a subtype of human breast cancer, unfortunately there is not an easy answer or single best choice. Examining co-clustering of human and mouse model tumors by gene expression [77] or predicted CNV [91] has revealed that many different models cluster with each of the subtypes of human breast cancer. MMTV-Myc is particularly instructive with varied histological subtypes and gene expression subtypes that individually cluster with most of the major subtypes of human breast cancer [77]. While this confounds the choice of model system, it underscores how sample to sample heterogeneity of gene expression in human breast cancer is reflected in the majority of mouse model systems.

Ultimately, the choice of mouse model system is a multifactorial one. This choice must take into account the initiating oncogene, latency, progression characteristics, gene expression similarities to human cancer, cell signaling pathway use, and whether copy number variation is relevant. Moreover, once a model is chosen, the

Table 1 Table contains a number of popular mouse models for the study of breast cancer, with their phenotypic and molecular characteristics. The lists of promoters and mouse backgrounds are not exhaustive, but try to represent the more common elements with the particular genes of interest

Mouse model	Promoter	Background	Latency	Metastasis	Histology
PyMT	MMTV; other promoters include RCAS	FVB; other backgrounds include RF/J, C57BL/6JNIcr, C58/J, many others	~50 days in FVB background. Ranges ~40–80 days other backgrounds	>90% tumor bearing mice in FVB background. No occurance in RF/J and C58/J	Varied, including squamous, papillary, microacinar
Neu	MMTV; other promoters include WAP	FVB	~200 days	Pulmonary ~70% tumor bearing mice	Varied, including microacinar
Myc	MMTV; other promoters include WAP	FVB	~10.5 months	~20%	40% microacinar, others include squamous, papillary, EMT, adenocarcinoma
Wnt	MMTV	FVB	8 months	Pulmonary and Lymph Node metastasis occurs in unknown percentage	Adenocarcinoma
$Rb^{-/-}$/p107	Conditional Knockout, MMTV-cre	Mixed genetic backgrounds	18.7 months	Pulmonary metastasis occurs in unknown percentage	Adensquamous and adenocarcinoma
$Trp53^{-/-}$	Transplant from P53 null mice into cleared fat pad	BALB/c	Fat pad filled 8–10 weeks after transplantation	Not reported	Adenocarcinoma
$Brca1^{\pm}$;$p53^{\pm}$	MMTV or WAP	Black Swiss	6–8 months	Not reported	Papillary, Solid
DMBA	N/A	Varied, including C57BL/6NCr X DBA/2NCr	Highly variable, median ~200 days	None in provided background	adenomyoepithelial, and myoepithelial
Stat1 KO	N/A	Mixed C57BL/6 J and 129/Sv	65% tumor development at 23 months	Not reported	Adenocarcinoma
SV40 Large T antigen	C3 WAP	FVB	3 months	Occasional metastasis to lung	small ducts and acini
MET	MMTV, other promoters include metallothionein	FVB	Overexpressed WT MET has 9% tumor burden at 475 days. Y1003F/M1248 T overexpressed MET has 40% tumor burden at 381 days	Not reported in WT MET overexpression.	50% tumors have solid histology with remaining being papillary, scirrhous, or adenosquamous
IGF-IR	MMTV, doxycyclin induced	FVB	75 days	Do not occur	Varied, including adenomyoepithelioma, and glandular adenocarncinoma
Stat5, constituatively active	Beta-lactoglobulin	FVB	~25% tumor formation at 10 months	Not reported	Adenocarcinoma
$Brg1^{+/-}$	Homologous recombination inactivation	Mixed, C57BL/6 J, 129/Sv, and CD1	~12% tumor development between 7 and 19 months	Tumor cells reported in blood vessels. No macroscopic metastatic lesions	Papillary, squamous
H-Ras	MMTV	BALB/c	4–10 months	Not reported	Adenocarcinoma
APC	K14 Cre mediated heterozygous Conditional Knockout	C57BL/6	~50% tumor development at 12	Lung metastases occur	Varied, including Acinar, basosquamous, and differentiated pilar

Table 1 (continued)

Mouse model	Promoter	Background	Latency	Metastasis	Histology
Autotaxin	MMTV	FVB	~50% tumor development at 12–24 months	low at ~5%	Squamous metaplasia
Notch1 intracellular domain	MMTV	CD1	6–15 months	Do not occur	Papillary fronds

Mouse model	Histological Expression Signature [76]	ER/PR Status	Cluster with Human PAM50 [4]	References
PyMT	Varied, including squamous, solid, microacinar, and papillary	Starts ER+, late stage ER-	Luminal A, Luminal B, Basal	[77, 111]
Neu	Varied, including microacinar, papillary, and solid	ER-/PR-	Luminal A	[112]
Myc	Extremely Varied, including squamous, EMT, microacinar, papillary, solid, and adenomyoepithelial	Not reported	Highly Variable	[45]
Wnt	Varied, including squamous, microacinar, solid and papillary	ER-/PR+	Luminal A, Normal-Like	[113, 114]
$Rb^{-/-}$/p107	Squamous, EMT	Not reported	N/A	[115]
$Trp53^{-/-}$	Largely varied, including squamous, EMT, and microacinar	Not reported	Highly Variable	[116]
$Brca1^{\pm}$;$p53^{\pm}$	Extremely Varied, including squamous, EMT, microacinar, papillary, solid, and adenomyoepithelial	Not reported	Claudin-Low, Luminal A, Normal-Like	[117]
DMBA	Squamous	Not reported	Highly Variable	[35]
Stat1 KO	Solid, Adenomyoepithelial	ER+ PR+	Her2, Luminal A, Luminal B	[118, 119]
SV40 Large T antigen	Not reported	Not reported	N/A	[120]
MET	Squamous, EMT	Not reported	Highly Variable	[110]
IGF-IR	Largely varied, including squamous, EMT, and microacinar	30–40% cells ER+/PR+	Varied, including Luminal A, Basal, Claudin-Low	[121, 122]
Stat5, constituatively active	Largely varied, including squamous, EMT, and microacinar	Not reported	Closest to Claudin-Low	[123]
$Brg1^{+/-}$	Largely varied, including squamous, EMT, microacinar, and solid	Not reported	Varied	[124]
H-Ras	Microacinar, Solid	Not reported	Luminal A, Luminal B	[125]
APC	Microacinar	ER-/PR-	Basal, Her2, Normal-Like	[126]
Autotaxin	Solid, Adenomyoepithelial	~60% cells ER+	Her2, Basal, Claudin-Low	[127]
Notch1 intracellular domain	Papillary	Not reported	Basal	[128]

resulting tumors must be characterized to determine how the tumor to tumor heterogeneity that is present in the various models has been altered with the experimental manipulations.

Discussion

Numerous genomic perturbations, and a cascade of protein interactions and regulatory pathways all function together to initiate and maintain oncogenic transformation. Given this complexity, the mouse model is highly suited to study breast cancer. The in vivo nature of mouse models allows the complexity of cancer to be studied more accurately than cell culture and other in vitro experiments alone. Numerous types of mouse models, including carcinogen induced, PDXs, and GEMMs recapitulate certain aspects of the disease. While their usefulness is dependent on the research question, GEMMs are perhaps the most comprehensive due to their ability to closely mimic the initiating oncogenic event that occurs in a number of cancers while maintaining an appropriate tumor microenvironment and functioning immune system.

On an expression and histological level, GEMM tumors are as complex as the human tumors they attempt to mimic. Just as a wide array of histologies are seen within human tumors, tumor histological differences can be seen within single GEMMs. Classifying histological subtypes on their expression profile also shows relevancy to human breast cancer. Since the initial characterization of human breast cancer into intrinsic subtypes, an increasing amount of data has been generated showing mouse subtypes that mimic each. While little whole genome sequencing data has been generated for GEMM tumors, the data available has shown that like human tumors, mouse tumors display a large array of genomic rearrangements, including single nucleotide variants, copy number alterations, and translocations. The histological, expression, and sequencing similarities between human and mouse breast tumors show that when used correctly, genetically engineered mouse models can be an accurate method for studying human breast cancer.

Given the complexity of both human breast cancer and the numerous mouse models used to study it, choosing the correct mouse model is essential for the experimental question. Initial examination of expression based analysis and the human based subtypes that are mimicked through large scale gene expression experiments is critical [3, 4]. Depending on copy number alterations in the gene, it is also beneficial to examine the mouse models for similar changes [91]. Whether through GSEA or a signature based approach, signaling pathways should also be examined [4, 95] to ensure that the appropriate model is used. Recent examples of drug screening in mouse models have taken these parameters into account [129, 130] in important demonstrations of the integration of bioinformatics analysis of mouse models with wet lab experiments.

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