Commentary

Models of breast cancer: *quo vadis*, animal modeling?

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Abstract

Rodent models for breast cancer have for many decades provided unparalleled insights into cellular and molecular aspects of neoplastic transformation and tumorigenesis. Despite recent improvements in the fidelity of genetically engineered mice, rodent models are still being criticized by many colleagues for not being 'authentic' enough to the human disease. Motives for this criticism are manifold and range from a very general antipathy against the rodent model system to well-founded arguments that highlight physiological variations between species. Newly proposed differences in genetic pathways that cause cancer in humans and mice invigorated the ongoing discussion about the legitimacy of the murine system to model the human disease. The present commentary intends to stimulate a debate on this subject by providing the background about new developments in animal modeling, by disputing suggested limitations of genetically engineered mice, and by discussing improvements but also ambiguous expectations on the authenticity of xenograft models to faithfully mimic the human disease.

Keywords: breast neoplasms, gene targeting, genetically engineered mice, genetic models, genetic techniques, xenograft

Introduction

In the present issue of *Breast Cancer Research*, Kim and colleagues [1] demand the development of 'an authentic breast cancer model'. Based on suggested shortcomings of genetically engineered mice (GEM), the authors conclude that this goal can merely be achieved by improving xenograft models. In this commentary, I will briefly highlight the latest technological advances in the generation of mouse models for breast cancer and will dispute some of the suggested limitations of GEM. Based on recently published findings, I will also critically review the proposed species-specific function of genes (in particular, the role of telomerase) during carcinogenesis in humans and rodents. Furthermore, I will discuss technical improvements of xenografting human epithelia along with appropriate stromal components into immunocompromised rodents. In this context, I will also list five arguments to caution unrealistic expectations on the authenticity of these newly developed xenograft models to faithfully mimic the human disease.

For a basic understanding of the concepts and terminology of xenograft models please refer to the article by Kim and colleagues [1] in the present issue. I would also like to draw the reader's attention to a more comprehensive review by Van Dyke and Jacks [2] that discusses in greater detail the technical advances in manipulating the murine genome.

Models and reality: a dialectic liaison

When can we expect to have an authentic mouse model for breast cancer? This frequently asked question clearly

Brca1/2 = breast and ovarian cancer gene 1/2, early onset; Cre = site-specific recombinase from bacteriophage P1 (catalyzes recombination between *loxP* sites); ER = estrogen receptor; GEM = genetically engineered mice; H-ras = Harvey rat sarcoma virus oncogene; K14 = keratin 14; LoxP = locus of X-ing over (34 bp DNA recognition site of Cre recombinase); MCF-7 = metastatic human breast adenocarcinoma cells; pRb = retinoblastoma protein; SV40 = simian virus 40; T-47D = metastatic human breast ductal carcinoma cells; TERT = telomerase catalytic subunit.

illustrates that breast cancer is still considered one specific disease. However, various types of human breast cancer differ significantly in their morphology, their histopathology, their dependence on endogenous growth factors, their activation/inactivation of specific genes, and, most of all, their clinical outcome. For example, the latest studies distinguish at least four or five breast cancer types based solely on hierarchical clustering of gene expression profiles [3,4]. The term 'breast cancer' thus does not stand for a specific disease, which is genetically and phenotypically uniform. It is, therefore, a misapprehension to envision a single model system that could mimic all features of breast cancer. Based on this fact alone, it is likely there will be more than just one 'authentic model' to recapitulate human breast cancer(s).

The general definition of a model (Latin *modulus*, small measure) is that it reflects only certain aspects of the original. It might be just a philosophical exercise to argue whether a model as such can ever be 'authentic' or identical to the original. From an ideal animal model for human breast cancer we expect that it faithfully reflects the human disease on various levels such as etiology, pathology, and genetics, that cancer originates only in the mammary gland, that neoplasia occur with a 100% incidence in treated or modified animals, whereas control animals do not develop tumors, and that tumorigenesis should have a relatively short latency.

After careful consideration of all these expectations, one might ask whether it will ever be possible to establish ideal animal models. For instance, to model tumorigenesis in a relatively short period in the mouse we need to introduce shortcuts (i.e. precise mutations in the exact sequential order in which they might occur over several years or decades in the human). In the end we might obtain models that carry almost identical genetic alterations of oncogenic pathways as we can distinguish them today. But does each step toward neoplastic transformation recapitulate the morphology, pathology, and etiology of the progressing disease? For now, we do not even have a complete map of all genetic alterations that occur in specific forms of breast cancer, nor do we have a genuine concept about the precise sequence of events that take place during cancer progression. The generation of models for breast cancer that accurately reflect all the characteristics mentioned is therefore a distant goal that does not even seem to emerge on the horizon. Nevertheless, one should be aware of the fact that we will always read about models that are highlighted as being 'authentic'. Whether such claims are reasonable, however, depends largely on how we interpret a model in its capability to reflect certain features of breast cancer, and whether we accept imperfections when we have a closer look at all other characteristics.

Main categories of mouse models for human breast cancer

Mouse models for human breast cancer can be categorized into three main groups: xenograft models; chemically induced, virally induced, or ionizing radiation-induced models; and GEM such as transgenics and knockouts. Certain models rely on a combination of techniques used to generate these three main types of cancer models. One example is a knockout mouse that was treated with a carcinogen or with ionizing radiation. To date, genetically engineered mouse strains are used primarily as tools to study the biological function of genes during neoplastic transformation and tumorigenesis. The US Food and Drug Administration is, however, considering altering the guidelines on preclinical testing for the carcinogenicity of pharmaceuticals (see the earlier article by Durso [5]), and specific strains are now being used in selective chemoprevention and chemotherapy trials (for examples, refer to Van Dyke and Jacks [2]).

Among the mouse models for breast cancer, xenografts still play the dominant role in preclinical trials. The reasons for this are manifold. First, xenograft models are relatively inexpensive and easy to generate. Also, the subcutaneous or orthotopic injection of tumorigenic human breast cancer cells such as the metastatic human breast adenocarcinoma cells (MCF-7) or the metastatic human breast ductal carcinoma cells (T-47D) into immunocompromised recipients results in the formation of solid tumors after a relatively short latency. Third, xenograft models that utilize estrogen receptor (ER)-positive cancer cell lines are currently indispensable for preclinical testing of inhibitors of steroid receptor signaling and drug resistance studies. Approximately 50% of all human breast cancers are ER-positive, but the vast majority of mammary lesions in GEM are ER-negative. Most GEM thus do not precisely recapitulate steroid receptor signaling during neoplastic transformation. Based on the studies by Medina and colleagues (for references, see [6]), however, it is established that steroid and peptide hormones have a considerable effect on the initiation of mammary tumorigenesis in mice.

As an alternative to human breast cancer cell lines and xenograft models, studies on steroid signaling and mammary cancer are being performed in rat models that, unlike mice, exhibit a significantly higher frequency of ER-positive lesions. The latest developments in the targeted manipulation of the rat genome [7] hold great promise for the improvement of rat models to study estrogen signaling and tumorigenesis *in vivo*.

Of mice and men

Xenograft models seem to gain importance for mechanistic studies since it has been proposed that, compared with murine cells, human mammary epithelial cells have to alter additional molecular pathways to achieve neoplastic

transformation. This theory, recently advocated by Hahn and Weinberg [8], was highlighted in the article by Kim and colleagues [1] in the present issue. Based on the conclusions by Hahn and Weinberg it seems essential to develop new xenograft models for breast cancer that reflect the suggested differences to the rodent system. The initial findings by Hahn and Weinberg were significant but, based on newer findings, it is necessary to revisit the proposed differences and to challenge overly simplistic views on molecular events leading to tumorigenesis in humans and mice.

The theory of a 'one-hit kinetics of tumorigenesis' attributed to GEM by Kim and colleagues [1] is in sharp contrast to current views about the hallmarks of cancer initiation and progression [2,9]. Unlike in immortalized cell lines that lack important tumor suppressor pathways, no oncogene alone is able to instantaneously transform normal mouse mammary epithelial cells *in vivo*. This is indicated by two lines of evidence: tumors arise in GEM after a certain latency period; and, despite a widespread expression of the oncogene throughout the entire gland, tumors arise as focal lesions, suggesting that multiple genetic alterations have to occur to promote neoplastic transformation in transgenic models. The latter argument is also supported by the fact that transformation is reversible at defined stages of tumorigenesis when the oncogene is turned off [10–12]. Again, as in humans, mammary epithelial cells in rodents need multiple genetic defects before uncontrolled cell growth and tumorigenesis can occur *in vivo*.

In their initial study, Hahn and colleagues [13] use defined genetic elements to transform primary human fibroblasts. The same strategy was used recently by Elenbaas and colleagues [14] to generate human breast cancer cells *in vitro* that form tumors in immunocompromised animals. The necessary genetic elements to transform human cells were defined as simian virus 40 (SV40) large T antigen, as the Harvey rat sarcoma virus oncogene (H-ras), and as the telomerase catalytic subunit (TERT). Large T antigen inhibits a broad spectrum of oncogenic pathways, most of all p53 and Rb, and it is known that this protein can inactivate various members of the Rb gene family such as retinoblastoma protein (pRb), p107, and p130. Despite these refinements of transforming human cells *in vitro*, one should recognize that the new xenograft model by Elenbaas and coworkers cannot serve as a genuine model for human mammary neoplasia. First, SV40 large T antigen has not been linked to the etiology of human breast cancer. Also, like any other conventional xenograft model, the cancer cells are able to form tumors without the appropriate microenvironment. Third, despite inclusion of human fibroblasts that seem to support the multiplication of the tumorigenic cells, the histopathological appearance of this xenograft model does not resemble human breast cancer (see later). In conclusion, it would be a necessity for the development of new xenograft models to introduce defined mutations into tumor susceptibility loci of immortalized human epithelial cells as they occur *in vivo* in human carcinoma.

It is not absolutely clear to date what these defined mutations and genetic elements are. One genetic pathway has already been overlooked in the primary study by Hahn and colleagues [13]. Other workers found that the defined genetic elements mentioned earlier were not sufficient for the transformation of primary human cells. For instance, the inactivation of p53 and pRb using human papillomavirus E6 and E7 oncoproteins instead of SV40, in combination with H-ras and TERT, did not result in neoplastic transformation [15]. Although Elenbaas and colleagues predicted the presence of a small t protein in addition to the large T antigen expressed by the SV40 early region, it was a year later when Hahn and colleagues [16] recognized the essential role of small t antigen as the transforming agent by inhibiting isoforms of the protein phosphatase 2A. There are now five suggested pathways that are important for neoplastic transformation (p53, pRb family, TERT, H-ras, and protein phosphatase 2A), and one might ask whether there are more to be identified since the 'defined' elements used to transform cells are not directed against one particular member of these pathways.

Another important question remains: how different are these pathways in human versus murine cells? All the pathways already listed have been linked both individually and in combination to mammary tumorigenesis in the mouse. Appropriate mouse models have been genetically engineered and analyzed in great detail. For a narrative of various models, see the February 2000 special issue of *Oncogene*. The prime paradigm that is frequently used to exemplify the 'fundamental' differences between these two species is the function of telomeres and telomerase (TERT) to limit the life span in human cells [17]. It is often stated (see also the article by Kim and colleagues [1]) that, in contrast to the human cells, murine cells have very long telomeres and a basal telomerase activity. As one hallmark of neoplastic transformation, human cells have to (re)activate TERT to achieve immortality. (Of note, one should know that the generalized statement about the telomere length in mice is incorrect. Unlike *Mus musculus* that we currently use to generate GEM, *Mus spretus* has telomeres of approximately the same length as humans [18]. Species-specific variations in telomere length therefore cannot serve as an indicator for an expected lifespan.)

Several lines of evidence exist to suggest that telomeres and TERT also play an important role in mammary tumorigenesis in GEM. It has been reported that the activity of telomerase was elevated in mouse mammary tumor virus-neu-derived tumors compared with normal mammary

epithelia [19]. Second, the overexpression of TERT in transgenic mice resulted in the development of invasive mammary carcinomas [20], suggesting that there are cancer-promoting, telomere-independent functions of TERT. This conclusion is supported by the fact that a mutant of TERT lacking the capability of extending telomeres still retains its tumorigenic properties in cells that use alternative mechanisms of telomere maintenance [21]. Third, the lack of telomerase function greatly inhibits tumorigenesis in cancer-prone *Cdkn2a* knockout mice [22]. Also, the targeted mutation of the *Terc* gene, which encodes the RNA subunit of TERT, results in telomere shortening that, in turn, leads to end-to-end fusions and subsequent chromosome breaks and translocations. In the absence of functional p53, these knockout mice have a higher susceptibility to develop carcinomas. Like human cancers, these lesions possess nonreciprocal translocations [23]. In summary, telomere and telomerase function is critical for tumorigenesis in both human and murine cells.

The final issue that I would like to discuss in this context is whether the re-activation of TERT is really a prerequisite for the multiplication of tumor-initiating cells ('breast cancer stem cells') in the human breast. Hanahan and Weinberg [9] did not completely reject the possibility that growth arrest of human cells in culture might be a technical artifact. According to the study by Elenbaas and colleagues [14], it is obligatory to introduce TERT into mammary epithelial cells in culture to bypass senescence. This seems to be a logical approach since, with the exception of stem cells, most other somatic cells in humans do not express telomerase. It is, however, interesting to note that human mammary epithelial cells can apparently be propagated for at least five passages without signs of senescence and without exogenous TERT expression under culture conditions that maintain cells in an undifferentiated state [24]. According to the latter study, these special culture conditions, in which epithelial cells are grown in a nonadherent fashion as mammospheres, allow the enrichment of early progenitor or stem cells. It would be logical to assume that these mammary epithelial progenitor cells have active telomerase and, therefore, an extended replicative potential. However, a difference in TERT expression between these progenitor cells and differentiated cells was not reported.

An interesting subject of future studies is to examine whether mammary stem cells in the human breast express telomerase. Without TERT expression how do they otherwise maintain their replicative potential? The idea that undifferentiated progenitors are the cancer-initiating cells in the human breast is currently a very popular theory that is based on various findings in humans and mice [25–28]. If 'breast cancer stem cells' have residual expression of telomerase, this might explain why these cells are prime targets for transformation. In this case they would not need an additional mutation to reactivate the TERT gene during tumorigenesis. Since we already know that many somatic cells in mice express TERT, the stem cell theory of human breast cancer would logically imply that cancer-initiating cells in both species have the same extended or limitless replicative potential. A confirmation of this hypothesis would probably be the end of the discussion about fundamental differences of TERT function in mouse and human cells.

Modeling molecular events for initiation of mammary tumorigenesis

Kim and colleagues [1] state that tumors occurring in mice must have an accelerated program of progression since mice have a shorter lifespan than humans. This general statement does not just apply for GEM, but it is also relevant for xenografts. Xenograft models with rodents as recipients could not be established if it would take many years or decades for a human premalignant lesion to progress into metastatic breast cancer. This issue needs to be addressed when new objectives are articulated to develop 'authentic' xenograft models on the basis of immortalized or precancerous human epithelial cells. Most human cells that are used for xenotransplantation today are already fully neoplastic and form tumors in immunocompromised animals after a short latency without the appropriate stromal environment.

Wild-type mice do not develop mammary tumors during their lifetime unless they are inbred strains that carry mouse mammary tumor virus or other selected mutations. In all types of mouse models, we introduce mutations to initiate and speed up neoplastic transformation. Transgenic strains that express oncogenes under mammary-specific promoters were the first generation of GEM for breast cancer [29,30]. In a subset of these strains, pregnancy hormones greatly induce the expression of a particular oncogene, leading to an increased tumor incidence in parous animals. This is not necessarily a limitation of these early mouse models since humans also show a transient increase in breast cancer risk after a term pregnancy. The protective effect of pregnancy on breast cancer that Kim and colleagues [1] mention in their article is a long-term effect, which becomes statistically significant several years *post partum*. For more information on this subject see the summary report of a recent workshop on pregnancy and breast cancer (http://www.cancer.gov/cancerinfo/ere). To avoid pregnancy-induced oncogene expression, newly developed transgenic strains now use the native promoter of an oncogene [31] or hormonally nonresponsive promoters [32]. It is interesting to note that some of these new strains seem to develop neoplastic lesions that express ER [32], suggesting that it might be technically possible to model ER-positive breast cancer in GEM (see earlier discussion).

The second generation of GEM comprises conventional knockouts with targeted mutations of tumor susceptibility genes. *p53*, *Rb*, and *Cdkn2a* knockout mice are well-known examples. However, these strains develop a distinct tumor spectrum that differs from humans with inherited germline mutations in these tumor suppressors. In particular, most p53-deficient mice succumb to lymphoid neoplasia before they develop carcinomas. Since ductal elongation and branching morphogenesis occur primarily in pubescent animals, we can perform a transplantation of mammary epithelia into wild-type recipients to establish mice lacking p53 specifically in the mammary ducts [33].

Early embryonic lethality of other important mouse models for breast cancer, such as breast and ovarian cancer gene 1 and gene 2 (*Brca1* and *Brca2*) knockouts, led to the development of the third and most current generation of GEM: conditional knockout mice [34,35]. This major refinement in manipulating the murine genome utilizes the Cre recombinase from bacteriophage P1, which catalyzes the excision of an essential element of a target gene (for instance a coding exon) located between two *loxP* (locus of X-ing) recognition sites. This technology enables us to delete a tumor susceptibility gene from virtually any cell type at any given time point during development. This also includes the deletion of any given gene in cells that have undergone neoplastic transformation to study tumor progression or to mimic a therapeutic approach with genetic means. More importantly, this technology can also be used to model hereditary breast cancers as well as sporadic mutations in a limited number of somatic cells. For instance, the Cre-lox-mediated deletion of *Brca1* from developing mammary epithelial cells resulted in tumorigenesis. In addition, these conditional mutants allowed us to examine important functions of p53 in preventing an early onset of the disease [35].

In summary, many of the technical limitations that were innate in earlier generations of GEM (see Kim and colleagues [1]) are already a relic of the past. Nevertheless, it still needs to be established whether these newly developed GEM resemble important features of human breast cancer such as histopathology, dependence on endogenous growth factors, and formation of metastases. The consensus report of the Annapolis Meeting on histopathological features of mouse models for breast cancer was primarily focused on transgenic mice alongside chemically induced and virally induced mammary tumor models [36]. It is therefore desirable to assemble a panel of comparative pathologists to validate these newly developed strains.

The importance of the stroma

There are obvious differences between rodents and humans regarding mammary morphogenesis, and this distinction is not just noticeable on the anatomical level (e.g. number and location of glands). One important distinction between mice and humans is the composition of the mammary stroma. Pathologists use this feature to distinguish human and murine mammary lesions. While the mouse mammary stroma largely consists of adipose tissue, the human stroma contains a relatively high amount of fibrous cells surrounding the epithelial compartment. This may be one reason why particular lesions in GEM do not closely resemble human breast cancers on the histopathological level. The difference in the stromal environment may also be considered a reason why primary human breast cancers are difficult to grow in immunocompromised mice. Normal and transformed cells do not just need an adequate amount of the right hormones, but also need an appropriate microenvironment, which supports epithelial cells with local growth factors. In turn, epithelial cells signal back to the stroma, which then becomes competent to support epithelial proliferation and differentiation [37]. For a summary of the main functions of the mammary stroma and the role of various paracrine factors, refer to a recent review by Wiseman and Werb [38].

Novel xenograft models that utilize fibroblasts derived from the human mammary gland attempt to address the issue of generating the correct microenvironment for human epithelial cells [39]. These features will undoubtedly significantly improve existing xenograft models that use untransformed epithelial cells. The co-transplantation of the correct stroma is, however, only needed when normal and preneoplastic cells still depend on local growth factors. The vast majority of human breast cancer cell lines that are used in xenograft models today lack that requirement. The newly designed breast cancer model described by Elenbaas and colleagues [14], which utilizes *in vitro* transformed cells, shows some increase in the efficiency of tumor formation when these cells were co-implanted with normal human fibroblasts, suggesting that local growth factors have an effect on the proliferation of these neoplastic cells. Tumorigenesis in this model is, however, not completely dependent on the human stroma. Nevertheless, one important requirement of newly designed xenograft models is that they, unlike many GEM, mimic human breast cancer on the histopathological level (see Kim and colleagues [1]). This is clearly not the case in the model of Elenbaas and colleagues. The addition of fibroblasts resulted in tumors that were histologically similar to those tumors arising when malignant cells were injected alone.

In addition to these technical issues that obviously need to be resolved, xenograft models have other, more important limitations. The shortage of applicable techniques to introduce precise mutations into endogenous genes of non-immortalized and untransformed primary mammary

epithelial cells as they might occur during human breast carcinogenesis was discussed earlier.

Second, in order to generate a xenograft model, immortalized human cells (stroma and epithelia) need to be transplanted into immunocompromised animals. The lack of a normal immune response against tumor cells is a common weakness for using xenograft models in preclinical trials since a number of cancer therapies rely directly or indirectly on an intact immune system. In particular, immunotherapy uses strategies to suppress tumor growth by attempting to promote rejection of the tumor through cell-mediated immunity in the host. To generate such a complex mouse model one would have to reconstitute the matching human immune system in addition to the human stroma–epithelia graft in the mammary gland.

Also, human precancerous cell lines are difficult to establish and maintain in a stable condition. Genetic drift will affect precancerous human epithelial cells and immortalized stroma cell lines in the same way as existing models that utilize MCF-7 or T-47D cells.

A fourth limitation is that the tissue-recombination approach might reconstitute a correct epithelial–stromal interaction in the primary mammary cancer in the xenograft model. Therapeutic regimens have been proposed to target the cancer-associated stroma in addition to cancer cells. The use of this strategy clearly becomes inappropriate for the treatment of metastasizing breast cancer in this xenograft model. Assuming that neoplastic epithelial cells, but not normal fibroblasts, invade other organs, one should expect that, unlike in the primary tumor, cancer cells interact with the murine stroma in lung or bone metastases. In this regard, improved xenograft systems might not be different from conventional models. The use of transformed stromal cells that might metastasize along with malignant epithelial cells as suggested by Kim and colleagues [1] has not been proven to work and, more importantly, this strategy does not mimic what happens in human breast cancer.

Finally, it is assumed that the tissue recombination approach will model all necessary growth factors to support normal proliferation and differentiation of epithelial cells. This strategy focuses on local growth factors that act mostly in a paracrine fashion, but it does not reflect species-related incompatibilities of systemic factors produced by the host with the corresponding receptors of the graft. The implementation of this condition in the model design requires the expression of human hormones at near-physiological levels in the immunocompromised host. Before this long-term goal can be achieved it will be necessary to examine what effects such a 'hormone replacement' with ligand-receptor incompatibility will have on the general physiology and reproductive capability of the animal model.

Some current challenges

Several shortcomings of GEM and xenograft models have been discussed. While most existing cancer models today represent early stages of mammary tumorigenesis, many of them do not recapitulate advanced human breast cancers; in particular, the frequency and location of metastases. The establishment of mouse models that show a high frequency of metastasis to the bone is an important aim that needs to be addressed in the future. To achieve this goal it is necessary to re-examine existing animal models, in particular, the newly developed GEM and xenografts, for their ability to develop micrometastases in the bone. In this context, new imaging technologies will allow us to visualize tumors noninvasively, to quantify their progression including neovascularization, to guide us precisely to locations of distant metastases for histopathological examination, and to assess responses to therapeutic regimens.

Another future goal is the targeted modification of the murine stroma to achieve a more human-like appearance without compromising the normal function of the gland. The nature of pregnancy-related, permanent changes in the gland is another important subject of current investigations. It has been shown that pregnancy results in a persistent alteration of the expression profile of various genes [40,41], and not all cells that express advanced differentiation markers undergo apoptosis during involution at the end of lactation [42]. Interestingly, these surviving cells are unique for parous females and have certain properties of multipotent stem cells. A closer examination of these cells might explain the differences in gene expression mentioned earlier and the variations in tumor susceptibility between mammary tissues of nulliparous and parous females [43].

The identification and characterization of multipotent stem cells in normal breast tissue and animal models for breast cancer is another hot topic. While discussing the function of telomerase in breast cancer, I mentioned several open questions regarding the involvement of stem cells during tumorigenesis. The identification of cancer-initiating epithelial subtypes (i.e. 'cancer stem cells') is of utmost importance to understand the process of tumorigenesis. In turn, it is essential to target the correct epithelial subtype to study the function of tumor susceptibility genes. Various GEM use different regulatory elements to target a gene of interest to diverse epithelial subtypes in different compartments of the gland. In addition, it also needs to be considered that some oncogenes function as paracrine factors, suggesting that the cells expressing the oncogene might not necessarily be those that become malignant. Hence, without knowing what are the cancer-initiating cells in particular models, it is difficult to directly compare one with another and to draw conclusions about the relevance of certain signaling pathways.

The following is an example that might illustrate the importance of targeting the correct epithelial subtype. Based on the expression of keratin 8, keratin 18, and keratin 19 in breast cancers, it is generally accepted that luminal epithelial cell types contribute mainly to neoplastic transformation in the human breast [44–46]. Many observations in mouse models for breast cancer support this paradigm [36]. Keratin 14 (K14) is predominantly expressed in myoepithelial cells in the adult breast, and these rarely contribute to malignant lesions [44]. The deletion of *Brca2* from luminal epithelial cells using whey-acidic-protein-(WAP)-Cre mice is entirely sufficient to render these cells susceptible to neoplastic transformation [47]. The majority (16 out of 23) of Brca2-deficient tumors were p53-positive and $p21^{Cip1}$-positive. In contrast, the excision of the *Brca2* gene from the 'mammary gland' using K14-Cre mice did not cause mammary tumorigenesis [48]. Only in combination with a *p53* null mutation did K14-Cre *Brca2* floxed mice develop tumors. Hence, the phenotype of both Brca2-deficient models and the mechanistic implications about p53 function are strikingly different. Unfortunately, the latter report did not provide adequate information about whether the tumors arose solely from K14-positive myoepithelial cells to draw a final conclusion about the cellular origin of these lesions.

In summary, the generation of animal models for breast cancer is becoming increasingly complex. It is therefore essential that one pays close attention to the details of the experimental design, such as targeting the desired epithelial subtype, when utilizing genetic tools to model mammary carcinogenesis.

Conclusions

Mouse models for breast cancer are valuable to study molecular pathways of neoplastic transformation and tumorigenesis *in vivo*, and they serve as tools for selected preclinical trials. Breast cancer is not genetically and phenotypically uniform, and therefore one model system will never be enough to recapitulate various forms of the disease. Whether a model system is 'authentic' to human breast cancer is determined by its capability to reflect certain features of the disease, but it is unrealistic to expect that one model can mimic all aspects of human breast cancers. The superiority of one model over another largely depends on the scientific hypothesis, on experimental design, and on the type of study that one wishes to perform.

The majority of animal models are readily available for biological studies at academic institutions around the world. The Mouse Model for Human Cancer Consortium even distributes the newest cancer models free of charge (for more information, see http://mouse.ncifcrf.gov). Scientists are also encouraged to enter supplementary information about their cancer models into a central database (http://cancermodels.nci.nih.gov). This resource might be a valuable tool to decide whether a certain model has the desired features of breast cancer that one plans to examine and whether a model is suitable for testing therapeutic strategies.

Competing interests

None declared.

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