Commentary

Models of breast cancer: is merging human and animal models the future?

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Abstract

Survival rates of patients with early breast cancer in the United Kingdom and in the United States have improved steadily over the past 15 years. The only way to continue or even accelerate this progress, however, is the discovery and development of new preventative and therapeutic strategies. With the massive explosion in potential therapeutic strategies becoming available, in the postgenomic era, better and more representative breast cancer models are urgently required for preclinical trials. Development of better *in vivo* models of human breast cancer are thus of crucial importance in the development of new cancer therapeutics.

Keywords: breast cancer, immunodeficient mice, *in vivo*, stroma, xenograft

Introduction

Breast cancer is the most commonly diagnosed form of cancer and the second leading cause of cancer death in Western women. Between one out of eight and one out of 10 women will develop breast cancer during her lifetime, with the disease being a leading cause of mortality in women over the age of 35 years. Survival rates of patients with early breast cancer in the United Kingdom and in the United States have improved steadily over the past 15 years [1], largely as the result of advances in and improved access to early diagnosis and more effective therapy. Additional gains, however, will require new preventative and therapeutic strategies that require better understanding of the genetics and biology of human breast cancer. Such knowledge, which is rapidly accruing as the result of postgenomic technologies such as proteomics and transcriptional profiling, must be translated into a setting in which potential clinical responsiveness can be evaluated. This in turn requires better *in vitro* and *in vivo* models of human breast cancer.

Although *in vitro* culture of established breast cancer cell lines is probably the most widely used model for such preclinical evaluation, it is limited in so far as it contains no stromal cells and, as generally used, lacks three-dimensional structure. These limitations make it poorly representative of real cancers. Animal models in which stroma and structure are present should, if they are to be useful, possess genetic and other biomarker abnormalities similar to, if not identical to, their human counterparts. The most direct way to achieve this is to merge human and animal models in the form of heterotransplanted tissues, implanted either heterotopically (subcutaneous) or orthotopically (mammary fad pad). This commentary discusses the basic concepts of current 'xenograft' models and outlines some of their limitations and potential, as compared with syngeneic and genetically engineered (transgenic) rodent models.

Syngeneic and genetically engineered mouse models

With the recent introduction of syngeneic mouse tumour models, the choices of animal models have improved [2]. However, the most widely used animal models have a limited role in cancer research because the biology of rodents and their tumours differs significantly from that of

GEM = genetically engineered mice; SCID = severe combined immunodeficiency.

humans and human cancer. The differences in developmental programmes of mouse and humans manifest in many ways, with size being an obvious example. Cellular targets for oncogenic transformation consequently differ in number, in their degree of maturation and in their differentiation in mouse tissues compared with their human counterparts. In the mammary gland, for example, full glandular maturation is contingent on pregnancy in rodents, but not in humans. This has significant implications with regard to the presence, or absence, of multipotential stem cells, and their role in mammary carcinogenesis.

The shorter lifespan of rodents means that observable tumours must have a rapid programme of progression as mice can develop very malignant tumours showing multiple genetic alterations within a relatively short time period (6–18 months). Although the basic mutation frequency is similar in both species, cells of rodent origin are much easier to transform *in vitro* by oncogene transfection or chemical carcinogens. Possible explanations for the easier transformation include less efficient DNA repair, poorer control of genetic stability, and or altered control of gene expression through processes such as DNA methylation [3]. Another difference lies in immortalization, which is a key step in tumour progression [4], and the ease with which rodent cells become immortalized [5,6]. Mouse and primary human cells have major differences in telomere dynamics and telomerase regulation. Telomeres are significantly longer in laboratory mice (40–60 kb) compared with in humans (10 kb), and the enzyme telomerase is widely expressed in adult mouse tissues. In contrast, human cancer cells have acquired the capacity to maintain telomeres through the reactivation of telomerase or other mechanisms to avoid replicative senescence.

Although rodents are intrinsically more susceptible to carcinogenesis, sporadic cancers are quite rare in wild-type rodents. Many decades have been devoted to selective inbreeding to enhance the incidence of specific tumours to useful levels in syngeneic mice, thus altering the genetic background in each strain. Mouse strains susceptible to mammary cancer were isolated many years ago, with vertical transmission (Bittner or milk factor) subsequently shown to be due to a mouse mammary tumour virus. Viruses have yet to be convincingly implicated in human breast cancer, except as possible cofactors [7]. Chemical carcinogenesis has been used in rats to enhance mammary tumour formation, again with no direct human parallel, and with enhanced chemically induced mutations, some of which can result in a partial immune response in the incompletely inbred rat strains.

Overall, a smaller number of genetic changes, in comparison to humans, are required for rodent cell transformation *in vitro* [8], and this is probably also true for rodent tumours *in vivo*. This may contribute to the notable differences in tumour biology and pathology observed between the species. For example, about one-half of human breast cancers are hormone responsive at diagnosis, while the vast majority of mouse tumours are hormone independent with much lower levels of oestrogen/progesterone receptors than human tumours [9]. Although similar morphological patterns can be seen in lesions in both species, the detailed morphology of most mouse tumours do not resemble the common human breast cancers and cannot be classified in an equivalent manner to the standard human tumour pathology grades and types [10,11]. Rat tumours are likewise distinct, and differ from both mouse and human counterparts in detailed histology. The metastatic patterns between the species are also different.

Breast cancer in humans usually spreads lymphatically, starting with local lymph glands, followed by distant metastasis predominantly to the bone, the brain, the adrenal gland, the liver and the lung. In contrast, mouse mammary cancers metastasize almost exclusively to the lung via the haematogeneous route [12]. One other major, but infrequently mentioned, difference between rodent and human cells, whether *in vitro* or *in vivo*, relates to their respiratory quotient. Small animals, such as mice and rats, consume greater amounts of oxygen on a per-cell basis than larger animals. This will result in very different cellular microenvironments, particularly in relatively avascular and hypoxic tumours, where hypoxia-induced genes may play an important role in growth and differentiation [13]. Larger experimental animals can provide potentially better models of human breast disease in this and other respects, but are seldom used for a variety of nonscientific reasons.

Genetically manipulated animals generated by transgenic and gene-targeting (knockout) technology have undoubtedly contributed tremendously to our understanding of gene function and regulation at the molecular level in the context of the whole organism. However, genetically engineered mice (GEM), like syngeneic rodent models, also present fundamental differences at the level of the organism and the cell. GEM are designed to reproduce very specific aspects of tumour formation and progression, usually but not invariably based on knowledge of human tumour genetics. When using transgenic mice, the extent and type of genetic abnormalities that cause disease must be assessed in relation to those that cause human disease, to decide whether they differ to a degree that makes them an unacceptable model. The precise genetic background on which the abnormal genes are either overexpressed or underexpressed within the tumour cells is also important as it may influence their effects or penetrance.

To date, most oncogene-bearing transgenic mice and tumour suppressor gene knockouts have had a whole-body phenotype, in which all tissues and cells bear the same defect. They thus do not mimic sporadic tumours

that arise from an initiating mutation affecting a single cell in an otherwise normal microenvironment. These models are effectively the rodent equivalent of human familial cancer syndromes. This problem has to some extent been rectified with the use of cell-type specific promoters to limit gene expression to specific target tissues, and with the use of promoter-specific recombinase-based (cre-lox) mechanisms for eliminating transgenes from specific tissues. However, these methods themselves generate other limitations in respect of mammary tumours, as they frequently depend on hormone-sensitive promoters such as the mouse mammary tumour virus long terminal repeat and whey acidic protein promoter. These promoters have hormone-regulated enhancer elements that are not the natural promoters for the activated oncogenes in human breast cancer. This can lead to inappropriate responses, for example enhanced mammary tumourigenesis caused by pregnancy, whereas pregnancy is protective in humans [11].

Despite these limitations, molecular events that occur in human breast cancer can be reproduced in mice, with the same genes triggering the same molecular events. Interestingly, the mammary tumours that are produced in GEM have phenotypes dissimilar to those in mouse mammary tumour virus-induced or chemically induced mammary tumours, and may have greater similarities with human breast cancers. Examples of human genes triggering similar molecular events in mice include a splice variant of erbB2 in humans, which mimics the transmembrane domain mutations that activate the murine c-erbB2 as the oncogene *neu*, as well as conditional mutants of the tumour suppressor gene *BRCA1* that produce mammary gland tumours in mice [14,15]. These syngeneic and GEM models have thus contributed significantly to our understanding of the fundamental aspects of breast cancer genetics, but do not provide sufficient similarity with human tumours for preclinical drug testing [16].

Xenograft models

The fact that some human breast cancer cell lines form tumours in immunodeficient mice was first reported by Isaacson and Cattanach in 1962 [17]. However, the complexity of the procedures used to render wild-type mice immunodeficient, a combination of surgery (thymectomy) radiation and/or drug treatment, meant that this approach was not widely used until the introduction of the mutant nude mouse. Today the nude (*Foxn1*) mice and severe combined immunodeficiency (SCID) mice, which have naturally occurring single gene mutations that affect their immune system, are the most commonly used research models in xenograft experiments. Nudes have a chromosome 11 autosomal recessive mutation that causes failure of hair growth and other defects, including thymic epithelial dysgenesis, which renders them T-cell deficient [18]. The SCID mouse has a spontaneous mutation inactivating DNA protein kinase resulting in lack of functional T cells and B cells [19,20]. Immunodeficient strains have been developed from other species, including the rat, but are not widely used.

Human breast cancer is one of the more difficult tumours to transplant directly into experimental animals, including nude mice and SCID mice. The reported success (take rates) for invasive human breast cancer is 7–20% [21], with differences accounted for by site of implantation (orthotopic being better), the age and strain of mice used, and whether hormonal supplementation is used (nude mice have low oestrogen levels, compared with humans). Serially transplantable xenografts are much rarer. Paradoxically, better success has been reported with preinvasive disease samples (ductal carcinoma *in situ*) [22,23]. It has very recently been reported that subsets of immunophenotypically distinct ($CD44^+/CD24^-$) cells within primary breast tumours have an enhanced take rate as xenografts [24]. Directly established mammary tumour xenograft lines with the capacity to metastasize were not developed until the early 1990s [25], although prior and subsequent to this a number of established *in vitro* lines have been adapted to xenograft cultures. Some such lines are able to locally invade or metastasize, sometimes as the result of further genetic engineering to a more aggressive phenotype.

There are currently many human xenograft models available for use in breast cancer research (Table 1), most derived from both established cancer cell lines and spontaneously or genetically engineered immortalized normal breast epithelial cells. Among the more commonly used are the MCF10AT and MCF-7 systems, probably because of their ease of use and the wealth of information available on these lines from previous *in vitro* studies. However, the use of established cancer lines as the source of xenograft models raises a number of questions. Cancer cells that have been adapted to grow in culture are likely to have different environmental requirements to primary breast tumour cells. *In vitro* establishment is a rare event, found in no more than 1% of primary cancers [26] and almost certainly involving further selection of an 'establishment' phenotype. Thus, cell selection in conversion to continuous culture line, changes in later generations of cell lines (genetic drift) as well as viral or *Mycoplasma* infection, mislabelling of individual cell lines, and/or doubts as to their actual tumour of origin are factors that impact on the validity of such models.

By contrast, much less effort has been directed at improving primary tumour engraftment. It has recently been reported that histomorphologically intact primary human breast lesions and cancers can be grown in athymic mice. An experimental model system has been developed in which dissociated cells from surgical breast cancer

Table 1

Commonly used breast cancer xenograft models

Human breast cancer xenograft model	Description	Reference
MCF-7	Breast adenocarcinoma cell line, oestrogen receptor-positive	[37]
MCF-7/6	Oestrogen sensitive	[38]
MCF-7 BAG	Immunodeficient xenograft model	[39]
MCF-7/hVEGF	hVEGF overexpressing	
MCF-7-TAM LT	Long-term tamoxifen-stimulated breast tumour model	[40]
MCF-7/neu	Overexpressing oncogene *neu*	[41]
MCF-7-MIII	Oestrogen sensitive	[42]
MCF-7Ca	Transfected with human aromatase gene	[43]
MT2	MCF-7 tamoxifen-stimulated tumour with Asp351Tyr mutant oestrogen receptor	[44]
MT2 TAM	MCF-7-derived tumour serially passaged with tamoxifen	[40]
MT-1	Oestrogen and progesterone receptor-negative in nude rats	[45]
MT-3	Oestrogen receptor-negative	[45]
LY2	Anti-oestrogen-resistant variant of MCF-7	[46]
UMB-1Ca	Oestrogen-independent variant of MCF-7	[47]
MDA-MB-231	Oestrogen independent	[48]
MDA-MD-231 BAG	Immunodeficient xenograft model	[39]
MDA-MB-361	Brain metastasis-derived breast adenocarcinoma cell line	[49]
MDA-MB-435	Oestrogen receptor-negative	[48]
MDA-MB-435A	Ascites model	[50]
MDA-MB-435S	Spindle-shaped strain of parent line	[51]
MDA-MD-435 BAG	Immunodeficient xenograft model	[39]
MDA-MB-453 BAG	Fibroblast growth factor receptor overexpressing	[39]
MDA-453/LCC6		[52]
MDA-MB-468	Oestrogen receptor-negative metastasis-derived cell line	[49]
MDA-MB-453	Breast adenocarcinoma cell line	[53]
MCF10AT	Preneoplastic and proliferative model; nontumourigenic	[54]
MCF10AneoN	Transfected with neomycin-resistance gene	[55]
MCF10AneoT	T24-Ha-ras-transformed derivative of MCF10A	[55]
MCF10DCIS.com	Comedo ductal carcinoma *in situ*	[56]
MC-2		[57]
MC-5		[57]
MC-18		[57]
SK-BR3	Breast adenocarcinoma cell line that overexpresses oncogenic protein $p185^{HER2}$; oestrogen receptor-negative	[58]
SK-BR3/hVEGF	VEGF overexpressing cell line	[58]
BT-20	Oestrogen receptor-negative	[37]
BT-474	Erb2 overexpressing breast tumour	[59]
ZR-75-1	Oestrogen-dependent breast carcinoma	[60]

Continued overleaf

Table 1 ***Continued***

Commonly used breast cancer xenograft models

Human breast cancer xenograft model	Description	Reference
SUM149	Inflammmatory breast cancer cell line	[61]
SUM159PT	Oestrogen independent	[62]
T47D	Oestrogen receptor-positive	[63]
T47D-E2	Tamoxifen naïve tumour	[63]
KPL-1	Oestrogen receptor-positive	[64]
KPL-4	Oestrogen receptor-negative	[65]
MaTu	Oestrogen receptor-negative solid human mammary carcinoma cell line	[45]
MC4000	Oestrogen receptor-negative	[45]
HT-39	Oestrogen receptor-negative	[66]
HX99		[67]
T61	Oestrogen receptor-positive ductal carcinoma	[68]
B37		[69]
BO	Oestrogen receptor-positive	[70]
Br10		[71]
SE		[71]
WIBC-9	Inflammmatory breast cancer cell line	[57]
Met-1	Metastatic breast cancer cell line	
MAXF401		[72]
MX-1		[73]
MAXF 499	Solid ductal	[74]
NCI/ADR or MCF-7/ADR	Multidrug-resistant MCF-7 cell line that overexpresses P-glycoprotein	[75]
4296	Oestrogen receptor-positive	[76]
4049	Oestrogen receptor-negative	[76]
4151		[75]
4134		[75]
3366		[77]
4000		[77]
CAL51	Metastatic model of adenocarcinoma	[78]
MA-11	Oestrogen and progesterone negative receptor in nude rats	[79]
H31		[80]
MARY-X	Inflammatory breast cancer model	[81]
HBT 3477	Adenocarcinoma	[82]
Hs578T	Carcinosarcoma derived, epithelial in origin	[82]
C8161	Breast cancer line with high levels of spontaneous metastasis	[83]
$M24_{met}$	Breast cancer line with high levels of spontaneous metastasis	[83]
CaMa 15	Primary infiltrating ductal breast carcinoma	[37]
MaNo 4	Medullary breast carcinoma	[37]
GI-101	Metastatic breast tumour line	[25]
UISO-BCA-NMT-18	Primary breast carcinoma	[84]

VEGF, vascular endothelial growth factor.

specimens, after mixing with extracellular matrices, have been transplanted into nude mice. These transplanted cells undergo morphogenesis that reflects their original phenotype, and they provide a much more relevant model for studying primary human breast lesions and cancers *in vivo* [27].

However, even these models that are derived directly from clinical samples have their limitations. Overall, xenografts contain fewer stromal cells and the stroma that does exist is murine in origin, resulting in a chimeric tumour. The biology of chimeric rodent/human tumours can differ significantly from that of humans and can result in unpredictable growth, differentiation or metastatic properties [16]. Another limitation inherent to all xenograft models is the lack of an immune response against the tumour cells. However, there are several potential solutions to the immune response problem in the context of modelling immunotherapies. For example, it has been shown that nondisrupted pieces of tumour biopsy tissues implanted into SCID mice resulted in the coengraftment of tumours plus tumour infiltrating lymphocytes, with tumour infiltrating lymphocytes within the tumour graft remaining functional and responding to lymphocyte cytokines [28]. Human peripheral blood lymphocytes, injected subcutaneously with a human lung tumour into SCID mice, also engraft and display antitumour cytotoxic activity [29]. One could envisage the use of mice that combine the immunodeficiency phenotype of the nude/SCID with engraftment of human bone marrow stem cells.

Future progress

Better understanding of breast cancer biology has lead to the realization that tumour stromal interactions, including desmoplasia and neo-angiogenesis, are of major importance in cancer biology. Understanding these reciprocal interactions offers the possibility of new potential therapeutic strategies, including those that target breast cancer stroma itself. Tumour fibroblasts, which have an activation phenotype different to that of resting tissue fibroblasts, thus offer a potential target for antitumour therapy [30]. Also, recent reports have shown that cancer stromal alterations precede the malignant conversion of tumour cells [31]. In the light of this new evidence, therapeutic targeting of stromal cells as opposed to (or as well as) epithelial cells is now considered an appropriate strategy [32–34]. Developing better model systems representing both human stromal and epithelial cells will enable these emerging therapies to be tested more critically.

This requirement has long been recognized, but attempts to date have often floundered on the lack of readily available human stroma in a form that can be easily manipulated. Ideally, these xenograft models should represent both stromal and epithelial cells with normal, premalignant, preinvasive malignant, invasive malignant and metastatic phenotypes. A novel three-dimensional cell–cell interaction model was recently xenografted into immunodeficient mice. This comprised normal breast fibroblasts derived from reduction mammoplasties, plus normal human umbilical vein endothelial cells in combination with normal and preneoplastic human breast epithelial cells derived from clinical samples [35]. However, the model has some deficiencies. Key among these is the difficulty in assembling such cell combinations on a long-term and reproducible basis. Normal cell types have a limited lifespan *in vitro*, and will undergo senescence-related changes if extensively passaged. Reproducibility is also an issue if the cells are freshly isolated for each preparation from different donors. Also, umbilical vein endothelial cells differ from their mature vascular counterparts.

The cells used for such mix-and-match combinations should ideally be derived from the breast, be capable of being generated without donor or passage-related differences, and be available in limitless quantities. With the recent development of immortalized human adult mammary stromal cells [36], it has now become possible to satisfy these criteria and to perhaps develop a fully 'humanized' breast cancer model in immunodeficient mice. Both endothelial cells and fibroblasts were immortalized using a combination of retroviral transduction of the catalytic subunit of human telomerase plus mutant variants of the SV40 T-antigen gene. Despite its name, the large T protein does not transform the stromal cells, but it does unlock their indefinite proliferation, provided that telomeric erosion is prevented by the telomerase activity present in the cells. Neither gene singly was capable of full immortalization of these cells.

The availability of cells that are conditionally immortalized (temperature sensitive) as well as nontemperature-sensitive variants from the same individual donor stocks enables different combinations of quiescent and proliferatively active cells to be generated. In this way, the response of tumour cells to continued stromal proliferation (equivalent to desmoplasia and neo-angiogenesis) can be examined, as well as the response of quiescent stromal cells to the presence of proliferating tumour. Preliminary experiments have shown that multicellular spheroids composed of mammary epithelial, endothelial and fibroblastic cell types can be created *in vitro* using 'zero gravity' culture vessels, as a step towards the engraftment of such aggregates in nude mice. We envisage that such models will initially comprise combinations of xenograftable tumours derived directly from primary clinical material, rather than pre-adapted cell lines, in combination with the immortalized stromal cells; however, such combinations could substitute purified primary tumour cells from invasive or *in situ* carcinoma types.

Conclusion

Good models for preclinical testing must not only reproduce the pathology and behaviour of human tumours, but must also be highly reproducible with predictable endpoints. To enable mouse xenograft models to be used in routine screening of preventative and therapeutic strategies, they must reflect the cellular composition of 'real' tumours but also be simple to construct and preferably not too costly. Barriers to progress include an attitude that animal model and tumour cell line development is not critical research, restricted access to existing animal models and, finally, difficulties that pertain to the direct access and use of fresh clinical materials on a routine basis. Although considerable difficulties will be encountered in the generation and use of such complex models, their potential value in the longer term is such that every effort should be made to develop them.

Competing interests

None declared.

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