

## Importance of microenvironment in preclinical models of breast and prostate cancer

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### Abstract

The majority of cancer drugs entering clinical trials fail to reach the market due to poor efficacy. Preclinical

efficacy has been traditionally tested using subcutaneous xenograft models that are cheap, fast and easy to perform. However, these models lack the correct tumor microenvironment, leading to poor clinical predictivity. Selecting compounds for clinical trials based on efficacy results obtained from subcutaneous xenograft models may therefore be one important reason for the high failure rates. In this review we concentrate in describing the role and importance of the tumor microenvironment in progression of breast and prostate cancer, and describe some breast and prostate cancer cell lines that are widely used in preclinical studies. We go through different preclinical efficacy models that incorporate the tissue microenvironment and should therefore be clinically more predictive than subcutaneous xenografts. These include three-dimensional cell culture models, orthotopic and metastasis models, humanized and transgenic mouse models, and patient-derived xenografts. Different endpoint measurements and applicable imaging techniques are also discussed. We conclude that models that incorporate the tissue microenvironment should be increasingly used in preclinical efficacy studies to reduce the current high attrition rates of cancer drugs in clinical trials.

**Key words:** Tumor microenvironment; Breast cancer; Prostate cancer; Preclinical; Efficacy

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**Core tip:** It is today a recognized major problem in cancer drug development that the vast majority of drugs entering clinical trials fail to reach the market due to poor efficacy. One important reason for this is the wide use of subcutaneous xenograft models that are cheap, fast and easy to perform, but lack tumor microenvironment. Concentrating on breast and prostate cancer, we explain why the presence of tumor microenvironment is important, and describe different types of preclinical efficacy models that incorporate

tumor microenvironment. We state the importance of using these models to reduce the high failure rates in clinical trials.

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## INTRODUCTION

During the course of multistep tumorigenesis of breast and prostate carcinomas, neoplastic epithelial cells are in a continuous interplay with mesenchymal cells that form the tumor-associated stroma. This tumor microenvironment is constituted by endothelial cells, pericytes, myoepithelial cells, osteoblasts, immune cells, fibroblasts, cancer stem cells, and many other cells that secrete growth factors and cytokines<sup>[1]</sup>. While complex interactions between these different cell types reshape the surrounding extracellular matrix (ECM) as cancer progresses, also neoplastic and stromal cells undergo constant changes. Endpoint of this extreme plasticity is that a tumor almost never contains two completely identical cells<sup>[2]</sup>. While tumor heterogeneity remains a major obstacle to effective cancer treatment and personalized medicine, it can also be used as a biomarker to predict the risk of progression and therapeutic resistance<sup>[3]</sup>.

An optimal preclinical model mimics these plastic genetic and phenotypic changes that occur within human disease, is heterogenic, and results in appropriate tumor growth and spread<sup>[4]</sup>. Mouse (*Mus musculus*) has emerged as the main species of *in vivo* tumor biology due to its basic physiology and genome size that are similar to human<sup>[5]</sup>. Other advantages for using mice include the ease of genetic manipulation, low maintenance cost, and short gestation period<sup>[6]</sup>. Here we rationalize how mouse models of breast and prostate cancer can help us to understand the interaction between microenvironment and cancer cells in neoplastic progression. Major differences between human and mouse tissue architecture and different research models will be discussed.

## MOUSE VS HUMAN BREAST AND PROSTATE TISSUE

In mammals, the morphology of mammary gland changes throughout the entire reproductive life. Ductal morphogenesis, as well as carcinogenesis, are regulated by steroid and polypeptide hormones and growth factors that act as local epithelial-mesenchymal inductive signals. The glandular part of the human and murine mammary tissue is composed of major

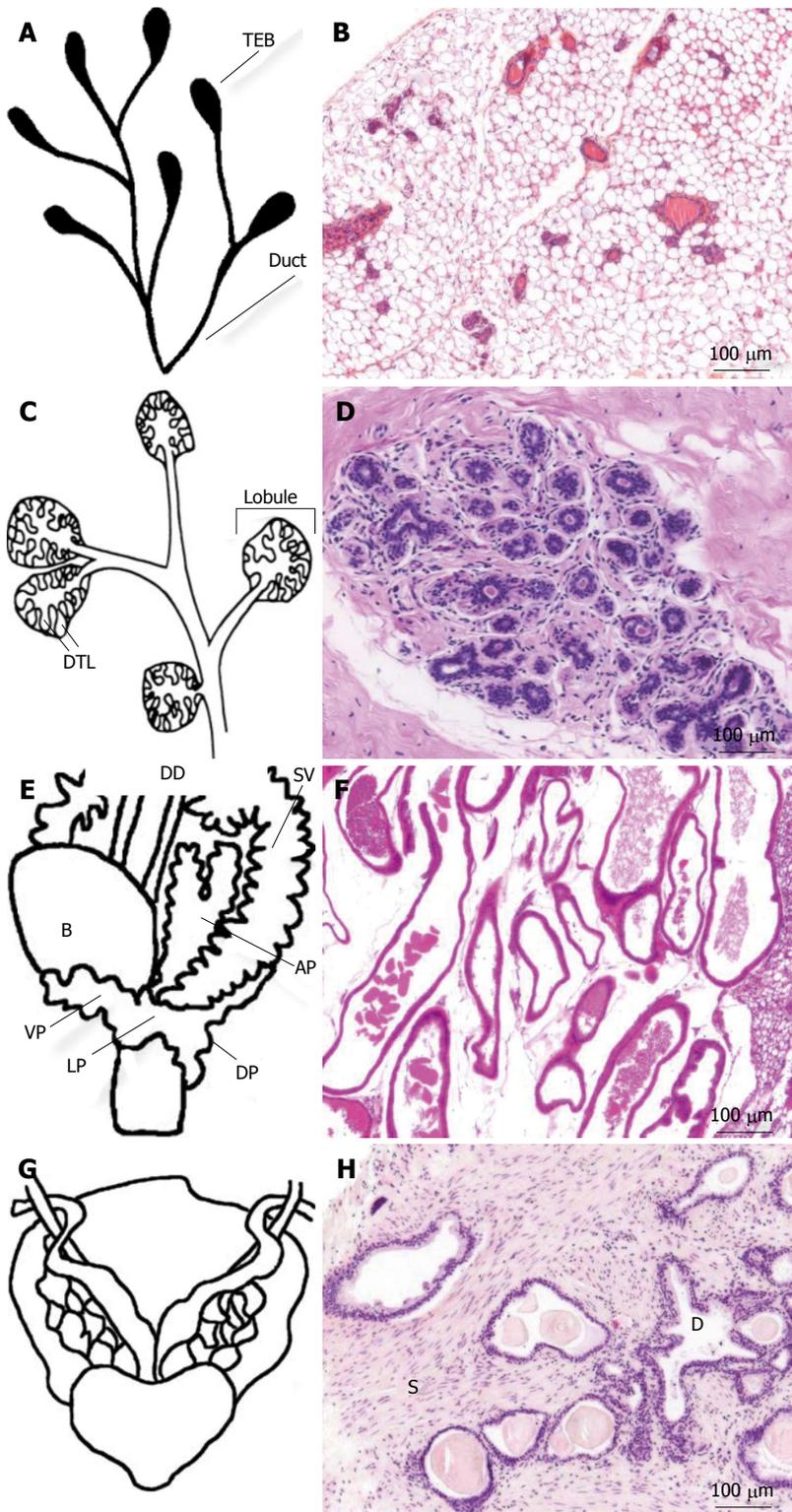
lactiferous ducts that arise inside the nipple, branch into terminal ducts, and end up in acini that are embedded in the intralobular stroma<sup>[7,8]</sup>. The acini are composed of a bilayer of inner milk producing luminal cells and outer myoepithelial cells<sup>[9]</sup>. The human acini with the surrounding intralobular stroma are termed terminal ductal lobular unit. It is comprised of a small group of lobules, resembling a cluster of grapes at the end of a stem<sup>[10]</sup>. The murine mammary tissue is organized differently. The corresponding functional units are termed lobuloalveolar units. Unlike in human, the individual ducts branch minimally and end in single bulbous terminal end-buds (Figure 1)<sup>[11]</sup>.

Breast cancer usually originates from the epithelium, but the stroma has a profound effect on tumor growth, invasion, metastasis, and drug resistance<sup>[12]</sup>. The mouse mammary stroma is histologically different from the human stroma<sup>[13,14]</sup>. Human mammary epithelium is surrounded by fibrous connective tissue, whereas mouse tissue consists of larger number of adipose cells and smaller proportion of connective tissue (Figure 1). Also, the human breast contains fat, but it is not in contact with the epithelium<sup>[11]</sup>.

Both human and murine prostates are muscular glands that surround urethra. The prostate is covered with a capsule, and it is in close contact with accessory sexual glands such as coagulating gland in mice, bulbourethral gland in humans, and seminal vesicles in both. The obvious difference in gross anatomy between human and murine prostates is that murine prostate is composed of separate ventral, dorsal, and lateral lobes, whereas human prostate is a single nut-shaped gland that is divided into lobes or zones according to their location and function. In humans, there are two lateral lobes in the anterior end of the gland. The anterior lobe is located behind the lateral lobe, anterior to urethra. It is constructed of fibromuscular tissue, and activates during ejaculation. On the posterior to the urethra there is an area called median lobe, and on the posterior to the median lobe a very thin area called posterior lobe. The human prostate can also be divided into an anteriorly located central zone, an urethra surrounding transition zone, and a peripheral zone, which is the largest zone and the most common location of a tumor<sup>[4,15]</sup>.

The prostatic tissue is composed of exocrine glands, ducts, and fibromuscular stroma. The human and mouse prostates contain similar cell types, but the proportion of stroma is larger in the human prostate (Figure 1). Of the mouse prostatic lobes, the dorsolateral lobe resembles most the human prostate histologically and biochemically<sup>[4,16]</sup>. Therefore, the dorsolateral prostate is an appropriate inoculation or implantation site in xenograft models.

During carcinogenesis, the stroma undergoes extensive changes in gene expression, and often proliferates actively<sup>[17]</sup>. The stroma co-evolves with its tumor and adapts to the needs of the tumor<sup>[18]</sup>. For



**Figure 1 Anatomical and histological comparison of mouse and human mammary gland (A-D) or prostate (E-H).** A: Schematic representation of pubertal mouse mammary tree ducts, which end in club shaped terminal end buds (TEBs); B: Hematoxylin & eosin (H&E) stained section of mouse breast tissue, showing ducts imbedded in a stroma composed of adipose tissue; C: Human mature nulliparous terminal ductal lobular unit, 30-50 ductules (DTL) are present in each lobule; D: H&E stained section of human mammary gland showing a terminal ductal lobular unit comprised of ducts and acini in a fibrous connective tissue stroma; E: Mouse prostate surrounds urethra and has distinct lobes: ventral lobe (VP), dorsal lobe (DP) and lateral lobe (LP); F: H&E stained section of mouse ventral prostate; G: Human prostate is a nut shaped gland which also surrounds the urethra; H: The proportion of stroma in human prostate is larger compared with mouse prostate, H&E staining shows secreting ducts (D) and stroma (S). B: Bladder; DD: Ductus deferens; SV: Seminal vesicle; AP: Anterior prostate.

example the amount of collagens increases in tumor ECM, which makes it thicker and may act as a physical or cell attachment - based barrier to drugs. Despite

the differences in organization of the stroma between humans and mice, similar gene activation as in patients is seen in the stroma of transgenic and xenograft-

**Table 1** Classification of the most commonly used human breast cancer cell lines

Name	Histopathological classification	Immunohistochemical classification
MCF-7	Luminal A	ER <sup>+</sup> , PR <sup>+</sup> , Her2 <sup>-</sup>
SUM185	Luminal A	ER <sup>+</sup> , PR <sup>-</sup> , Her2 <sup>-</sup>
T47D	Luminal A	ER <sup>+</sup> , PR <sup>+</sup> , Her2 <sup>-</sup>
BT-474	Luminal B	ER <sup>+</sup> , PR <sup>+</sup> , Her2 <sup>+</sup>
ZR-75	Luminal B	ER <sup>+</sup> , PR <sup>-</sup> , Her2 <sup>+</sup>
SKBR3	Her2-positive	ER <sup>-</sup> , PR <sup>-</sup> , Her2 <sup>+</sup>
MDA-MB-453	Her2-positive	ER <sup>-</sup> , PR <sup>-</sup> , Her2 <sup>+</sup>
MDA-MB-468	Basal	ER <sup>-</sup> , PR <sup>-</sup> , Her2 <sup>-</sup>
SUM190	Basal	ER <sup>-</sup> , PR <sup>-</sup> , Her2 <sup>+</sup>
BT-20	Basal	ER <sup>-</sup> , PR <sup>-</sup> , Her2 <sup>-</sup>
MDA-MB-231	Claudin-low	ER <sup>-</sup> , PR <sup>-</sup> , Her2 <sup>-</sup>
HS-578T	Claudin-low	ER <sup>-</sup> , PR <sup>-</sup> , Her2 <sup>-</sup>
Cal-51	Claudin-low	ER <sup>-</sup> , PR <sup>-</sup> , Her2 <sup>-</sup>

Adapted from a review of Holliday and Speirs 2011<sup>[24]</sup>. ER<sup>+/−</sup>: Estrogen receptor-positive/negative; PR<sup>+/−</sup>: Progesterone receptor-positive/negative; Her2<sup>+/−</sup>: Human epidermal growth factor-positive/negative.

**Table 2** Classification of the most commonly used human prostate cancer cell lines

Name	Site of origin	Hormonal status	PSA expression
PC-3	Bone	AR <sup>-</sup>	No
DU-145	Brain	AR <sup>-</sup>	No
LNCaP	Lymph node	AS	Yes
C4-2B	Subline of LNCaP	AI	Yes
VCaP	Bone	AS	Yes
CWR22	Prostate	AS	Yes
22Rv1	Subline of 22Rv1	AI	Yes
PC-346	Prostate	AS	Yes

AR<sup>-</sup>: Androgen receptor negative; AS: Androgen sensitive; AI: Androgen independent.

bearing mice<sup>[19,20]</sup>.

## HUMAN BREAST AND PROSTATE CANCER CELL LINES

BT-20 was the first commercial breast cancer cell line. It was established in 1958, followed by the still very popular MD Anderson series (MDA), and MCF-7 cell lines 20 years later<sup>[21–23]</sup>. Breast cancer, as well as prostate cancer, is a very heterogeneous disease, and until today there are no comprehensive models available to study them. However, human breast cancer cell lines (summarized in Table 1) are available that represent the main categories of breast cancer<sup>[24]</sup>.

Table 2 summarizes the most commonly used human prostate cancer cell lines. PC-3 and DU-145 cells were originally cloned from bone and brain metastases of prostate cancer, respectively<sup>[25,26]</sup>. Their tumorigenicity is high and they form metastases when inoculated into immunodeficient mice<sup>[27]</sup>, and they can thus be considered as models of advanced disease. However, these very popularly used cell lines lack expression of androgen receptor (AR) and prostate

specific antigen (PSA), which are both characteristic for hormone-responsive prostate cancer. LNCaP cells express AR and secrete PSA, but they have limited tumorigenicity and respond aberrantly to androgen therapy because of a mutated AR, and they are also sensitive to other sex steroids<sup>[28]</sup>. Some newer prostate cancer cell lines respond to androgens and secrete PSA, including VCaP cells<sup>[29–31]</sup>, 22Rv1 cells<sup>[32]</sup> and PC-346 cells<sup>[33]</sup>. A panel of transplantable human-derived xenografts (CWR, MDA Pca, LuCaP, and LAPC series) have interesting characteristics that mimic human disease<sup>[26]</sup>. Their benefit is the relevant tissue architecture with stromal support, which improves tumor growth and metastasis.

## THREE-DIMENSIONAL CELL CULTURE MODELS FOR STUDYING THE IMPACT OF MICROENVIRONMENT

Currently, *in vitro* drug testing is mostly based on traditional two-dimensional (2D) monoculture models that utilize immortalized cancer cell lines in systems that cannot incorporate the tissue microenvironment. However, 3D cell cultures have raised considerable attention in recent years because of their potential to deliver higher quality and more accurate information that is more representative and predictive of drug responses *in vivo*. Currently, the main applications of 3D cell cultures include cancer therapy and studies of cell-to-cell and cell-to-matrix interactions. It is known that both cancer cells and normal cells cultured in 3D in the presence of ECM components show differences in gene expression, differentiation and proliferation when compared to cells cultured as monolayer in 2D. The importance of the microenvironment was highlighted by Mina Bissell's research group, who were the first to recognize that normal mammary epithelial cells grown in monolayers divided exponentially through several passages, but when the cells were grown in 3D Matrigel culture, they responded to microenvironmental signals by reducing proliferation and differentiating into nearly normal-sized mammary acinar structures<sup>[34]</sup>. An interesting finding was also that when cultured in the presence of a matrix that contained a combination of reconstituted basement membrane proteins, including type I collagen and normal breast fibroblasts, MCF-7 cancer cells were induced to near-complete tumor phenotype reversion<sup>[35]</sup>.

The most widely used 3D culture structures are spheroids that can be formed by multiple different approaches, including scaffolds such as hydrogels, and as floating structures formed either by hanging drop method or by low attachment coatings. The spheroid systems allow co-culturing of different cell populations for studying the role of cell-to-cell or cell-to-ECM interactions, and therefore provide an improved approximate of the *in vivo* tissue architecture. Multiple cell types, such as stromal fibroblasts, nerve

ganglia or endothelial cells, have been seeded within a matrix gel to influence spheroid growth and define specific roles or interactions with prostate cancer cells, including DU-145, LNCaP and PC-3 cells<sup>[36]</sup>. Also, co-culture of bone stromal derived HS5 cells and PC-3 cells in Matrigel scaffold displays up-regulated invasion and proliferation, along with altered expression of epithelial-to-mesenchymal and chemokine protein constituents involved in metastatic progression<sup>[37]</sup>. Additionally, multiple cells, including PC-3, osteoblasts and endothelial cells, have been seeded into hanging drops to form heterogeneous aggregates recapitulating the *in vivo* growth behavior of cancer cells within the bone metastatic prostate cancer microenvironment<sup>[38]</sup>. In breast cancer, the surrounding microenvironment, including stromal fibroblasts, is believed to promote the progression of ductal carcinoma *in situ* (DCIS) to invasive ductal carcinoma<sup>[39-43]</sup>. Indeed, human mammary fibroblasts cultured in a 3D matrix have been shown to secrete more paracrine signaling molecules than in 2D monolayer cultures, increasing the invasive progression in MCF10-DCIS.com cells<sup>[44]</sup>. Even though the role of the matrix in regulating fibroblast behavior has been studied, the consequences of modified fibroblast behavior with cancer cells remains poorly understood.

## XENOGRAFT AND SYNGENEIC ANIMAL MODELS

The term xenograft implies transplantation of material between species. Most commonly, human cells or tissue implants are grafted into immunodeficient mice. If the transplanted material is from genetically nearly identical individuals, it can be transplanted into immunocompetent mice to produce syngeneic tumors. Syngeneic models allow to study the role of adaptive immunity in tumor progression, which is a benefit compared with xenografts. However, the fact that the cells are from murine origin and very rarely respond to hormonal therapy may hamper the results. There are several good syngeneic models for breast cancer, such as Balb/cC3H-originated 4T1 subline grafted into Balb/c mice<sup>[45]</sup>, S115 cells grafted into DD/Sio mice<sup>[46]</sup>, and Py8119 cells grafted into C57BL mice<sup>[47]</sup>. Until now, there are only few syngeneic models for prostate cancer such as RM1 cells or TRAMP-C2 cells in C57BL mice<sup>[48,49]</sup>.

## SUBCUTANEOUS, ORTHOTOPIC AND METASTASIS MODELS

Subcutaneous inoculation of tumor cells is a popular and inexpensive way to perform xenograft models. However, these models can be used only in studies of primary tumor growth because of restricted spread and formation of metastases due to incomplete blood and lymphatic vasculature<sup>[50,51]</sup>. This, and the fact

that these models lack the correct microenvironment for the tumor cells, leads to poor clinical predictivity. The correct tumor microenvironment is important not only for the processes of tumorigenesis, invasion and metastasis, but also for its potential effects on efficacy of tested drug candidates. The correct microenvironment can either improve the efficacy of tissue-specific targeted therapies, or protect the cancer cells from the therapy<sup>[12]</sup>. The wide use of subcutaneous xenografts and relying on the obtained results is probably one important reason why a very high number of cancer drug candidates fail in clinical trials due to poor efficacy<sup>[52]</sup>. However, many other reasons such as non-enhanced patient groups, tumor heterogeneity, and low number of clinically relevant events also contribute to the high failure rates.

Clinically much more relevant xenograft models are orthotopic models, where breast cancer cells are inoculated into the mammary fat pad, and prostate cancer cells into the prostate. In these models the cancer cells form primary tumors in the relevant tumor microenvironment and interact with the mouse stromal cells<sup>[53-55]</sup>. Orthotopic models can also include formation of metastases, depending on the characteristics of the used cell line<sup>[56,57]</sup>. Typically, orthotopic breast and prostate tumors metastasize into local (inguinal or iliac and sacral, respectively) lymph nodes, liver and lungs<sup>[58,59]</sup>. Bone metastasis is a common and deadly complication of both breast and prostate cancer. Some breast and prostate cancer models produce bone metastases, but macroscopic bone tumors are rarely, if ever, observed using orthotopic models<sup>[56]</sup>. By inoculating tumor cells into the bone marrow cavity of the mouse tibia, tumor cell-bone interactions can be studied. Although several steps of the metastasis cascade remain unstudied in this model, the intratibial tumors provide valuable information about the tumor-bone interaction.

Tumor cells can also be inoculated directly into the tail vein or the left cardiac ventricle in order to mimic metastatic disease<sup>[60,61]</sup>. These models are clinically highly relevant, since at the time of diagnosis of breast and prostate cancer, dormant tumor cells can be found in bone marrow cavity<sup>[62]</sup>. The models are based on Paget's seed and soil-hypothesis, where a small number of tumor cells have evolved towards metastatic phenotype after a series of somatic mutations<sup>[63]</sup>. Some laboratories have succeeded in enrichment of bone- or lung-seeking tumor cell populations, and created sublines of some commonly used cell lines. Examples of such breast cancer sublines are bone-seeking MDA-MB-231(SA) and MDA-MB-231(B02) cells<sup>[64,65]</sup>, and MDA-MB-231(LM) cells that form tumors in lungs when inoculated into the blood stream<sup>[59]</sup>.

## HUMANIZED MICE

The major limitation of using xenograft models with immunocompromised mice is the lack of immune cells

in the tumor microenvironment. The use of human stroma may be a solution to this problem. Kuperwasser *et al.*<sup>[66]</sup> injected human mammary stromal and epithelial cells into cleared murine mammary fat pads. This chimeric mouse “humanized mammary fat pad” was found to be similar to that of humans and allowed genetic manipulation of the human stroma. Currently, there are no xenograft models where bone metastases are formed from orthotopic tumors with a relevant rate. Several laboratories have introduced a humanized mouse, where human bone tissue is first grafted into immunodeficient mice and after inoculation of the human breast or prostate tumor cells, metastases have been formed into human bone instead of mouse bone<sup>[67-69]</sup>, underlining the importance of species-specificity of the microenvironment in metastasis formation. However, the effect of possible differences in bone metabolism of the transplant vs normal bone cannot be ruled out, since there is clear evidence of higher bone metabolism connected to higher metastasis rate<sup>[70]</sup>. Challenges of the model include the availability of human bone, donor-related variance, immune reactions, and difficulties in implant functionality and viability<sup>[71,72]</sup>.

## TRANSGENIC MOUSE MODELS

Genetically engineered mouse models are physiologically relevant models to study tumor progression, because they include natural microenvironment and immune competence. However, most transgenic breast and prostate cancer models are hormone-independent and do not respond to hormone therapy<sup>[73,74]</sup>. Also, mouse tumors are often mesenchymal instead of epithelial origin<sup>[75]</sup>, and none of the transgenic models include the entire heterogeneity and plasticity of human carcinogenesis.

When an oncogene is overexpressed in mammary gland or prostate epithelium, the most commonly used promoter elements are the mouse mammary tumor virus (MMTV) long terminal repeat, human cytomegalovirus and ubiquitin promoters, the rat probasin gene, the rat C3 prostate steroid-binding protein gene, the human PSA gene, and the mouse cryptic gene<sup>[76-79]</sup>. Hruska *et al.*<sup>[80]</sup> created an estrogen receptor overexpressing conditional mouse line that developed mammary adenocarcinomas, which responded to estrogen and had similarities to human breast cancer histology. The transgenic adenocarcinoma mouse prostate (TRAMP) model was established in 1995, and TRAMP mice have been widely used in oncology<sup>[78,81]</sup>. In the TRAMP model, SV40 small and large T-antigens inactivate tumor-suppressor proteins and enhance the development of neoplasia<sup>[78,82]</sup>. TRAMP mice develop prostate adenocarcinoma and metastasize into para-aortic lymph nodes and lungs, and occasionally to distant sites<sup>[78]</sup>. Disadvantage of the model is that metastases develop at a relatively low frequency<sup>[4]</sup>. In addition, Chiaverotti *et al.*<sup>[83]</sup> have shown that the background of TRAMP

mice (FVB instead of C57/BL) influenced the tumor type. FVB mice frequently developed neuroendocrine-type prostate tumors, while C57/BL mice developed adenocarcinomas. In addition to TRAMP mice, a popular transgenic model is c-Myc overexpression<sup>[84]</sup>. A structural variation of the c-Myc gene is common in cancer, and accordingly the increased copy number of c-Myc results in a homologous gene-expression profile with human c-Myc-overexpressing cancer, such as disappearance of NKX3.1 during tumorigenesis<sup>[85]</sup>.

Alternatively, the role of specific genes in breast and prostate tumorigenesis can be studied using knockout mice. Since ablation of important genes often leads to embryonic or early fatality, genetically modified mice with conditional knockouts have been developed. Germline mutations in oncogenes BRCA1 and BRCA2, in which DNA repair function is interrupted, account for the majority of familial breast cancers. In order to study the role of BRCA1 in breast cancer, MMTV-cre mice have been created, and used to produce conditional mammary BRCA1 knockout mice<sup>[86]</sup>.

Inactivation of the tumor suppressor gene *PTEN* is associated in approximately 70% of advanced human prostate cancers<sup>[87]</sup>. *PTEN*<sup>+/-</sup>, *PTEN* hypomorph, and *PTEN* conditional knock-out models have been established to study prostate cancer progression<sup>[87-89]</sup>. Conditional *PTEN* knock-out leads to prostate cancer with lymph node and lung metastases<sup>[88,89]</sup>. In addition to the cre-loxP system, tissue-specific, conditional knock-out models have been created using the tetracycline promoter system under the regulation of tet operator promoter. In this model, the specific gene is expressed only under doxycycline supplementation<sup>[80]</sup>.

## PATIENT-DERIVED XENOGRAPTS

While cell line based models have provided invaluable knowledge of cancer progression, the utility of these systems is diminished in the light of the findings that patient derived tumor cell lines have significantly different gene expression patterns when compared to the original cell lines or the xenografted tumors<sup>[90-92]</sup>. Patient-derived xenografts (PDXs) are recent advances in personalized medicine. These models use mouse avatars, where fresh tumor tissue from the patient is grafted in order to study which therapies are most effective for an individual cancer patient. A large number of drugs or drug combinations can then be screened in the mice, which increases the likelihood that a given treatment will benefit the patient. In addition to clinics, PDX models are used increasingly as tumor models in drug development. An obvious benefit of PDX models vs traditional cell line - based subcutaneous xenografts is that they possess the natural tissue architecture and composition<sup>[93]</sup>.

However, PDX models have many challenges. The success rate for implanting human tumors in mice is low and depending on the tumor type, engraftment efficiencies vary a lot. In clinical use, it takes more

**Table 3 Comparison of different types of breast and prostate cancer xenograft models**

Type	Relevant ME	Metastases	Costs	Ease	Ref.
Subcutaneous	No	No	Low	Easy	[51]
Orthotopic BrCa	Yes	Yes	Low	Easy	[45]
Orthotopic PCa	Yes	Yes	Medium	Difficult	[55]
Intratumoral	Yes	Yes	Medium	Difficult	[56]
Intravenous/cardiac	Yes	Yes	Medium	Medium	[60]
Humanized	Yes	Yes	High	Difficult	[67]
PDX	Yes	No	High	Difficult	[94]

ME: Microenvironment; BrCa: Breast cancer; PCa: Prostate cancer; PDX: Patient-derived xenograft.

than six months to generate PDXs and screen potential therapies, and many patients die before they can benefit from the results. Although the patient tumor is engrafted along with human stromal components and is sustained during several passages<sup>[94]</sup>, murine stroma may gradually replace the human stroma and lead to confounding results. High cost of PDX technology also limits their use. However, increased use of PDX systems with modern molecular biology techniques will continue to improve the methodology and may help more patients in the future.

There are several companies that offer breast cancer PDX models, but none that offer prostate cancer PDX models. Human prostate cancer xenografts have been implanted in immunodeficient mice subcutaneously or under the renal capsule to study, maintain, or even expand the tumor tissue<sup>[95]</sup>. This technique has been particularly tested for the propagation of the tumor tissue from castration resistant prostate cancer, which is available for research only in very limited amounts from biopsy samples.

## ENDPOINTS AND IMAGING

Experimental tumors are evaluated using immunohistochemical markers and histomorphometry that are already established in clinic. The major obstacle of comparing experimental tumors with clinical specimens is the mouse background, which may hamper immunohistochemical stainings. Also the need for an experienced disease model pathologist may be an obstacle.

The classical endpoint in subcutaneous xenograft models is tumor dimension measurement by caliper, where tumor volume can be calculated using the formula  $V = a \times b^2/2$ , "a" being the biggest dimension of the tumor and "b" the perpendicular dimension<sup>[96]</sup>. If the tumors are dissected the formula of three dimensions can be used, where  $V = \pi/6 (a \times b \times c)$ <sup>[97]</sup>. Naturally, caliper measurements can only be used if the tumors are palpable. The rapid evaluation of novel drugs in animal models requires developing clinically translatable noninvasive imaging strategies, which are discussed below.

Optical imaging is based on a signal produced by

a reporter protein. The signal can be produced by constitutive expression of a fluorescent protein<sup>[98]</sup>, or by enzymatic activation of an inactive substrate<sup>[99]</sup>. In both options, tumor-producing cell lines need to be transfected with a reporter molecule. A popular method of transfection is the use of genome-integrated viruses. However, they contain a risk of genotoxicity and unpredicted effects due to random integration, which may directly affect the expression levels of not only surrounding but also distant genes. Also, both plasmid and virus based methods can modify the cell behavior indirectly because they typically contain unmethylated or hypomethylated CpG sequences that act as ligands for Toll-like receptor 9, and therefore activate the immune system<sup>[100,101]</sup>. The third obstacle is that cells may spit out the redundant reporter material during the course of the experiment<sup>[102]</sup>. In a recent study, these problems were avoided by transfecting cells using non-integrated, episomal CpG-depleted lentivector with a scaffold/matrix-attachment region that acts as an initiation point of replication during mitosis, and enables efficient and stable production of labelled cell lines<sup>[103,104]</sup>.

In addition to optical imaging, bone metastases can be imaged and quantitatively analysed using radiography, micro-computed tomography (CT)<sup>[105,106]</sup>, or micro-magnetic resonance imaging (MRI)<sup>[107]</sup>. Multimodality functional imaging approach effectively combines the advantages of optical imaging, CT and MRI to analyze breast or prostate cancer bone lesions. Soft tissue metastases can be detected using ultrasound imaging<sup>[108]</sup>, MRI<sup>[107]</sup>, or *ex vivo* by histology and quantitative polymerase chain reaction<sup>[56]</sup>. Micro-ultrasound imaging can be used to image the surrounding tissue at 3 cm depth, which is usually sufficient for detecting tumors in mice, but difficult for detecting metastases due to their small size. Micro-MRI combined with a contrast agent that specifically attaches to prostate specific membrane antigen receptor, a marker implicated in prostate tumor progression and metastasis, may prove to be a sensitive technique<sup>[109]</sup>.

Today, popular methods of functional imaging are single-photon emission CT and positron emission tomography combined either with CT or MRI. Although clinical use of these techniques is increasing in oncology for diagnosis and image guided radiotherapy planning, their use in preclinical studies is still limited due to their poor resolution and because they are very expensive<sup>[110]</sup>.

## CONCLUSION

There are several types of xenograft models available for breast and prostate cancer research (summarized in Table 3). Subcutaneous models are most widely used because they are cheap, fast and easy to perform, but they lack the correct tumor microenvironment. The presence of tumor microenvironment is very

important and necessary for obtaining results that are clinically predictive. It would be important to use preclinical efficacy models that incorporate tumor microenvironment instead of or in addition to subcutaneous models to decrease the very high number of cancer drugs that fail in clinical trials due to poor efficacy.

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