



# Animal models of bone metastatic prostate cancer

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Metastatic disease is a main cause of mortality in prostate cancer and remains to be incurable despite emerging new treatment agents. Development of novel treatment agents are confined within the boundaries of our knowledge of bone metastatic prostate cancer. Exploration into the underlying mechanism of metastatic tumorigenesis and treatment resistance will further expose novel targets for novel treatment agents. Up to date, many of these researches have been conducted with animal models which have served as classical tools that play a pivotal role in understanding the fundamental nature of cancer. The ability to reproduce the natural course of prostate cancer would be of profound value. However, currently available models do not reproduce the entire process of tumorigenesis to bone metastasis and are limited to reproducing small portions of the entire process. Therefore, knowledge of available models and understanding the strengths and weaknesses for each model is key to achieve research objectives. In this article, we take an overview of cell line injection animal models and patient derived xenograft models that have been applied to the research of human prostate cancer bone metastasis.

**Keywords:** Bone neoplasms; Neoplasm metastasis; Prostate cancer

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## INTRODUCTION

Prostate cancer is the second most common cancer and fifth leading cause of cancer death in men, with a low mortality rate in the non-metastatic stage (15-year cancer specific mortality rates for ranging between 0.5% and 5.7%) [1,2]. However, unlike localized prostate cancer, metastatic prostate cancer has a poor prognosis (5-year survival rate, 29.8%) [3,4]. Therefore, in the future, inhibiting and treating the progression to metastatic prostate cancer will be an effective strategy to increase the overall survival rate of prostate cancer along with novel treatment agents that are currently on the way to even further enhance survival in the latter stages of prostate cancer.

An effective treatment strategy to prevent metastatic disease will be to target detachment, migration and infiltra-

tion of cancer cells to metastatic lesions and their migration through the lymphatic or vascular system. Especially, targeted therapies directed to dormant cancer cells may alleviate treatment resistance, recurrence and metastasis of prostate cancer to some degree.

Animal models of bone metastatic prostate cancer are essential tools to understand the characteristics of prostate cancer and to find novel targets for future treatment agents. However, there are no sole bone metastatic animal model that can cover the full spectrum of metastatic disease from cancer cell migration and infiltration to progression and acquisition of treatment resistance. Every model has its own strengths and weaknesses associated to its model design and will bypass certain steps of the metastatic cascade. For example, subcutaneous injection of prostate cancer cell line into the flanks of mice or rats may be suitable for studying

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primary tumor lesions but will rarely progress to metastatic disease. In addition, direct injection of prostate cancer cell lines into the blood stream can be used to study hematogenous metastasis but inevitably omits the initial stages of metastasis.

Patient derived xenograft (PDX) models are clinically invaluable tools that reflect natural human prostate cancer genetics and cellular heterogeneity of patients by direct engraftment of cancer tissue into immunodeficient mice. These models have been used to better recapitulate the cellular heterogeneity and characteristics of human prostate cancer. However, a model that accurately replicates the whole spectrum of metastasis remains elusive.

In this review, the changes occurring in the intraosseous microenvironment of bone metastatic prostate cancer is described from a molecular biological perspective. In addition, models of bone metastatic prostate cancer are reviewed, and future directions of next-generation bone metastatic prostate cancer models are presented.

## STAGES OF BONE METASTASIS

After the initial stages of proliferation of prostate cancer at the primary site, detachment and migration of cancer cells to distal sites of metastasis occur through the lymphatics or peripheral vessels. Normal prostate cancer epithelial cells form cohesive connections with adjacent cells and the extra-cellular matrix. In contrast, prostate cancer cells have weakened connections with adjacent cells and extracellular matrix (ECM) that result in the detachment and migration of cancer cells. These changes have been described in theory and so called epithelial-to mesenchymal transition (EMT). EMT consequently reduces the cell-to-cell adhesion and promotes intravasation. This is presumed to be mainly due to mutations in the cytoskeletal protein [5], the decrease in the expression of E-cadherin which is a cell adhesion molecule, and the increase in the expression of N-cadherin [6].

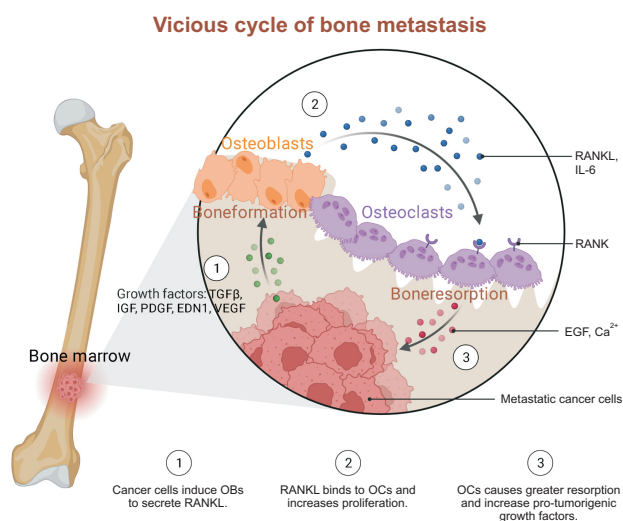
Chemotaxis of prostate cancer cells to the bone marrow are mediated by CXC-chemokine receptor 4 [7,8] which bind with CXC-chemokine ligand 12 secreted by bone marrow epithelial cells, stem cells and bone marrow derived interstitial cells [9]. Integrins such as  $\alpha V\beta 3$  and  $\alpha 2\beta 1$  mediate prostate cancer cell adhesion to bone marrow endothelial cells and ECM [10-12].

Matrix metalloproteinases produce a bone niche to harbor prostate cancer cells that interact with the bone microenvironment. Factors that induce the migration of prostate cancer cells to the bone marrow are derived from osteoclasts [13,14]. In the process of metastasis, successful migration and

metastatic progression are achieved in a limited portion of cells that detach from the primary site [15,16]. The low rate of successful metastasis of detached cancer cells is due to the low survivability and failure of growth initiation in the site of metastasis.

Most of the non-progressive and solitary metastatic cancer cells remain in a cell cycle arrest or dormant status which is known to contribute to treatment resistance and further metastatic progression when latter activated. The underlying mechanism behind dormancy is uncertain. There have been reports identifying prostate cancer cells to remain in dormancy in the bone microenvironment where osteoblastic cells are abundant and relatively scarce of hematopoietic stem cells (HSCs) due to competition of prostate cancer cells and HSCs for the endosteal HSC niche; until conditions are favorable for reactivation and cancer progression [17]. Reactivation of dormant cancer cells are assumed to be triggered by reactivation of osteoclasts or by self-induction of cancer cells [18].

Interaction of cancer cells with the bone microenvironment gradually proliferates abnormal bone formation (Fig. 1). Prostate cancer cells secrete factors that activate osteoblasts to generate abnormal bones which sequentially induces activation of osteoclasts through receptor activator of nuclear factor  $\kappa B$  ligand (RANKL) secretion by osteoblasts. Activated osteoclasts in turn resorb bone which releases growth



**Fig. 1.** Vicious cycle of cancer proliferation in the bone microenvironment. Tumor cells secrete several factors such as transforming growth factor beta (TGF $\beta$ ), insulin growth factor (IGF), platelet-derived growth factor (PDGF), endothelin-1 (EDN1), vascular endothelial growth factor (VEGF) that activate osteoblast (OB)s. Activated osteoblasts secrete receptor activator of nuclear factor  $\kappa B$  ligand (RANKL) and interleukin 6 (IL-6) to activate osteoclast (OC)s which in turn resorb bone and release growth factors and calcium ions to accelerate cancer cell proliferation. Created with BioRender.com.

factors such as transforming growth factor  $\beta$  (TGF $\beta$ ) that promote further tumor growth in a vicious cycle that accelerates progression. Similarly, bone metastatic breast cancer studies have shown to produce osteolytic bone lesions where tumor cells secrete factors that stimulate osteoclast differentiation [19]. In addition, expression of parathyroid hormone-related proteins, jagged1, tumor necrosis factor, interleukin (IL)-6e, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF) in tumor cells may contribute to osteoclast differentiation and bone resorption directly or indirectly through activation of osteoblasts which in turn secrete factors such as IL-6, GM-CSF or RANKL [19-21]. Skeletal demineralization results in release of growth factors, particularly TGF $\beta$ , insulin growth factor (IGF) and calcium ions which stimulate tumor growth [20,21]. However, prostate cancer bone metastatic lesions are osteoblastic in nature, causative of unstably woven bone [22]. It is presumed that osteoblastic bone lesions are induced through Dickkopf-related protein 1 which suppresses the Wnt signaling pathway [23]. In addition, Wnt ligands, bone morphogenetic proteins, fibroblast growth factors (FGFs), IGF secreted by tumor cells stimulate the differentiation and activation of osteoblasts at the surface of the bone marrow [20,24,25].

The interaction between the microenvironment and cancer cells in bone metastases is a highly complex process, and it is assumed that various cells participate in the process of tumor proliferation and suppression of cancer cells. Although the process of bone metastasis in prostate cancer has been studied for decades, the full spectrum of the disease and its underlying mechanism is still poorly understood. To comprehend the full scope of the disease, innovative and effective preclinical models must be developed to further shed light on the mechanism and participating factors that can arise as future targets for novel treatment agents.

## BONE METASTATIC ANIMAL MODELS

### 1. Early animal models

The Dunning rat is one of the early animal models of prostate cancer, and succeeded in isolating and preserving cancer cells named R3327 cells, and was the first model to reinject tumor cells subcutaneously into other rats [26-29]. However, subspecies of R3327 only metastasized to the lymph nodes and lungs and did not form bone metastasis, limiting its usefulness in research of bone metastatic disease [30]. Dogs were also found to spontaneously acquire prostate cancer, and studies found that prostate cancer in dogs were not only rare, but lacked the membrane androgen receptor (AR) [31,32]. As a result, prostate hyperplasia seen in dogs

always occur independently of ARs, which limits its application to human prostate disease research [33,34].

### 2. Animal models using human cell lines

Prostate cancer models using cell lines account for the majority of current *in vitro* and *in vivo* models. In 1991, Wang and Stearns first developed a mouse model of bone metastatic prostate cancer, which was developed by injecting the highly invasive PC3 cell line into the lateral tail vein of severe combined immunodeficient (SCID) mice [35]. Through the repetitive process of extracting cancer cells from the metastatic lesion and re-injecting them into the lateral tail vein of a new mouse, extraction of a new strain of PC3 cell line that caused bone metastasis in more than 80% was developed [36].

Techniques for extracting cell lines exhibiting high metastatic potential *in vitro* or *in vivo* have become commonplace, and numerous human prostate cancer cell lines currently exist. Haq and colleagues devised a model by intracardiac injection of R3327-Mat-LyLu cells, a subspecies of the Dunning cell line, into Copenhagen rats [37]. Intracardiac injection of cell lines have been reported to cause spinal metastases in 100% of inoculated mice, eliminating the need for continuous inoculation and invasive cell selection as in conventional mouse models [37]. Bone metastatic lesions with intracardiac injection is hypothesized to occur by bypassing the pulmonary capillaries through the left ventricle of the heart [37]. This inoculation technique is one of the most commonly used methods for developing bone metastasis in mice, and is more advanced than the lateral tail vein injection with vena cava occlusion [38,39]. In addition, bone metastasis induced by intracardiac injection are pathologically more similar to human bone metastasis than conventional lateral tail vein injection and vena cava occlusion models [40]. Since the most common sites for bone metastasis are the central skeleton and spine, which are rich in red bone marrow, the intracardiac injection model has advantages of similarly replicating bone metastasis observed in humans.

Preclinical models of prostate cancer bone metastasis are made by directly injecting an appropriate cell line into immunodeficient animals. If the origin of the cell line is different from that of the host, the use of immunodeficient animals is essential [41]. In general, a measure of the model's success is the tumor take rate, which is determined by the type of cell line, mouse strain, and injection method. Each cell line has its own unique genetic characteristics such as hormone sensitivity, metastatic ability, and antigenicity. The most commonly used human prostate cancer cell lines are the PC3, DU145, and LNCaP cell lines. They differ from

each other in biochemical characteristics such as AR dependence and expression of prostate-specific antigen (PSA); thus selection of an appropriate cell line should be determined based on the objectives of the experiment.

The PC3 cell line was identified in 1979 from a patient with bone metastatic prostate cancer [42]. It is highly metastatic, hormone resistant and PSA negative [43-46]. In addition, the PC3 cell line has stronger characteristics of neuroendocrine or small cell carcinoma. Currently, derivatives of various PC3 cell lines exist, which have been identified through several generations of serial injection and extraction of cancer cells from metastatic lesions. For example, in 1984 Kozlowski et al. [47] injected PC3 cells into the spleen of nude mice and harvested cells from liver nodules, and these metastatic strains were named PC3M cells [48]. In 1996, in a study by Pettaway et al. [49], PC3M cells were collected from metastatic lymph nodes to establish the PC3M-LN4 cell line. All cell lines derived from the PC3 cell line form osteolytic bone lesions [50]. Considering that human prostate cancer bone metastases are generally osteoblastic, the PC3 cell line does not fully reflect the nature of human prostate cancer. Nonetheless, PC3 cell lines are still one of the most commonly used cell lines, because of its highly aggressive nature to rapidly grow *in vivo* [51].

The DU145 cell line originated from a brain metastatic lesion of human prostate cancer [52]. Like PC3 cells, DU145 cells are hormone refractory and do not express PSA [41,52]. In addition, the DU145 cell line produces osteolytic bone lesions when injected via intratibial or intracardiac [50]. While the PC3 cell line has strong characteristics of neuroendocrine cancer or small cell carcinoma, the DU145 cell line has characteristics of adenocarcinoma and is advantageous for prostate cancer research because it can generate bone metastases *in vivo*. However, similar to the PC3 cell line, when bone metastasis is formed, it mainly causes bone degradation, which varies from the osteoblastic lesion observed in bone metastatic lesions of human prostate cancer.

The LNCaP cell line is a commonly used cell line in pre-clinical prostate cancer models. Unlike PC3 and DU145 cells, LNCaP cells are hormone sensitive (AR positive) and express PSA. It is similar to the phenotype of prostate cancer observed in humans [41,53]. LNCaP cells also express EGFR, TGF $\alpha$  receptor, FGF receptor, and IGF1 receptor, and have wild type p53 and nonfunctional PTEN [54-57]. In addition, AR expressed in LNCaP cells have a T877A mutation that allows it to have specificity for various androgens in addition to testosterone [58]. A subspecies of LNCaP cells, C4-2B, was cultured in castrated mice and then passaged by repeated injection into castrated mice until cells could be harvested

from the bone metastatic lesions [59]. LNCaP C4-2B cells are hormone refractory, have superior metastatic ability than the original LNCaP cell line, and produce osteoblastic or osteoblastic-osteolytic mixed bone lesions inside immunodeficient mice upon intraosseous or intracardiac injection [41]. This is very similar to the most undifferentiated state of prostate cancer.

All naturally occurring tumors and their metastases have molecular and cellular heterogeneity [60]. However, in the process of developing a cell line, cell culture and selection over several generations results in loss of the original molecular and cellular heterogeneity. Despite shortcoming of losing tumor heterogeneity, the use of cell lines guarantees reproducibility and predictability. In addition for research purposes, cell line animal models have the advantage of utilizing *in vivo* imaging through the attachment of specific luciferases and the expression of fluorescent marker genes. However, since genetic mutations can possibly occur over time, testing for changes in the characteristics of cancer cells through *in vivo* experiments should be performed before use.

In order to select a model suitable for the purpose of the experiment, the characteristics and limitations of each model should be considered (Table 1) [50,61-67]. For example, since the method of directly injecting a cell line into the long bones of rodents omits the process of bone metastasis, it can be used to study the interaction with the microenvironment in the state of bone metastasis. Similarly, the method of injecting cell lines into the systemic circulation will enable the study of extravasation and development of metastatic tumors. The choice of cell line should also be carefully considered (Table 2) [47,68-75]. The PC3 cell line would fit the phenotype of an aggressive late-stage prostate cancer, more like a neuroendocrine differentiated tumor than the commonly observed adenocarcinoma. On the other hand, the DU145 cell line preserves the characteristics of adenocarcinoma, and the experimental results obtained using it have the advantage of better predicting the clinical effects of human subjects than the PC3 cell line. However, both cell lines are androgen insensitive and have the disadvantage of forming osteolytic lesions which limits their use in the research of hormone sensitive prostate cancer and osteoblastic bone lesions.

### 3. PDX models

A PDX model, also known as a tumor graft model, of prostate cancer is made by directly transplanting patient tumor tissue into immunodeficient mice. The success of the PDX model is highly dependent on the implantation site. Commonly used implantation sites include the subcutane-

**Table 1.** Strength and weaknesses according to inoculation methods and sample types

	Advantages	Disadvantages
<b>Inoculation sample type</b>		
Human cell line	Numerous established human derived prostate cancer cell lines are available to match research objectives (prostate-specific antigen expression, androgen sensitivity, bone lesion type, etc.) Bioluminescent imaging through luciferase tagged cell lines are available Maintenance and preservation of cell line is relatively simple	Limited in reflecting the heterogenous characteristics of naturally occurring prostate cancer Requires immunodeficient mice strains, thus inappropriate for studying immunobiology
Mouse derived cell line	Can use immune intact mice allowing study of immunobiology of prostate cancer	Poorly reflects the human disease
Patient derived xenograft	Heterogeneity of the original tumor is well maintained [61] Reflects tumor pathology observed in patients [62] Genetic integrity of parental tumor is preserved after serial engraftments [63]	Requires <i>in vivo</i> cell culture for maintenance Cryopreservation could be an alternate option Requires immunodeficient mouse strains
<b>Inoculation method</b>		
Subcutaneous	Useful for primary tumor studies Technically simple to inject and monitor	Rarely metastasizes to bone
Orthotopic	Best model for primary tumor research	Injection technique requires surgery Rarely metastasizes to bone
Subrenal capsule	Provides the best take rate (gold standard) High vascularization, good interstitial fluid pressure, high lymphatic flow ensure even nutrient, growth factor and oxygen supply [64]	Although the technique is straight forward, may require surgery
Tail vein	Earliest form of injection resulting in bone metastasis Technically simple Studies the circulation and growth of metastatic cancer cells	Does not bypass the lungs, thus primary metastasis to lungs are mainly observed [65]
Intracardiac	Bypasses the lungs and produces vertebral metastasis Studies the circulation and growth of metastatic cancer cells	Requires high technical proficiency; only limited number of cancer cells can be injected at one time [50] Cancer cells are preferably delivered to organs other than bone, such as the lungs and liver; often develop into lethal cancers causing early termination of bone metastatic study
Intraosseous	Controlled bone metastatic lesions can be formed reliably Good for investigating interaction of cancer cells with bonemicroenvironment	Technically difficult and possible bone damage may interfere with results Limited to longbone metastasis, whereas prostate cancer usually occurs in vertebrae
Intra-arterial	Uses intrafemoral and intracaudal arteries as access sites; targets bone marrow of hind limbs Studies the circulation and growth of metastatic cancer cells Reduced incidence of lethal metastasis in other organs [66]	Technically challenging due to small size of artery Success rates are variable and need further investigation [67] Intrafemoral artery injection may cause necrosis to the leg due to major vessel injury [67]

ous space, the prostate, and the subcapsular space of the kidney. Implantation into the kidney subcapsular space has the highest take rate due to high blood flow, and have been used to study the process of progression to castration-resistant prostate cancer following hormone deprivation therapy [76]. In addition, the degree of immunodeficiency has been reported to influence the success rate of PDX models [77,78]. The more severely immune-deficient mice are better suited

for PDX generation [79]. In general, commonly utilized mouse strains include nude mice (lacking functional T cells), SCID and NOD-SCID mice (lacking functional T and B cells) or NOD-SCID/IL2γ-receptor null (NSG) mice (lacking functional T, B, and NK cells). Among the existing prostate cancer models, the PDX model most closely reproduces prostate cancer observed in humans and have been used to provide predictive insights when evaluating the efficacy of novel



**Table 2.** Characteristics of commonly used prostate cancer cell lines

Cell line	Origin	Type of bone metastasis	Androgen sensitivity	PSA expression	Reference
PC3M	Human	Osteolytic	-	-	[47]
PC3	Human	Osteolytic	-	-	[68-71]
LNCap	Human	Mixed	+	+	[69,71]
LNCapC4-2B	Human	Mixed	-	+	[69,71]
DU145	Human	Osteolytic	-	-	[70,71]
Ace-1	Dog	Mixed	-	N/A	[72]
RM-1	Mouse	Mixed (predominantly osteolytic)	-	N/A	[73]
R-3327	Rat	N/A	+	-	[74,75]

PSA, prostate-specific antigen; +, positive; -, negative; N/A, not available.

cancer therapies and are invaluable tools that may provide personalized medicine in the future [80]. However, the current state of PDX models have a limited role in the clinic due to its time consuming nature.

In the case of the PDX model, tumor specimens are cryopreserved or must be continuously cultured *in vivo*. For example, the LuCaP prostate cancer model was established by individually identifying the metastatic sites of 21 patients, and is traditionally maintained by subcutaneous injection into mice [35,81]. The tumor heterogeneity is well preserved in the PDX model, however, with repeated culture over generations, a portion of the heterogeneity can be lost [39]. Nevertheless, because external forces do not act to favor or detriment specific cells, a significant portion of tumor heterogeneity is preserved [78,82]. PDXs are known to retain the morphology and have the same immunohistochemical profile of the original human prostate cancer donor [79,80,83]. As is the issue with cell line derived animal models, in order to create a spontaneous bone metastatic prostate cancer PDX model, repeated inoculation of extracted cancer cells over several generations is mandatory [43]. In a study by Wang and colleagues [43], patient tumor samples were transplanted into the subrenal capsule of SCID mice for cell culture, and then reinjected into the prostate of mice until the mice developed lymph node metastasis. Cancer cells retrieved from the metastatic lymph nodes thereafter showed to spontaneously develop metastasis to various organs including the bone when inoculated into the prostate of mice [43]. However, creating a bone metastatic PDX model in this fashion can result in the disruption of the original tumor heterogeneity due to selection of highly aggressive cancer cells [39].

Unlike other PDX models, the PCSD1 model was created by direct injection of tumor cells into the femur of immunodeficient mice [44]. Despite the fact that this process omits the earlier stages of metastatic development, this method can preserve the original tumor heterogeneity observed in

patients and provide insight into the mechanism of metastatic progression in the bone [44]. In addition, intra-femoral injection of tumor cells may be used to investigate the underlying mechanism of treatment resistance observed in bone metastatic prostate cancer patients. When treated with anti-hormonal agents such as bicalutamide, PSA and AR expressions were significantly reduced [44]. However, caution must be taken as direct injection of tumor cells into the bone niche may inadvertently cause damage to the bone resulting in local inflammatory responses which may influence experimental results [45].

In conclusion, the PDX model of bone metastatic prostate cancer are powerful tools that have the potential to recapitulate most if not all of the disease observed in humans. It has the advantage of preserving tumor heterogeneity and is highly applicable to human prostate cancer research. However, the take rate may vary depending on the method of tumor injection and site of transplantation, and results may vary depending on the skills of the technician. In addition, since experimental animals must be immunodeficient to prevent tissue rejection, it cannot be used for immunobiological studies of prostate cancer [82].

## FUTURE DIRECTION

Despite various types of animal models applicable to bone metastatic prostate cancer research, it is inevitable for most models to utilize immunodeficient animals to suppress rejection of xenografts limiting research in the field of immunobiology. In hand with the expansion and development of novel immunotherapy agents in the clinic, innovative models such as transgenic mouse models have been developed to elucidate the complex immunobiological interactions observed in cancers. Transgenic mouse models have been utilized to identify several molecular drivers of metastasis and treatment resistance explained by lineage plasticity, a

concept of the ability of a cell to substantially modify its identity and take on a new phenotype such as the transition of adenocarcinoma to neuroendocrine differentiated prostate cancer. Pten-knockout mouse model of prostate cancer with ablation of TGFβ type II receptor (TGFBR2) led to more proliferative and invasive phenotype enriched in early metastases, supporting a role for TGFBR2 as a suppressor of lineage plasticity [46]. Recent studies utilizing transgenic mouse models with combinant Pten/TRP53/Rb1 loss have suggested that Rb1 suppresses metastatic dissemination of prostate adenocarcinoma initiated by Pten loss and Trp53 mutation cooperates with Rb1 loss to confer a castration resistant phenotype [48]. However, there are still limitations in their application to human prostate cancer research due to their non-human origin. To conduct more in-depth studies on the efficacy of immunotherapy in bone metastatic prostate cancer, animal models utilizing human prostate cancer cells that mimic the metastatic cascade and form osteoblastic bone lesions while maintaining immune capacity are needed. Researches regarding immunodeficient mice with humanized immune systems are under development and may help to alleviate the hardships of studying immunotherapy with animal models [32].

Positive outcomes of preclinical drug screening via animal models are not always reproducible in clinical trials. Differences in the immune system of humans and animals have been reported to be a significant hurdle in translating outcomes [28,84]. Currently, many studies are trying to engineer the mouse immune system to resemble that of humans and use it as a model for preclinical drug testing [29]. In the future, immunodeficient animal models engineered to resemble the human immune system will allow further investigation into the immunobiology of prostate cancer and more accurately predict drug efficacy in clinical trials. Currently, a good alternative will be to use a transgenic mouse model that spontaneously progresses to bone metastasis. It provides a good overall representation of the metastatic process and enables the study of prostate cancer immunobiology.

## CONCLUSIONS

Among the currently available animal models of prostate cancer, no single model can fully reproduce the whole process of bone metastasis. Therefore, careful consideration into the strengths and weaknesses of each animal model should be taken prior to selecting an adequate model for pursuing research objectives. In addition, research into the immunobiology of prostate cancer using animal models are limited to using non-human cell lines or transgenic mice

because models that mimic the human immune system are technically difficult to obtain and not common place. However, transgenic mouse models are known to be relatively easy to maintain after the initial model formation. However, bone metastases occurring in transgenic mice are known to only develop neuroendocrine differentiated cells.

Cell lines are commonly used to create animal models of prostate cancer due to its ease in accessibility and maintenance. However, cell lines are homogenous and do not fully represent the complexity and heterogeneity of tumors observed in patients. In contrast, PDX models reflect tumor heterogeneity but are not easily accessible and must be cultured *in vivo* for maintenance. Nevertheless, the PDX model is an indispensable tool for prostate cancer research. Ultimately, a bone metastatic prostate cancer animal model with a fully intact or humanized immune system with preservation of tumor heterogeneity will yield a more accurate preclinical drug screening result and a more predictable outcome of clinical trials.

## CONFLICTS OF INTEREST

The authors have nothing to disclose.

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## AUTHORS' CONTRIBUTIONS

Research conception and design: Jong Hyun Tae. Data acquisition: Jong Hyun Tae. Drafting of the manuscript: Jong Hyun Tae. Critical revision of the manuscript: Jong Hyun Tae. Obtaining funding: In Ho Chang. Supervision: In Ho Chang. Approval of the final manuscript: all authors.

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