Animal Models for Prostate Cancer Research: A Mechanistic Outlook on the Challenges and Recent Progress

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Mayukh Chatterjee, Pramit Kumar Ghosh, and Amlan Ghosh

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Abstract

Molecular pathogenesis of prostate cancer (PCa) still remains poorly understood in human. Difficulties in understanding of prostate oncogenesis and lack of efficient noninvasive therapeutic measures are primary obstacles in PCa management. Among animal models of human prostate cancer, rat, mice, and canine models are mostly used. However, genetic differences of these model animals and anatomical as well as histological differences of their prostate with respect to human make the PCa research quite tricky. Also, stages of disease development from benign to malignant and the progression of malignant tumors to more

Mayukh Chatterjee and Pramit Kumar Ghosh contributed equally to this work.

M. Chatterjee · P. K. Ghosh · A. Ghosh (✉)
Genetics of Non-communicable Diseases, Department of Life Sciences, Presidency University, Kolkata, India
e-mail: amlan.dbs@presiuniv.ac.in

S. Pathak et al. (eds.), *Handbook of Animal Models and its Uses in Cancer Research*,
https://doi.org/10.1007/978-981-19-3824-5_20

aggressive forms in these animal models often vary substantially from those seen in human. Therefore, animal models of prostate cancer must be selected carefully with respect to the specific research purpose. In this context, present study aimed to summarize a thorough idea of purpose and efficacy of different animal models suitable for different aspects of prostate cancer research from the published literature. The information may serve as a reference in further research for better understanding of the disease pathogenesis and developing novel as well as effective therapeutic approaches.

Keywords

Prostate cancer · Challenges · Animal models · Oncogenesis · Castration resistance · Metastasis · Pathways

Introduction

Prostate cancer (PCa) is a malignancy of aged male, associated with higher mortality rate primarily because of insufficient knowledge on disease pathogenesis and lack of effective management (Dasgupta et al. 2012). Conventional treatment modalities of prostate cancer include surgery, chemo-radiation, and androgen deprivation that have limited efficiency for more aggressive and advanced disease stages (Litwin and Tan 2017). To understand molecular events associated with disease pathogenesis and progression, research works involving animal models of human prostate cancer are conducted, and for this purpose, rat, mice, and dog are mostly used (Russell and Voeks 2003). However, unlike other cancers, prostate cancer research comes with a set of problems, most notable of which are the heterogeneity in its oncogenesis and lack of an all-purpose animal model (Lamb and Zhang 2005). This leads to a fragmented research approach in PCa, where the utility of a given animal model gets restricted to a very limited number of study purposes. On the aforesaid background, present review work aimed to shed light on the current scenario of animal model-based PCa research and discuss how different existing animal models can be used in different aspects of the study to increase the efficiency of the research outcome (Hayashi et al. 1991).

Animal Models of Prostate Cancer; the Challenges

The aim of using animal models in PCa research is to replicate the steps of disease development in animal for understanding details of the disease pathogenesis and develop novel targeted therapeutics. Rodents are the conventional pick for PCa research due to their small size, ease of handling, and maintenance. Additionally, rodent genome can also be manipulated efficiently for experimental purposes. Apart from rodents, Canines, mainly dogs also serve as animal model for PCa research, although their maintenance cost is high and genetic modification is very difficult.

Table 1 Comparison of prostate characteristics between human male and animal models

Characteristics	Human	Mouse	Rat	Dog
Prostate structure				
Anatomy	Single gland five lobes	Four distinct lobes	Four distinct lobes	Bilobed
Functional differentiation	Heterogeneous	Partly heterogeneous	Partly heterogeneous	Homogenous
PCa development and progression				
Incidence of spontaneous PCa	Occurs naturally	Occurs rarely	Occurs in a few strains	Occurs naturally
Resistance to castration	In advanced stages	In advanced stages	In advanced stages	In early stages
Bone metastasis	Frequent	Rare	Rare	Frequent

The usage of canine animal model is restricted for studying mainly bone metastasis, an aggressive phenotype frequently seen in human PCa (Simmons et al. 2014b). However, using animal models for PCa research itself has problems, which makes the management of animal model-based study quite convoluted.

Human prostate differs from that of rodent and canine anatomically and histologically in several aspects (Table 1). Rodents have four anatomically different prostate lobes while canines have two that are homogenous with respect to cellular differentiation. On the contrary, human prostate gland can be subdivided into five lobes (anterior, posterior, right lateral, left lateral, and median) and functionally differentiated into three distinct zones (peripheral, transitional, and central). Rodents have compound ductules in prostate and in dog ducts are branched into alveolar glands, whereas characteristic acinus is present in human prostate (Lamb and Zhang 2005). Discontinuous basal layer of prostate in dogs and rats is a norm while in human, the criteria can rather be an indicator of PCa (Hayashi et al. 1991; Hameed and Humphrey 2005).

In dogs, both benign and malignant prostatic lesions develop spontaneously and more likely in aged animals similar to human (Waters et al. 1997). Whereas, although a few rat strains can develop spontaneous PCa (Nascimento-Gonçalves et al. 2018), the phenomenon seems to be a rare one in mice. In rodent, malignant tumor of prostate progresses to adenocarcinoma, sarcoma, and neuroendocrine carcinoma while it mostly progresses to adenocarcinoma in human (Grabowska et al. 2014). Similar to humans, rodent PCa may acquire androgen independence in advanced stages. On the other hand, canine PCa show pathological and molecular criteria similar to androgen-independent subtype from very early stage of tumor development (LeRoy and Northrup 2009). Rodent PCa does not naturally metastasize to bones, but the phenomenon is frequent in canine PCa and is very similar to that of human (Cornell et al. 2000).

Testing the efficacy of novel therapeutics for PCa on animal models is also challenging. Rodents are smaller in size, and their life span is shorter than humans; hence in preclinical trial with rodents, the data on effective dosage, toxicity measurements, and retentions of a given therapeutic is expected to differ in humans and

therefore needs extrapolation during clinical trial. Canines might be a better research model on this background, although here, the latent period of PCa is relatively longer than human (Ryman-Tubb et al. 2022).

Purposefulness of Animals Models in Prostate Cancer Research

Several approaches are followed to create different types of animal models suitable for achieving different specific purposes of human PCa research.

Animal Models to Understand the Stages of PCa Development

To understand the pathogenesis of human PCa, it is essential to study all developmental stages of the disease namely benign prostatic hyperplasia (BPH), prostatic intraepithelial neoplasia (PIN), and prostate adenocarcinoma as well as its more aggressive subtypes separately. In human, availability of tissue samples from all the developmental stages seems to be limited indicating the emergence of animal model in PCa research.

Benign Prostatic Hyperplasia

Benign prostatic hyperplasia (BPH) is the enlargement of prostate gland due to increase in both cell number and size. Studying animal models of BPH will provide information about the key changes of mainly prostatic epithelial cells leading to their uncontrolled proliferation and hyperplastic growth of the organ as a whole. Existing animal models generated through genetic manipulation for studying exclusively BPH are listed below.

p27 Knockout mice model: This model was generated by targeted knockout of p27 through vector-mediated standard protocol at the embryonic stage of C57BL/6 mice. Histological findings showed incidence of prostatic hyperplasia in acinar epithelial cells of 14-month-old mice of this model, and this is also comparable to human BPH (Cordon-Cardo et al. 1998).

ERβ knockout mice model: Mice model lacking oestrogen receptor β (ERβ) was generated by knocking out the *ESR2* gene through inserting a neomycin resistance genetic element into exon 3 of the coding region in embryonic stem cells. Aged male mice showed hyperplastic growth in prostate and bladder. In prostate of ERβ (-/-) mice, increased AR level was seen and the tissue contained multiple hyperplastic foci (Krege et al. 1998; Abdulkadir and Kim 2005).

Mice model of prolactin deregulation: In this model, a construct of rat prolactin (PRL) gene was cloned under the metallothionein-1 (Mt-1) promoter of MtbGH 2016 plasmid and was microinjected into C57BL/6JxCBA-f2 mice embryos to overexpress prolactin. Although prolactin overexpression is neither prostate specific nor aggressive, nonetheless it causes enlargement of prostate gland in the model animal comparable to the BPH lesions of human (Wennbo et al. 1997).

Mice model of Int2 and kgf deregulation: In this model, mouse mammary tumor virus (MMTV)-regulatory sequence from pMMTV-neu-NT plasmid was inserted between the SV 40 promoter and murine Fibroblast growth factor 3 (int2) gene cloned in a pKC3–9 plasmid. Following the same approach, keratinocyte growth factor (kgf) was inserted under the MMTV regulator sequence. In either case, the recombinant plasmid (pKC3-9) was microinjected into female pronuclei of FVB mouse strain following implantation in the foster mother. In the transgenic animals, overexpression int2 and kgf resulted in the development of benign prostatic hyperplasia (Muller et al. 1990; Kitsberg and Leder 1996).

Prostatic Intraepithelial Neoplasia

Prostatic intraepithelial neoplasia (PIN) is the first sign of prostate malignancy, and here the neoplastic growth is restricted to epithelial cells within the ducts or acini of preexisting benign prostatic lesion. An understanding of molecular events associated with the development of PIN will be helpful in identifying the driver steps of PCa oncogenesis.

TGFBR2knock out model: In this model, the gene-encoding TGFβ receptor 2 (TGFBR2) is inactivated in mice fibroblasts by crossing $Tgfbr2^{floxE2/floxE2}$mice with mice having fibroblast-specific protein 1 (FSP1) gene under a cre-recombinase system. The resulting transgenic mice were nonresponsive to TGFβ stimulation and neoplastic lesions comparable to the PIN developed in their anterior and dorsolateral prostate lobe at the age of 5–7 weeks. This model may be useful for studying PIN and understanding the role of TGFβ as a tumor suppressor in PCa development (Bhowmick et al. 2004).

Rb knockout model: Mice homozygous for lox introduced retinoblastoma (Rb) gene were crossed with heterozygous mice for cre-recombinase gene cloned under the rat probasin (PB) promoter (−426/+28 region). The filial generation was then inter-crossed to obtain the concerned model in which Rb gene was deleted selectively in prostate gland under the influence of PB promoter resulting in development of PIN lesions. In this model, development of prostate adenocarcinoma was not found probably because of redundant function of other Rb family members such as p130 that might reverse the effect of Rb deletion (Maddison et al. 2004; Abdulkadir and Kim 2005).

ERG/ETV1 deregulated model: In this model, a TMPRSS2-ERG fusion construct was produced by inserting noncoding exon-1 of transmembrane protease serine 2 (*TMPRSS2*) gene adjacent to the exon-2 of ETS-related gene (ERG). The resulted shortened ERG product was then put under the control of a modified PB (ARR_2PB) promoter through recombination events. Finally, the construct was microinjected into fertilized egg pronuclei and following their implantation into FVB surrogate mice, consecutive crossings were performed to establish the model. Total 12–14-weeks-old mice acquired PIN in their ventral lobe of prostate (Tomlins et al. 2008).

Akt deregulated model: The coding sequence of Akt1 along with a myristoylation sequence and hemagglutinin epitope sequence from pCDNA3 plasmid was cloned into pBSK plasmid under rat PB promoter. This construct was

linearized and microinjected into fertilized oocytes of FVB mice. The resulted transgenic model showed constitutive expression of Akt in prostate and developed PIN lesions. This model is also known as murine prostate AKT (MPAKT) model (Abdulkadir and Kim 2005; Cunningham and You 2015).

High-Grade Prostatic Intraepithelial Neoplasia

High grade PIN (HGPIN) is considered as the precursor stage of prostate adenocarcinoma and characterized by uncontrolled proliferation along with cytological abnormalities of glandular epithelial cells restricted to prostatic duct and acini. Studying HGPIN pathogenesis seems to be essential to understand the molecular events associated with gradual progression of PIN lesions toward the invasive adenocarcinoma.

NKX3.1$^{-/-}$; PTEN$^{+/-}$ knockout model: An inducible (conditional) double knockout model for NKX3.1$^{-/-}$ and PTEN$^{+/-}$ was generated by crossing PSA^{Cre}NKX3.1 with PSA^{Cre} PTEN conditional knockout mice. The resulted double knockout animals developed HGPIN lesions at a higher frequency which was shown to be 60% by 26 weeks and about 100% by 52 weeks in comparison to PTEN$^{+/-}$ knock-out models where lesion incidence was only 25% by 52 weeks (Valkenburg and Williams 2011).

Conditional PTEN knockout model: PTEN was conditionally knocked out in mouse prostate by crossing PTEN$^{loxP/loxP}$ mice with MMTV-cre mice. The transgenic model developed HGPIN (2 weeks) lesions with atypic papillary proliferation of epithelial cells (Backman et al. 2004).

Prostate Adenocarcinoma

Prostate adenocarcinoma is the final stage of prostate oncogenesis characterized by the malignant tumor. Studying prostate adenocarcinoma will be helpful to understand the key molecular events associated with invasion, migration, and other aggressive phenotypes of malignant cells.

Animal model by carcinogen/hormone treatment: Rats are commonly used to generate carcinogen-/hormone-treated animal models of prostate cancer. Subcutaneous application of carcinogen 3,2'-dimethyl-4-aminobiphenyl (DMAB) in F344 rat and intraperitoneal/ intravenous injection of N-methyl N-nitrosourea (MNU) in Sprague Dawley and Lobund-wistar rats alone or in combination with testosterone in silastic transplant was found to induce prostate adenocarcinoma. In WU rats, DMAB treatment followed by cyproterone acetate and testosterone propionate consecutively can induce mild adenocarcinoma in prostate lobes of dorsolateral and anterior regions. In various inbred rat strains, only hormonal treatment with testosterone orestradiol-17β can also induce prostate adenocarcinoma and thus providing an ideal model for studying the role of hormonal (gonadal) dysregulation in carcinogenesis of prostate (Bosland 1992; Nascimento-Gonçalves et al. 2018).

Transgenic animal model: Mice are generally used to generate this transgenic model in which ERG/Bmi gene is overexpressed or Gata3 gene is deleted under the background of conditional PTEN knockout resulting development of prostate adenocarcinoma. For overexpression of ERG gene, mice model carrying TMPRSS-

ERG fusion construct is used and crossed with $PTEN^{+/-}$mice; the resulted transgenic animal developed aggressive prostate adenocarcinoma within 26 weeks (Carver et al. 2009). Bmi1 is a component of polycomb-repressive complex 1 (PRC1) frequently upregulated in prostate cancer of human (Zhu et al. 2018). By crossing Bmi^{LSL} mouse having a Lox-STOP-Lox (LSL) sequence with PB-Cre4 strain, overexpression of Bmi1 allele is effectuated in the prostate gland of resultant transgenic model. Upregulation of Bmi1 under background of $PTEN^{+/-}$ haploinsufficiency resulted in development of invasive prostate adenocarcinoma (Nacerddine et al. 2012). GATA3 is a transcription factor frequently inactivated in human prostate cancer and the phenomenon under condition of PTEN deficiency favors tumor progression. Transgenic animal model carrying double PTEN; GATA3-mutant [$PTEN^{-/-}$; $GATA3^{-/-}$] was generated by crossing PB-Cre4; $PTEN^{flox}$ mice with $GATA3^{flox}$ one, and the model was shown to develop invasive prostate adenocarcinoma within shorter time in comparison to $PTEN^{-/-}$ one (Nguyen et al. 2013) (Fig. 1).

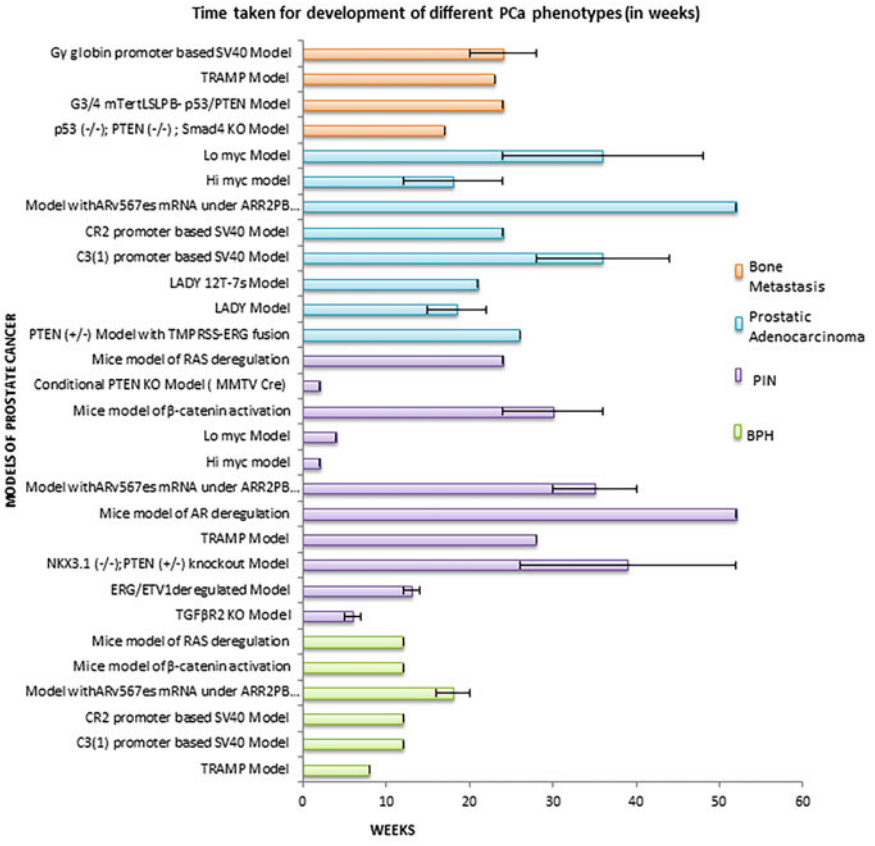

Fig. 1 Time taken for mice models to develop different phenotypes of PCa

Animal Models for Studying Aggressive Phenotypes of Prostate Cancer

In several instances, PCa shows aggressive phenotypes like androgen-independent progression and metastasis to distant places. Prostate cancer develops androgen independence after getting exposed to androgen deprivation therapy and then considered under the castration resistance category which is more aggressive in nature and associated with poor patient outcome. Among incidence of distant metastasis, bone is considered as the most affected organ in prostate cancer of human and the phenomenon was found to affect the disease outcome. Therefore, these aggressive disease phenotypes must be studied in detail to design effective therapeutic measures with an aim for better disease management.

Development of Castration-Resistance of Prostate Cancer

LNCaP/Neo xenograft model: LNCaP cell line derived from prostate adenocarcinoma of a 50-year-old Caucasian male metastasized to the left supraclavicular lymph node was infected with pLNSXNeo retrovirus overexpressing Neu oncogene. Resulting LNCaP/Neu cells were injected subcutaneously in castrated SCID mice (male) resulting in development of prostate tumor even in absence of androgens, which thus resembled the properties of CRPC in human. Additionally, the latent period for the development of this xenograft tumor was shortened and the level of PSA was also increased. This model can be useful for understanding the role of Her2/Neu-mediated signaling in the pathogenesis of CRPC (Craft et al. 1999).

Metastasis

Transgenic Models (Mice)

p53; Rb double knock out model: PB-cre4 male mice (C57BL/6xDBA2 background) were crossed with $p53^{loxP/loxP}Rb^{loxP/loxP}$ female mice (FVB/N;129 background), and the transgenic mice model of desired genotype was generated in the F3 generation. In the transgenic animals, PCa metastasized to lymph nodes, muscle, and blood vessels as well as to several other organs and tissues. This model provides information about potential sites where PCa can metastasize; however, the model is not appropriate for studying skeletal metastasis, a phenomenon more frequently found in humans (Zhou et al. 2006).

Smad4 knockout model on the background of $p53^{-/-}$; $PTEN^{-/-}$ genotype: Smad4 is a tumor-suppressor gene of TGFβ pathway showing frequent promoter methylation and concordant reduced expression in prostate cancer (Aitchison et al. 2008). The model was generated by crossing a $p53^{-/-}$; $PTEN^{-/-}$ mice with a Smad4-conditional knockout strain to test the effect of Smad4 deletion in prostate on the background of $p53^{-/-}$; $PTEN^{-/-}$ genotype. Total 12.5% of the resulted animals showed bone metastasis of prostate tumor with a very short period of survival (17 weeks) (Ding et al. 2012).

G3/4 $mTert^{LSL}$ PB- p53/PTEN: TERT gene encodes telomerase, aberrant activation of which was reported in malignancy of different organs including prostate

(Graham and Meeker 2017). In transgenic animal model, aberrant activation of TERT gene was achieved in prostate and the model was generated by crossing p53/PTEN conditional knockout mice under rat PB-promoter with $mTert^{LSL}$; Cre mice continued up to 3rd/fourth generation. Among 25% of the model animals at the age of 24 weeks, PCa metastasized to lumbar spines, although the type of metastatic phenomena with respect to the effect on target sites (osteoblastic or osteolytic) was not determined (Ding et al. 2012).

Xenograft model: In this model, prostate cancer cell lines are transplanted to the host animal and allowed to grow into solid tumor. However, xenograft tumors as derived from a single malignant cell type are more or less homogenous in nature and therefore may not be representative to the primary tumor with respect to tumor heterogeneity and associated microenvironment.

Xenograft Models of Rat Origin

MATLyLu cell line: This PCa cell line, known as Metastatic Anaplastic Tumor Metastasizing to Lymph node and Lungs (MATLyLu), was derived from a poorly differentiated androgen-insensitive prostate carcinoma of a 22-month-old inbred Copenhagen male rat. After introducing this cell line into Copenhagen rats by intravenous (tail) and intracardiac injection, osteoclastic bone metastasis of this xenograft tumor was noticed (Simmons et al. 2014b).

PA-III cell line: The PA-III androgen-insensitive cell line was derived from spontaneous prostate carcinoma of a Lobund-Wistar rat. When this cell line is implanted over the calvaria/scapula of rodents after periosteal disruption, mixed osteoblastic/osteolytic metastatic lesions are formed in bones (Koutsilieris 1992).

Xenograft Models Canine Origin

DPC-1 cell line: This cell line was developed from poorly differentiated prostate adenocarcinoma of an 11-year-old Doberman Pinscher. DPC-1 is used to develop xenograft tumor following its orthotropic seeding in the prostate tissue of immunosuppressed dogs, and the incidence of mixed osteoblastic/osteolytic metastasis is frequently resulted in the pelvic bones (Simmons et al. 2014b; Chevalier et al. 2015).

Leo cell line: This cell line established from primary prostate carcinoma of a 5-year-old mixed breed dog can metastasize to the brain, spinal cord, and long bones (characterized by loss of cortical and trabecular bone) in nude mice after intracardiac injection. This is an almost exclusive model for studying brain metastasis of PCa (Thudi et al. 2011a; Simmons et al. 2014b).

Probasco cell line: The Probasco cell was developed from the primary prostate carcinoma of a 10.5-year-old mixed-breed dog that underwent castration and then received palliative radiation therapy and metronomic chemotherapy (piroxicam, cyclophosphamide, toceranib phosphate, and chlorambucil) afterward as treatment measures. After intracardiac injection in nude mice, these cells primarily metastasize to the appendicular skeleton leading to the development of metastatic osteoblastic tumors. Probasco cells transfected with parathyroid hormone-related protein were

found to result increase growth of the metastatic tumor with higher extent of osteolysis (Simmons et al. 2014a, b).

Multipurpose Animal Models to Study Prostate Cancer

Transgenic adenocarcinoma of mouse prostate (TRAMP) model: The Simian Virus 40 T/t antigen (SV40 Tag) from pSV plasmid was cloned to pBSK plasmid under the minimal rat PB promoter (-426/+28 region) and the recombinant plasmid was microinjected into pronuclei of fertilized mice oocytes. Among the transgenic mice, the strain showed higher SV40 Tag expression in dorsal and ventral lobes of prostate was designated as TRAMP. Mice from this model developed epithelial hyperplasia at eighth week of age that progressed to PIN at 28th week, and all of the lesions progressed to lymphatic metastasis among which 66% displayed pulmonary metastasis. Skeletal metastasis has also been found at around 23rd week of age. Castration of 12-week-old TRAMP mice resulted in development of androgen independence of the PCa showing more aggressive hyperplasia as well as metastatic potential than that of noncastrated mice (Abdulkadir and Kim 2005; Grabowska et al. 2014). This model is also useful in preclinical trials of several targeted and chemopreventive therapeutics (Abdulkadir and Kim 2005). TRAMP-derived tumor cells can also be introduced in immunocompromised and syngeneic mice for serving specific purposes, i.e., subcutaneous injection of TRAMP-C2 castration-resistant PCa cells in RAG mice is useful for assessing antitumor activity of TGF-β-insensitive $CD8^{+}$T cells (Zhang et al. 2006).

LADY models: LADY models are generally constructed by ligating SV 40 small tag deletion mutant under long PB promoter (-11,500/+28 region) followed by microinjection of the construct into the pronuclei of fertilized mice oocyte. The resulted transgenic mice developed prostate adenocarcinoma in 15–22 weeks with no instances of metastasis. The LADY 12T-7s (slow progressing PCa) model was crossed with PB-hepsin mice to express SV40 T antigen and the hepsin transgene specifically in the prostate of the next generation animals. As a result, by the age of 21st week, male mice developed invasive adenocarcinoma followed by distant metastasis to liver, lymph nodes, and bones. The 12T-7f/MT-DNIIR is another LADY model important for studying metastasis. LADY model is also used to study therapeutic potential of antioxidants like vitamin E, lycopene, and selenium for PCa management (Abdulkadir and Kim 2005; Grabowska et al. 2014).

Other SV40 models: Several mice models of PCa have been developed by overexpressing SV40 T/t antigen (Tag/tag) under promoters other than PB to study the stepwise process of development and aggressive phenotypes of the disease.

In C3(1) promoter-regulated model, the steps of disease development from low-grade PIN to invasive adenocarcinoma are well-characterized and consistent. Male C3(1)-Tag mice can develop BPH by 3 months and prostate adenocarcinoma by 7–11 months which metastasized to lungs occasionally (Yoshidome et al. 1998).

Intestinal epithelial cells (Paneth cells) produce antimicrobial peptides known as cryptdins. In absence of androgen, transgenic male mice expressing SV40 T antigen

in neuroendocrine cells of prostate under cryptdin-2 (CR2) promoter developed PIN by the age of 12th week. The lesion proceeded to locally invasive PCa by the age of 24 weeks followed by its further progression to the distant metastasis (Garabedian et al. 1998).

Transgenic mice overexpressing SV40 Tag and tag under embryonic Gγ globin promoter showed high incidence of neuroendocrine and epithelial prostate tumors with very short latency. The tumors developed androgen-independence at fourth to sixth week and later metastasized to kidneys, adrenal glands, and lymph nodes, also occasionally micrometastasized to thymus, lung, and bone at about fifth to seventh months (Perez-Stable et al. 1997).

Mouse Prostate Reconstitution (MPR) model: For development of prostate reconstitution model, fetal urogenital sinus tissue from a p53 knock out background was microdissected followed by enzymatic dissociation into mesenchyme and epithelium; next mesenchymal or epithelial or both cell types were subjected to retroviral transduction of RAS and MYC oncogenes, and subsequently grafted to renal capsule of immuno-compromised mice. This model showed 100% incidence of prostate cancer in both p53 homozygous and heterozygous backgrounds with very high frequency of distant metastatic deposit in lung, lymph node, bone, and liver (Buttyan 1997; Simmons et al. 2014b).

Mice model of AR deregulation: Androgen receptor (AR) is a marker as well as a primary determinant for the development of differentiated luminal epithelium in prostate. Osr1 promoter-mediated overexpression of human AR in mouse prostate leads to the development of PIN lesion in 50% and adenocarcinoma in 5% of animals (mice) by 52 weeks (Zhu et al. 2011). In another study, transgenic male mice overexpressing murine AR under rat PB promoter developed variety of prostatic lesions including BPH, PIN, and microinvasive HGPIN. Additionally, castrated transgenic mice overexpressing ARv567es mRNA (a transcript variant of AR lacking exons 5,6,7) under ARR_2PB promoter developed BPH in 16–20 weeks that progressed to PIN in 30–40 weeks and adenocarcinoma in 52 weeks (Liu et al. 2013). This animal model, as showed development of malignant prostate tumor in absence of endogenous androgen, can be considered as a novel model for studying CRPC.

Mice model of Myc deregulation: In this approach, myc-Pal (prostate antigen1) constructs were cloned under two types of PB promoter, and the constructs were then inserted into fertilized eggs of FVB mice followed by implantation into surrogate mother for establishment of transgenic animal models namely Hi-myc and lo-myc (Ellwood-Yen et al. 2003). In Hi myc model, where the *MYC* gene was put under ARR_2PB promoter, PIN developed at 2 weeks and progressed to adenocarcinoma at about 3–6 months. In the lo-myc mice model where the *MYC* gene was put under minimal rat PB promoter, PIN developed at 4 weeks of age and adenocarcinoma at about 6–12 months. In the Hi-Myc model, it has been found that increasing expression of myc and decreasing expression of NKX3.1 mark the transition from PIN to invasive adenocarcinoma. Additionally in this model, constitutive activation of the NF-κB pathway renders the adenocarcinoma castration resistant (Ellwood-Yen et al. 2003). Furthermore, transgenic mice overexpressing Z-Myc under PTEN and p53

knockout background [Z-Myc; PB-Cre4; PTEN$^{(-/+)/(-/-)}$; p53$^{(-/+)/(-/-)}$] was also generated and the animals developed very aggressive adenocarcinoma with very high incidence of lymph node metastasis (Valkenburg and Williams 2011; Grabowska et al. 2014).

Mice model of RAS deregulation: Rat sarcoma virus (RAS) is an important oncogene which is significantly activated/ overexpressed in various types of cancer. Although, in transgenic mice models where H-RAS-mutant G12V is expressed under rat PB promoter, malignancy of prostatic cells only progressed to PIN lesions (Valkenburg and Williams 2011). Another model has been established by crossing PB-cre mice with K-RAS$^{+/V12}$ mice which developed atypic hyperplasia at 3 months which progressed to low-grade PIN in 6 months. These models indicate that aberrant activation/expression of RAS may be important for occurrence of primary noninvasive lesions, but it does not seem to be sufficient for progression of the disease to the higher grades of PCa (Pearson et al. 2009).

Mice model of β-catenin activation: In this model, transgenic animal was developed by crossing between PB-cre4 mice with Catnb$^{flox(ex3)}$ mice, expressing truncated β-catenin (exon3 deleted) insensitive to proteasomal degradation specifically in the prostate epithelial cells. The transgenic animals developed BPH in 12 weeks that progressed to PIN at 6 months and finally to HGPIN at about 9 months. Following 2 weeks of castration, the prostate gland in the transgenic mice continued to grow indicating acquisition of androgen independence (Yu et al. 2009) (Tables 2 and 3).

Key Signaling Pathways Associated with Molecular Pathogenesis of Prostate Cancer

For studying the molecular pathogenesis of prostate cancer, transgenic animal models are frequently used. In transgenic models, organ-specific knockout or ectopic expression of selected tumor suppressor gene(s) or oncogene(s) was achieved to deregulate the associated signaling pathways that can be identified and characterized to explore the molecular pathogenesis associated with different disease parameters. Signaling pathways, deregulation of which was mostly studied in animal model of prostate carcinogenesis, are summarized from the available literature. The pathways found to be deregulated in animal models of PCa are portrayed in Fig. 2.

PI3K/Akt/mTOR Pathway

This pathway was found to exert an oncogenic effect in the pathogenesis of prostate cancer. Phosphatidylinositol 3 kinase (PI3K) is a membrane-associated enzyme which phosphorylates phosphatidylinositol-4,5-diphosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3) that activates Akt. Akt activates mTORC1 which phosphorylates its downstream targets like 4EBP1 and P70S6 Kinase (S6K) that in turn induce synthesis of proteins responsible for cell proliferation. PTEN is a well-

Table 2 Uses of animal models in PCa research

Purpose	Mouse	Rat	Dog
Carcinogenesis and oncogenesis	p27 knockout (Cordon-Cardo et al. 1998) TRAMP (Abdulkadir and Kim 2005; Grabowska et al. 2014) *TGFBR2* knockout (Bhowmick et al. 2004) MPAKT (Abdulkadir and Kim 2005) AR upregulation (Zhu et al. 2011) Myc upregulation (Ellwood-Yen et al. 2003) Rb knockout (Maddison et al. 2004) NKX3.1; PTEN knockout (Valkenburg and Williams 2011) RAS upregulation (Pearson et al. 2009) β-catenin activation (Yu et al. 2009)	Lobund-Wistar (Bosland 1992; Nascimento-Gonçalves et al. 2018) Sprague Dawley (Bosland 1992; Nascimento-Gonçalves et al. 2018) F344 (Bosland 1992; Nascimento-Gonçalves et al. 2018) WU (Bosland 1992; Nascimento-Gonçalves et al. 2018)	
CRPC	SCID (Craft et al. 1999) AR upregulation (Zhu et al. 2011; Liu et al. 2013) Myc upregulation (Ellwood-Yen et al. 2003) SV40/Gγ globin (Perez-Stable et al. 1997)		
Bone metastasis	TRAMP (Abdulkadir and Kim 2005; Simmons et al. 2014b) p53; Rb knockout (Zhou et al. 2006) Smad4; PTEN; p53knock out (Ding et al. 2012) MPR (Buttyan 1997; Abdulkadir and Kim 2005)	MATLyLu (Simmons et al. 2014b) PA-III (Koutsilieris 1992)	DPC-1 (Chevalier et al. 2015) Leo (Thudi et al. 2011a) Probasco (Simmons et al. 2014a)
Therapeutics	TRAMP (Abdulkadir and Kim 2005) LADY (Abdulkadir and Kim 2005; Grabowska et al. 2014) RAG (Zhang et al. 2006)		

Table 3 Modulated genes in animal models of PCa

Promoter	Induced gene	Model for BPH	Model for PIN	Model for adenocarcinoma	Model for metastasis	Model for CRPC	Reference
Metallothionein-1 (Mt-1)	PRL	✓	–	–	–	–	(Wennbo et al. 1997)
Probasin	Murine AR	✓	✓	–	–	–	(Zhu et al. 2011)
	RAS	✓	✓	–	–	–	(Valkenburg and Williams 2011)
ARR_2PB	MYC	✓	✓	–	–	–	(Ellwood-Yen et al. 2003)
	ERG	✓	✓	–	–	–	(Tomlins et al. 2008)
C3(1)	SV40 T-antigen	✓	✓	✓	–	–	(Yoshidome et al. 1998)
MMTV	Int2	✓	✓	–	–	–	(Muller et al. 1990)
	Kgf	✓	✓	–	–	–	(Kitsberg and Leder 1996)
Cryptdin-2	SV40 T-antigen	✓	✓	✓	✓	–	(Garabedian et al. 1998)
Gγ globin	SV40 T-antigen	✓	✓	✓	✓	✓	(Perez-Stable et al. 1997)
Long PB	SV40 large T-antigen	✓	✓	✓	✓	–	(Grabowska et al. 2014)

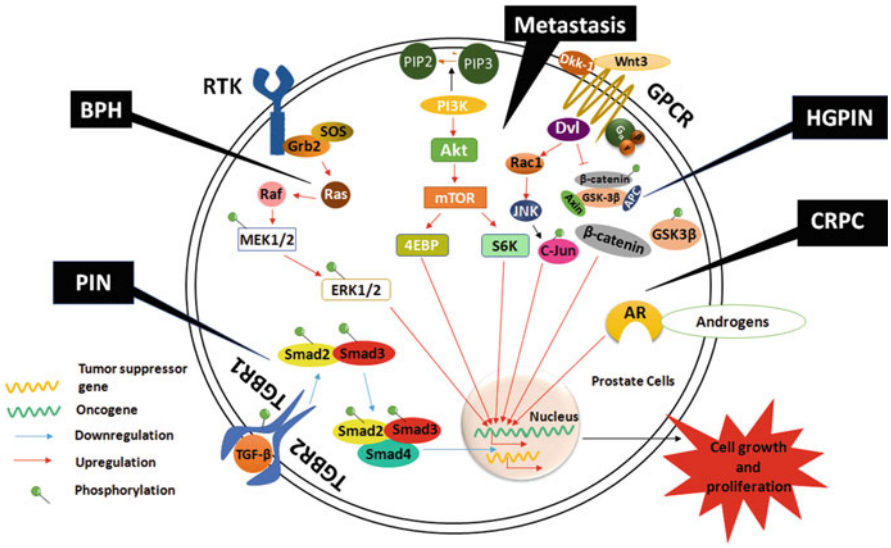

Fig. 2 Signaling pathways associated with animal models of PCa

known tumor suppressor which acts antagonistically to PI3K/Akt/mTOR pathway by de-phosphorylating PIP3 into PIP2. Conditional PTEN knockout in the prostate of aged mice displayed significant activation of PI3K/AKT/mTOR/S6K and PI3K/AKT/mTOR/4EBP1 axes leading to the development of neoplastic growth in the organ concern (Ma et al. 2005). This confirms that PI3K/Akt/mTOR pathway is crucial for cell growth and proliferation during prostate carcinogenesis. Additionally, in MPAKT mice model, activation of mTOR and S6K through ectopic Akt expression also led to the development of PIN, and treatment with the mTOR inhibitor RAD001 was found to cause reversal of the neoplastic phenotype through increase of apoptosis in epithelial cells (Majumder et al. 2003).

RTK/ERK Pathway

This pathway is involved in transducing signals mostly from extracellular growth factors and hence associated with cell growth and proliferation accordingly. Deregulation of RTK/ERK pathway involving aberrant activation of several components is frequently reported in malignancy of several organs including prostate. In RTK/ERK pathway, activation of RTKs (EGFR, FGFR, etc.) brings son of sevenless (SOS) protein (Guanine Exchange Factor or GEF) to RAS via adaptor protein Grb2 leading to its (RAS) activation through exchange of bound GDP by GTP. Activated RAS then binds to and activates Raf which initiates a cascade of phosphorylation reaction in the downstream leading to activation of mitogen-activated protein kinase (MEK) 1/2 first and then extracellular signal-related kinase (ERK)1/2. ERK1/2

phosphorylates and thereby activates transcription factors of several candidate genes actively associated with cell growth, proliferation, and migration (Chen et al. 2017). Transgenic mice with only RAS activation progressed to only PIN in male mice (Valkenburg and Williams 2011). Deregulation of RTK/ERK pathway through K-RAS upregulation in the background of PTEN knockout is frequently used to develop transgenic mice model for studying BPH, PIN lesions, and aggressive phenotypes of PCa (Mulholland et al. 2012).

TGFβ Pathway

Transforming growth factor-beta (TGF-β) pathway involves in the regulation of diverse cellular functions like proliferation, differentiation, motility, and apoptosis. Upon binding of the ligand (TGF-β), TGF-β receptor 2 (TGFBR2) recruits TGF-β receptor 1 (TGFBR1) which gets subsequently phosphorylated and in turn phosphorylates Smad2/3 that form a complex with Smad4; the Smad complex is then translocated into the nucleus leading to transcriptional upregulation of downstream target genes-regulating cell proliferation. Smad 6/7 act as inhibitors in this pathway through interfering the TGFBR-mediated activation of Smad2/3. Tumor-suppressive role TGF-β pathway in carcinogenesis of prostate has been documented in several studies (Tu et al. 2003). Downregulation of TGF-β pathway is achieved through partial knockout of *TGFBR2* intransgenic mice models leading to the development of PIN lesions and stromal inflammation (Bhowmick et al. 2004).

Wnt Pathway

Wnt signaling includes both canonical (Wnt/β-catenin pathway) and noncanonical pathways, regulating vital cellular functions like proliferation, differentiation, migration, apoptotic cell death, stem cell renewal, and so on (Many and Brown 2014). In canonical Wnt signaling, in absence of Wnt, β-catenin is phosphorylated in cytosol by a protein complex consisting adenomatous polyposis coli (APC), casein kinase 1α (CK1α), and glycogen synthase kinase 3β (GSK3β) leading to its proteasomal degradation. Upon binding of Wnt ligand to its receptor Frizzled (G-protein-coupled receptors; GPCR), GSK3β-mediated phosphorylation and subsequent degradation of β-catenin are inhibited. Now β-catenin after getting stabilized translocates into the nucleus and acts in cooperation with TCF/LEF to induce the transcription of several target genes (Pai et al. 2017). Among noncanonical Wnt signaling pathways that are operated independent of β-catenin and TCF/LEF, the planar cell polarity (PCP) is one of the best characterized candidates. In PCP pathway, Frizzled receptor after binding to Wnt activates RAC1 (a small GTPase) that in turn activates JNK as the downstream effector. JNK phosphorylates and thereby stabilizes c-JUN which then translocates into the nucleus leading to transcriptional upregulation of target genes (Kagey and He 2017). Aberrant activation of both canonical and noncanonical Wnt signaling has been reported in prostate cancer indicating importance of the pathways

in disease pathogenesis (Schneider and Logan 2018). In the development of animal model for HGPIN and CRPC in transgenic mice, de-regulation of canonical Wnt signaling was noted and found to be achieved through constitutive activation of β-catenin (Yu et al. 2009). Aberrant activation of canonical Wnt signaling was also noticed in TRAMP mice models (Shukla et al. 2007). When Dickkopf-1 (Dkk-1)-transfected Ace-1 (canine adenocarcinoma cell of prostate) was xenografted in athymic mice, upregulation of JNK signaling through noncanonical Wnt pathway was found leading to the development of solid tumor followed by the incidence of metastasis to bones (Thudi et al. 2011b).

AR Signaling Pathway

The development of prostate is hormonally regulated, and therefore in this process, androgen and associated signaling pathway seem to play an important role. In AR signaling, binding of androgens (testosterone, dihydrotestosterone, etc.) to androgen receptor (AR) in cytosol causes its dimerization followed by translocates into the nucleus. Active AR then binds to the androgen response elements (ARE) located in promoter regions of AR-regulated genes to induce their transcriptional expression. In prostate tumorigenesis, de-regulation of AR signaling pathway has been found to be achieved through AR overexpression mediated by Akt, TGF-β, and so on. Aberrant activation of AR signaling independent of androgen has also been reported in the disease progression more aggressive form. Thus, AR pathway seemed to contribute not only to the development of normal prostate but also to the development and progression of its malignant form (Lonergan and Tindall 2011). In transgenic mice models, aberrant activation of AR signaling is achieved through ectopic expression of AR leading to the development of malignant prostate tumor that showed stepwise progression to more aggressive form capable of growing in absence of androgen. Consequentially, all the pathways described above can modulate the each other (Gao et al. 2017) as well as AR signaling in PCa.

Conclusion and Future Perspectives

The pathogenesis and associated risk factors of PCa are not well characterized. PCa is a disease of aged male. Along with age, food habit and tobacco smoking are also considered as important epidemiological factors, and incidence of oxidative stress as well as inflammation has been well documented as associated cellular phenomena. However, to explore the disease pathogenesis in detail at molecular level, animal models of prostate cancer including rat, mice, and canine are commonly used. In the present chapter, a summarized view of commonly used animal models of prostate cancer categorized according to the study purposes was presented. Carcinogen models may help us to understand how chronic carcinogenic exposure particularly to the aged male in daily life can play a role in emergence of PCa. Transgenic and immunodeficient models are primly helpful to understand the details of molecular

pathogenesis associated with the PCa development and its progression to advanced stages. Castration-resistant rodent models and some canine models help in dissecting the driver molecular events leading to the development of androgen independence of PCa. Canine models are almost inevitable in studying bone metastasis; some bone metastasis-specific transgenic rodent models are also used in this purpose. Xenograft models of PCa may serve the purpose of preclinical studies for novel therapeutic measures. However, in developing effective therapeutics, it is important to keep in mind that dose-dependency and toxicity assays are subject to animal sizes and metabolic rates. So, the therapeutics approaches must be standardized for human body before incorporation. In conclusion, animal models of prostate cancer are of diverse types, each having specification to address research issues and limitations of their own. However, the genetic dissimilarities of the model animals from human may affect the bench side to bedside implementation of the research output, and to overcome this, the scientist may use more humanized animals for this purpose in future.

Acknowledgments We are thankful to the Hon'ble Vice Chancellor, Presidency University Kolkata, India. Financial support for this work was provided by the West Bengal State Govt. Sponsored Presidency University FRPDF funds to Dr. Amlan Ghosh.

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