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Review

In Vivo Models for Prostate Cancer Research

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Simple Summary: This review explores *in vivo* models of prostate cancer currently published in the literature, as well as novel prostate cancer models that have recently been proposed. The information that researchers currently have about such models is critical for the information that they hope to obtain from future studies. Therefore, it is important that the various models currently published in the literature are systematically brought together. With the benefits and drawbacks of various types of prostate cancer models provided in this review, combined with their relationships to different signaling pathways and stages of tumor progression, the researcher may tackle the question of which model or gene of interest associated with the development of prostate cancer bests suits their future studies.

Abstract: In 2022, prostate cancer (PCa) is estimated to be the most commonly diagnosed cancer in men in the United States – almost 270,000 American men are estimated to be diagnosed with PCa in 2022 [1]. This review compares and contrasts *in vivo* models of PCa with regards to the altered genes, signaling pathways, and stages of tumor progression associated with each model. The main type of model included in this review are genetically engineered mouse models, which include conditional and constitutive knockout model. 2D cell lines, 3D organoids and spheroids, xenografts and allografts, and patient derived models are also included. The major applications, advantages and disadvantages, and ease of use and cost are unique to each type of model, but they all make it easier to translate the tumor progression that is seen in the mouse prostate to the human prostate. Although both human and mouse prostates are androgen-dependent, the fact that the native, genetically unaltered prostate in mice cannot give rise to carcinoma is an especially critical component of PCa models. Thanks to the similarities between the mouse and human genome, our knowledge of PCa has been expanded, and will continue to do so, through models of PCa.

Keywords: prostate cancer; knockout mouse models; genetically-engineered mouse models; xenografts; patient derived xenografts; organoids; signaling pathways

1. Introduction

1.1. Historical Timeline of PCa Models

The level of genetic information we have obtained over past decades of research has guided the development of models that attempt to study PCa. When cancer cell lines were first developed in the late 1940s through the 1970s, information about their genetic makeup or mutations present in the malignant tumor cells was limited. Cancer cells were simply taken from either the primary tumor or metastasis and were grown *in vitro*, allowing researchers to obtain 'immortal' cells by passaging them over time. The first cell line, or the L929 cell line, was established by Dr. Wilton R. Earle in 1948 and was derived from fibroblasts in subcutaneous mouse tissue [2]. The first immortalized human cell line,

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the HeLa cell line developed by Dr. George O. Gay in 1951, used cells taken from epithelial cervix tissue from Henrietta Lacks [3]. Many more cell lines soon followed throughout the 1950s and 1960s that were taken from hamsters, canines, monkeys, and humans [4]. Important PCa cell lines – LNCaP cells that were taken from a lymph node metastasis [5], DU145 cells that were taken from a central nervous system metastasis [6], and PC3 cells that were taken from a bone metastasis [7] – were developed in 1977, 1978, and 1979, respectively. Despite the cancer researcher's ability to finally grow malignant, immortalized cells *in vitro*, little was known about the cells' gene expression or the mutations that promoted their tumorigenicity.

PCa models, as well as our understanding of several aspects of mammalian physiology, were significantly enhanced thanks to the research by Mario R. Capecchi, Sir Martin J. Evans, and Oliver Smithies in the late 1980s. In 2007, these scientists were jointly awarded The Nobel Prize in Physiology or Medicine for discovering how to introduce specific gene modifications in mice using embryonic stem cells [8]. Through homologous recombination between introduced DNA and endogenous DNA in embryonic stem cells, pure populations of cells carrying the target gene could be grown and injected into blastocysts [8]. The injected blastocysts are implanted into a surrogate mother where they develop into mosaic embryos, and then mosaic and normal mice mate to produce both gene targeted and normal offspring [8]. This powerful technique, known as gene targeting in mice, is often used to inactivate, or knock out, specific genes and thus elucidate the function of those genes (Figure 1a) [8]. Our current knowledge of PCa is owed to the numerous mouse knockout models made available through gene targeting.

Critical for the development of conditional knockout models is the site-specific recombinase technology known as Cre-Lox recombination (Figure 1b). This technology allows DNA modification – deletions, insertions, translocations, and inversions – at specific sites to be targeted to a particular cell type, or to be triggered by a particular external stimulus [9]. Derived from the bacteriophage P1, Cre recombinase is an enzyme that catalyzes a site-specific recombination event between two DNA recognition sites known as LoxP sites [9]. In 1994, the laboratories of Dr. Jamey Marth and Dr. Klaus Rajewsky reported that this system could be used for conditional gene targeting [9]. Since then, Cre-Lox recombination has been widely used to manipulate genes and chromosomes, creating genetic knock-out or knock-in mouse models [9]. In addition, the use of CRISPR (clustered regularly interspaced short palindromic repeats)/Cas 9 genome editing has further strengthened our understanding of the role of genes in cancer (Figure 1c). This technology can be designed to introduce RNA-directed, double strand DNA breaks or single strand 'nicks' using a mutated form of Cas9, a DNA endonuclease [10]. CRISPR is a simple and effective mechanism to introduce mutations both *in vitro* and *in vivo* that mimic somatic mutations, revolutionizing mouse models of cancer [10].

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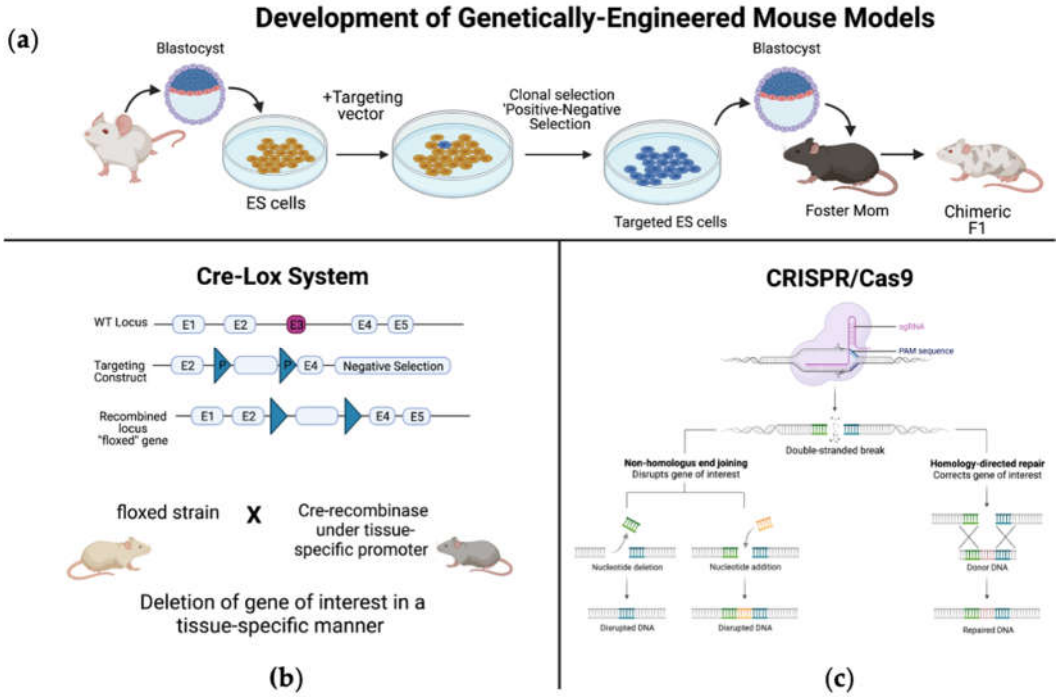

Figure 1. Summary of the development of genetically-engineered mouse models. (a) Genetically-engineered (GE) mice are used extensively in research as models of human disease. In the context of cancer, by deleting, or "knocking out", the function of a specific gene, one can determine the significance of that gene in either promoting or halting the progression of tumor development. The method to develop GE models begins with extracting blastocysts from the mouse, and culturing embryonic stem (ES) cells. A targeting vector, which is a DNA construct containing DNA sequences homologous to the gene of interest, is added. ES cells that successfully recombine with the genomic DNA are selected for, and these targeted ES cells are injected into the mouse blastocysts. (b) The Cre-Lox system is used to control site specific recombination events in genomic DNA, allowing one to control expression of a specific gene or to delete undesired sequences. Cre recombinase is an enzyme that is derived from the bacteriophage P1, and it catalyzes a site-specific recombination event between two DNA recognition sites known as LoxP sites [9]. The result is a recombined locus in which the gene of interest is deleted, creating a "floxed" gene. (c) In the CRISPR/Cas9 system, a foreign single guide RNA (sgRNA) seeks, matches, and binds a specific DNA sequence, and the nuclease Cas9 cuts the sequence at a precise binding site near a protospacer adjacent motif (PAM) sequence [10]. The double-stranded DNA break induced by Cas9 is repaired by the cell's DNA repair machinery. One method is via non-homologous end joining, either by deleting or adding a nucleotide, which does not require a template but disrupts the gene of interest. Or, homology-directed repair can be performed, which corrects the gene of interest, but a single stranded (ss) DNA template is required, which is provided by the CRISPR/Cas9 system [10].

Thanks to DNA sequencing and gene expression data in the 21st century, PCa research models and our understanding of PCa as a whole have expanded tremendously. In 2002, The International Mouse Genome Sequencing Consortium, which was part of the Human Genome Project, achieved a high-quality draft sequence of the mouse genome [11]. Drafts for the Human Genome Project were already established a couple of years prior to the draft of the mouse genome, and the final sequence mapping of the human genome was finished in 2003 [11]. Although mice and humans are separated by 75 million years of evolution, their genomes are about 90% similar and both share almost 30 thousand genes [11]. Thanks to their similarities, the field known as comparative genomics can explore how mouse genes and their human counterparts contribute to health and disease. Consequently, the genes, mutations, and signaling cascades discovered from studying tumor progression in the prostates of lab mice could be applied to enhance our understanding of human pathophysiology.

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2. Patient-derived Xenografts and Organoids of PCa

The shortage of clinically relevant, *in vivo* models is a large barrier to the understanding of tumor progression seen in PCa. Generating models that are derived from biopsy specimens and metastatic lesions from human patients is one means of mitigating this dilemma. One form of patient-derived models is xenografts (PDXs), which allow researchers to study and appreciate the tumor heterogeneity of prostatic diseases. It has been shown that populations of PDX mice upon passaging preserve most of the genetics of the original human tumors [12]. Extensive copy number alterations between human tumors and those in the PDX models were not found [12]. One type of PDX model, LuCaP PDXs, which harbor genetic alterations such as androgen receptor amplification, PTEN deletion, and TP53 deletion, demonstrate molecular heterogeneity in response to androgen deprivation therapy (ADT) and docetaxel treatment [13]. In PDXs studying hormone-naive prostate cancer, the Growth Factor Receptor Bound Protein 10 (GRB10) gene was found to be the most significantly upregulated, showing increased expression during and before the development of castration resistant prostate cancer [14]. Furthermore, PDXs were also established that are based on subrenal capsule grafting of patients' tumor tissue into nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice [15]. These PDXs preserve crucial properties of the original malignancies, including histopathological and molecular characteristics, reactions to androgen suppression, and tumor heterogeneity [15]. Consequently, PDXs represent valuable models not only for comprehending prostate tumor progression but also establishing drug screenings and therapies [15].

Despite their advantages, xenograft models are costly, time consuming, and require the use of immunocompromised mice. Three-dimensional (3D) patient derived organoids (PDOs) are perhaps a more efficient alternative – one that still maintains tumor heterogeneity and appropriate disease modeling. Organoids, or "mini-organs", are clusters of cells grown *in vitro* that self-organize and differentiate into functional cell types [16]. Like PDXs, PDOs can be derived from primary tissue materials such as needle biopsies [16], but it has also been demonstrated that they can be derived from PDXs themselves [17]. These PDOs can conserve the genotypic and phenotypic characteristics of the LuCaP PDX cells they are derived from and represent an effective model to study mechanisms of resistance to ADT in PCa [17]. Organoids can also be derived from stem cells, including those expressing Lgr5, which is a genetic marker of Wnt-dependent stem cells found in tissues including the prostate [18]. In addition, organoids have also been used to study uncommon variants of PCa, including neuroendocrine prostate cancer (NEPC) derived from four human patients [19]. In these PDOs, the epigenetic modifier histone methyltransferase enhancer of zeste 2 (EZH2) was found to show increased expression in NEPC compared to adenocarcinomas [19]. Overall, PDOs share a similar mutational landscape with PCa, and they recapitulate in situ histology both *in vitro* and *in vivo* [20].

3. Comparing and Contrasting Types of PCa models

The most common models used to study PCa in the lab include 2D cell lines, 3D organoids and spheroids, xenografts and allografts, PDXs, and GE mice, which are summarized in Table 1. GE mice include those with transgene expression and knockouts; knockouts may either be conditional or constitutional knockouts. The availability, ease of use, and cost of 2D cell lines and different patient derived tumor models, including novel lab-on-a-chip models, are provided in Figure 2.

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Table 1. Advantages and disadvantages of models for PCa studies.

Model	Availability, Ease of Use, Cost	Major Applications	Advantages	Disadvantages
2D Cell Lines	-High availability -Easy to use -Low cost	-Molecular and mechanistic studies -Drug screening studies -Validation experiments -Epigenetic studies	-Relatively quick results -Data may be made available online -Phenotypic analysis by microscopy studies	-Results may only apply to particular cell line, unless repeated in multiple cell lines -Lacks intra-tumor heterogeneity -Intrinsic effects due to high level passaging -Lacks phenotypic characteristics of parental tumors
3D Organoids, Spheroids	-High availability -Requires more time and elaborate methods for observations and experiments -Intermediate cost	-Molecular and mechanistic studies -Drug screening studies -Imaging and observational studies	-Data can be easily obtained in a relatively short time -Phenotypic analysis by microscopy studies -High content data such as drug screens -More relevant to human cancer -Easily accessible for DNA and mRNA sequence analysis -Presence of ECM	-Longer timescale than 2D cell lines -Requires more extensive analysis -Data obtained is dependent on environment, causing high variability
Xenografts, Allografts	-High availability -Technical expertise required to use -High cost	-Drug response studies and novel drug screening studies -Confirmation of molecular and mechanistic studies	-Comparable to *in vivo* context -Drug approval studies -Easily accessible for DNA and mRNA sequence analysis	-More time-consuming than 2D or 3D models -Drug response associated with genotype -Immunosuppression limits understanding the role of the immune system in tumor response (alleviated for allografts)
Patient Derived Xenografts (PDXs)	-Limited availability -Patient consent required to use -Technical expertise required to use -High cost	-Heterotopic injection or transplant -Understanding tumor heterogeneity -Personalized drug testing for effective therapy -Maintenance of tumor architecture -*In vivo* physiology	-Easily accessible for DNA and mRNA sequence analysis -Understanding of drug resistance response -May lead to specific identification for treatment target	-Lower potential for subsequent confirmation studies -Immunosuppression limits understanding the role of the immune system in tumor response
Genetically Engineered (GE) Mice -With transgene expression -Knockouts (KOs) -Constitutive -Conditional	-Limited number of models -Animal colony maintenance required -High cost	-Establishment of *in vivo* functions of oncogenes and tumor suppressor genes -Genetic interactions -Tumor progression studies -Epithelial-stromal interactions -Immunological studies -Platform for studying metastasis	-Definitive functional studies, including metastatic potential -Establishment of i*n vivo* functions in context of different tissues -Easily accessible for DNA and mRNA sequence analysis	-Long-term studies, with long tumor latency -Time and high cost associated with breeding skills and genotyping -Many common genotypes not represented -Patented strains unavailable -Phenotype may be influenced by strain -Spontaneous, strain-dependent

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	tumorigenesis independent from genetic engineering

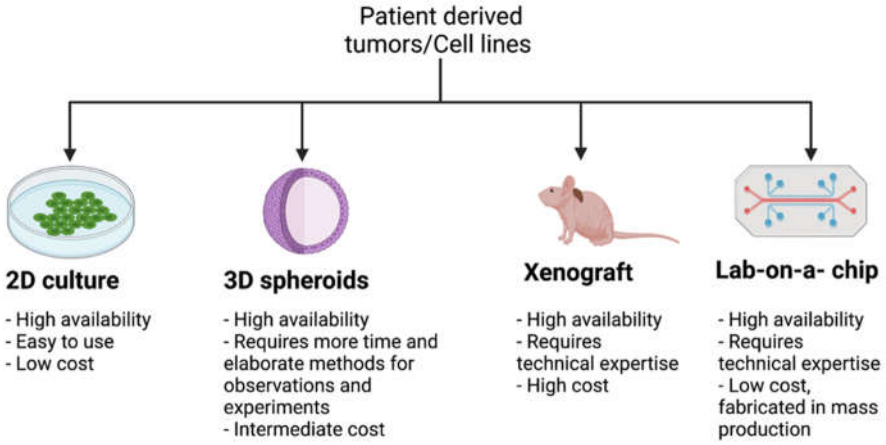

Figure 2. Summary of patient derived tumor models and cell lines. Various models of cancer are in use, each with their own applications, advantages, and disadvantages. 2D cultures are a widely used model for understanding cell biology and tissue morphology, as well as mechanisms of disease progression and actions of drugs [21]. Their high availability, ease of use, and low cost allow for high throughput data collection, although data from 2D cell lines alone is usually insufficient without further studies that more closely mimic *in vivo* conditions. 3D models, such as organoids and spheroids, overcome the 2D model's limitation of monolayer cell culture, and thus obtain a more accurate physicochemical environment that compares to *in vivo* conditions [22]. This comes with the disadvantage of requiring more time and more elaborate methods for observations and experiments compared to 2D cultures, as well as a higher cost. However, 3D models do not require the same level of time and technical expertise as xenograft models, nor do they require the use of immunocompromised mice. The major advantage of xenograft models is that, compared to 2D and 3D models, they are the most comparable to *in vivo* physiology, permitting more accurate analysis of molecular, mechanistic, and drug screening studies. Lastly, lab-on-a-chip models are novel devices that integrate laboratory studies onto a very small, single, integrated circuit, allowing for high throughput screening [23]. As they are fabricated in mass production [24], lab-on-a-chip models have a high availability and low cost. Tumor-on-a-chip or organ-on-a-chip models are currently being developed to perform drug screening studies and better emulate physiologic conditions [25].

4. Mouse Models based on Tumor Stage Progression

This section highlights the pathological stages of prostate cancer that mouse models can represent. Significant non-neoplastic and neoplastic changes to the mouse prostate epithelium are outlined, and the genetic lab models associated with each stage of tumor development are included. For a more expansive review of PCa progression, please see Shappell et al [26], which outlines prostate pathological changes in greater detail, including changes made to the prostate epithelia as well as the stroma.

4.1. Hyperplasia

Hyperplasia (Table 2) is defined as the proliferation of normal cells, and it is a non-neoplastic change to the prostate epithelium. Epithelial hyperplasia in the mouse can be diffuse or focal, and with or without small areas of nuclear atypia [26].

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Table 2. PCa models in which hyperplasia is documented.

Model	Alteration	Driver and/or Add. Genetic Alteration	Phenotype	Reference
p27^{Kip1}	Loss of expression	Created by gene targeting in embryonic stem cells	Hyperplasia of multiple organs, including prostate, testis, and thymus	[27]
Nkx3.1	Loss of expression	Genomic clones isolated from λFIXII library from 129Sv/J genomic DNA	Prostatic epithelial hyperplasia and dysplasia; decreased bulbourethral gland size	[28]
Rb^{flox}	Loss of expression	PB^{Cre4} driver Rb^{loxP} driver	Focal areas of epithelial hyperplasia; loss of basement membrane and smooth muscle layer integrity	[29]
IGF-1^{flox}	Gain of expression	PB^{Cre4} driver	Cell autonomous proliferation; hyperplasia	[30]
FOXA1	Loss of expression	PB^{Cre4} driver	Progressive hyperplasia with extensive cribriform patterning	[31]

[1] PB^{Cre4}: Probasin promoter, a frequently used promoter used to direct transgene expression of Cre recombinase to prostate epithelial cells.

4.2. Prostatic Intraepithelial Neoplasia

Mouse prostatic intraepithelial neoplasia (mPIN) (Table 3) is the focal proliferation of atypical cells, contained within the gland or duct [32]. mPIN is neither benign nor malignant, but is described instead as a neoplastic proliferation of premalignant potential [26]. mPIN appears to be the consequence of a clonal expansion of a single transformed cell [32]. mPIN can be subclassified as 1) mPIN with documented potential to invasive carcinoma or 2) mPIN without documented potential to invasive carcinoma [26]. Human PIN, however, can be subclassified as either low grade or high-grade PIN (HGPIN); increased nuclear size and more prominent nucleoli (macronucleoli) are characteristic of HGPIN [26].

Table 3. PCa models in which PIN is documented.

Model	Alteration	Driver and/or Add. Genetic Alteration	Phenotype	Reference
KDM5B	Gain of expression	Loss of Pten function	HGPIN	[33]
Sox9	Gain of expression	Hemizygous loss of *Pten* (germline heterozygous *Pten* allele)	HGPIN	[34]
Pten$^{flox/flox}$	Loss of expression	K14-Cre^{ERT2} driver	PIN development	[35]
Pten	Loss of expression	Mouse *Pten* disrupted by homologous recombination	PIN development; formation of aberrant embryoid bodies	[36]
Pten x p53	Loss of expression	Recombination of adult prostatic epithelium with embryonic rat seminal vesicle mesenchyme	HGPIN	[37]
Abi1	Loss of expression	PB^{Cre4} driver	PIN development	[38]
EAF2	Loss of expression	PB-CreER^{T2} driver	Luminal epithelial hyperplasia and mPIN	[39]
ACSS3	Loss of expression	Transfection of overexpressing lentivirus and sgRNA (CRISPR/Cas9)	PIN in anterior prostate; increased proliferation, migration, and invasion	[40]
CSF-1	Gain of expression	PB^{Cre4} driver	Immune cell infiltration into prostate; LGPIN	[41]

4.3. Microinvasive Carcinoma

Microinvasive carcinoma (Table 4) is the earliest recognizable form of invasive carcinoma, with penetration of malignant cells through the basement membrane of PIN-involved glands into the surrounding stroma [26]. Microinvasive carcinoma differs from

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invasive carcinoma by the greater extent of infiltration and destructive growth in the latter [26].

Table 4. PCa models in which microinvasive carcinoma is documented.

Model	Alteration	Driver and/or Add. Genetic Alteration	Phenotype	Reference
Timp3	Loss of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	HGPIN with microinvasion	[42]
mpAkt	Gain of expression	Myc gain of expression under control of PB driver	mPIN followed by microinvasive carcinoma, disruption of basement membrane integrity, stromal remodeling, and lymphocyte infiltration	[43]
Nkx3.1	Loss of expression	Myc gain of expression under control of CMV enhancer and β-actin promoter	HGPIN with microinvasion	[44]
Pten	Loss of expression	Myc gain of expression under control of CMV enhancer and β-actin promoter	Microinvasive cancer with disruption of smooth muscle actin	[45]

4.4. Invasive Carcinoma

Invasive carcinoma is described as the invasion of atypical, or malignant, epithelial cells into the surrounding stroma [32]. Most often, these epithelial cells are the luminal cells, and the increase in luminal cells is accompanied by a loss of basal cells [32]. The neoplastic growth pattern characterized by invasive carcinoma is incompatible with architecturally normal glands, and the greater extent of invasion in invasive carcinoma separates it from microinvasive carcinoma [26].

4.1.1. Adenocarcinoma

Most invasive carcinomas in human PCa are adenocarcinomas (Table 5). Many epithelia contain specialized cells that secrete substances into the ducts or cavities that they line. In the prostate, these epithelial cells are termed the luminal cells, and prostate adenocarcinomas are derived from malignant luminal cells. Adenocarcinomas are subclassified as well, moderately, or poorly differentiated, according to the extent of glandular formation [26].

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Table 5. PCa models in which adenocarcinoma is documented.

Model	Alteration	Driver and/or Add. Genetic Alteration	Phenotype	Reference
Nkx3.1	Loss of expression	Hemizygous loss of *Pten* (germline heterozygous *Pten* allele)	HGPIN with invasive adenocarcinoma	[46,47]
p27^{Kip1}	Loss of expression	Hemizygous loss of *Pten* (germline heterozygous *Pten* allele)	HGPIN with invasive adenocarcinoma	[48,49]
Aft3	Loss of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	HGPIN with invasive adenocarcinoma	[50]
Apc^{flox}	Loss of expression	PB^{Cre4} driver	HGPIN followed by local adenocarcinoma	[51]
Bmi	Gain of expression	Hemizygous loss of *Pten* (germline heterozygous *Pten* allele)	Locally invasive and highly vascularized adenocarcinoma, with frequent bladder outlet obstruction	[52]
Tsc2	Loss of expression	Hemizygous loss of *Pten* (germline heterozygous *Pten* allele)	Invasive adenocarcinoma; enhanced lymphoid proliferation; development of skin cancer	[53]
Phlpp1	Loss of expression	Hemizygous loss of *Pten* (germline heterozygous *Pten* allele)	Invasive adenocarcinoma at full penetrance with onset of 8 months	[54]
Chk1	Loss of expression	Hemizygous loss of *Pten* (germline heterozygous *Pten* allele)	Progression of HGPIN into invasive adenocarcinoma	[55]
PKCε	Gain of expression	Hemizygous loss of *Pten* (germline heterozygous *Pten* allele)	Invasive adenocarcinoma, preferentially in ventral prostate	[56]
Gata3	Loss of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Acceleration of invasive adenocarcinoma	[57]
Erg	Gain of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Foci of invasive adenocarcinoma with varying levels of *Erg* expression	[58]
Etv1	Gain of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma with homogenous *Etv1* expression	[58]
Junb	Loss of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma in anterior prostate, with strong histological similarity to human PCa	[59]
SPOP-F133V	Gain of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive, poorly differentiated, and highly proliferative adenocarcinoma	[60]
PSGR	Gain of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma featuring *Akt* activation and extensive inflammatory cell infiltration	[61]
Zbtb7a	Loss of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Highly penetrant invasive adenocarcinoma at 11 weeks	[62]
Hepsin	Gain of expression	*Myc* gain of expression under control of PB driver	Invasive adenocarcinoma lacking glandular prostate differentiation and clear basement membrane contour	[63]
MMP7	Gain of expression	Loss of Pten function	Invasive adenocarcinoma through induction of epithelial-to-mesenchymal transition (EMT)	[64]
Pten^{adcre+}	Loss of expression	Cre-expressing adenovirus via intraductal injection into anterior-posterior prostate	Invasive adenocarcinoma with onset of 8-16 weeks	[65]
Kindlin-3	Loss of expression	Xenograft	Subcutaneous prostate cancer tumor growth	[66]

4.1.2. Squamous Cell Carcinoma

Some epithelial sheets serve to seal the cavity that they line and to protect the underlying cell populations. Tumors that arise from epithelial cells forming these protective cell layers are termed squamous cell carcinomas (Table 6). In the prostate, this is seen in the form of keratinization and/or intercellular bridges [26].

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4.1.3. Neuroendocrine Carcinoma

Neuroendocrine (NE) carcinomas (Table 6) are characterized by the invasion of atypical neuroendocrine cells into the surrounding stroma. NE cells have traits of both nerve cells and hormone-producing endocrine cells. NE carcinomas do not exhibit well-defined glandular formation or extensive secretory differentiation [26].

4.1.4. Sarcomatoid Carcinoma

Sarcomatoid carcinomas, or spindle cell carcinomas (Table 6), are derived from atypical spindle cells [32]. Sarcomatoid is a term that means 'resembling sarcoma'. Sarcomas are tumors that are not of epithelial origin but rather of mesenchymal origin – connective tissue of the body such as stromal cells [32]. Sarcomatoid carcinomas arise from epithelial origin but resemble sarcomas.

Table 6. PCa models in which squamous cell carcinoma, neuroendocrine carcinoma, and sarcomatoid carcinoma are documented.

Model	Alteration	Driver and/or Add. Genetic Alteration	Phenotype	Reference
Pten$^{flox/flox}$	Loss of expression	Nkx3.1-CreER^{T2} driver	Microinvasive AD with areas of poorly differentiated AD; squamous metaplasia	[67,68]
RARγ	Loss of expression	C57BL/6 F1 background strain	Squamous metaplasia in prostate and seminal vesicles	[69]
MYCN	Gain of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma with neuroendocrine PCa (NEPC)	[70]
TRAMP	Gain of expression	PB promoter driving expression of SV40 early region	Androgen independent tumors are 100% synaptophysin positive, and metastases are 67% positive	[71]
LADY	Gain of expression	Large PB (LPB) promoter driving expression of SV40 large T-antigen (Tag)	Visceral metastasis; NEPC	[72]
LADY	Gain of expression	LPB driver, 12T-7s line; crossed with PB-Hepsin	Adenocarcinoma with neuroendocrine differentiation (NED); NE metastasis to liver, lung, and bone	[73]
LADY	Gain of expression	LPB driver, 12T-7s line; crossed with β-catenin	Adenocarcinoma with focal NED without apparent NEPC	[74]
Kras G12D	Gain of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma, sarcomatoid differentiation, with extensive metastasis	[75,76]
Pten and *p53*	Loss of expression	PB-Cre mediated deletion of *Pten* and *Trp53*; activation of ROSA-LSL luciferase reporter	Fast-growing, lethal sarcomatoid tumors; local invasive PCa	[77]
ALK and *N-myc*	Gain of expression	FVB/NJ and NSG background strains	Neuroblastoma development; metastasis with NED	[78]

4.5. Metastasis

Metastasis (Table 7) refers to the spread of cancer cells from the place where they first formed, or the primary tumor mass, to another part of the body through blood or lymphatic vessels [26]. Once the cells have reached a new organ or tissue, they undergo the process of colonization, or the growth of a micrometastasis into a macrometastasis. In mouse prostate cancer, metastasis usually occurs in the lymph nodes, visceral organs such as the lungs, and very rarely bones [32].

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Table 7. PCa models in which metastasis is documented.

Model	Alteration	Driver and/or Add. Genetic Alteration	Phenotype	Reference
Pten$^{flox/flox}$ (exon 5)	Loss of expression	PB^{Cre4} driver	Invasive adenocarcinoma with metastasis to lungs, rarely to lymph nodes	[79]
Nr2f2 (COUP-TFII)	Gain of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma with metastasis to lymph nodes	[80]
NCoA2	Gain of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma with metastasis to lymph nodes, lungs	[81]
NSD2 (Whsc-1)	Gain of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma with metastasis to lymph nodes, lungs, bone	[82]
Trp53	Loss of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma with metastasis to lymph nodes, spleen, liver, organs near GU tract excluding bladder	[83-85]
Rb	Loss of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma with metastasis to lymph nodes, lungs, liver that resembles NEPC	[86]
Jnk1/2	Loss of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma with metastasis to lymph nodes	[87]
Stat3 and IL-6	Loss of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Poorly differentiated cancer with metastasis to liver, lungs	[88]
NICD	Gain of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma with metastasis to liver, lungs	[89]
Smad4	Loss of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma with metastasis	[90]
Smad4 / p53	Loss of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma with metastasis to bone	[91]
HoxB13 / Myc	Gain of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma with metastasis to lymph nodes, liver, lungs	[92]
Braf ^{V600E}	Gain of expression	Homozygous loss of *Pten* (conditional *Pten* allele)	Invasive adenocarcinoma with metastasis to lymph nodes, bone marrow, lungs	[93]
p53^{flox}*Rb*^{flox}	Loss of expression	Homozygous loss of *Pten* (conditional *Pten* allele) PB^{Cre4} driver	Metastatic carcinoma, with distant metastases	[94]
NPK^{EYFP}	**N**kx3.1 loss of expression **P**ten loss of expression **K**ras gain of expression	Nkx3.1$^{CreERT2/+}$ Pten$^{flox/flox}$ Kras$^{LSL-G12D/+}$	Invasive adenocarcinoma with metastasis to bone	[95]
SIRT-6	Gain of expression	Luciferase expressing PC3M cells in an orthotopic xenograft mouse model	Metastasis to liver; upregulation of N-cadherin and vimentin, downregulation of E-cadherin *in vitro*	[96]
RIPK2	Gain of expression	Injection of RIPK2-KO 22Rv1 cells into male SCID/Beige mice	Invasive adenocarcinoma with metastasis to bone	[97]

5. Mouse Models based on Signaling Pathways

This section highlights the various signaling pathways characteristic of PCa, illustrating how PCa mouse models represent what happens in the context of human cancers. For each signaling pathway, the major players, or genes, that are affected are included, as well as the specific models that study those particular genes. Although the number of signaling pathways disrupted in PCa is essentially innumerable, the six below have been chosen to be investigated due to their significance in PCa pathology.

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5.1. AR Pathway

Almost all prostate cancers express the AR (androgen receptor), which is a nuclear receptor that binds to androgens such as testosterone and dihydrotestosterone [98]. Upon binding androgens in the cytoplasm, ARs translocate to the nucleus and undergo dimerization; the dimer then acts as a transcription factor by binding to sequences of DNA called hormone response elements [99]. ARs may also interact with other proteins, causing upregulation or downregulation of specific genes, as necessary for the maintenance and development of the prostate [99].

Examples of genes that are involved in the AR pathway include the Erg and Etv1 genes, which are both members of the ETS (erythroblast transformation-specific) family of transcription factors [100]. Conditional gain of expression of the Erg or Etv1 genes results in mice with invasive carcinoma, thought to be caused by enhanced AR signaling [58,101,102]. In addition, upon the gain of expression of the mutated SPOP F133V gene, an E3 ubiquitin ligase, invasive adenocarcinoma is observed in the SPOP F133V model due to AR signaling activation plus PI3K pathway activation [60]. On a different note, repression of AR signaling is cited as the proposed mechanism for causing invasive adenocarcinoma with NEPC, upon the conditional gain of expression of the MYCN gene [70]. It is important to note that the mouse models described above are also accompanied by the homozygous loss of the conditional Pten allele. With the loss of a tumor suppressor gene in Pten, combined with the activation of oncogenes such as Erg, Etv1, SPOP F133V, and MYCN, the result is activated AR signaling and thus tumor growth and proliferation.

5.2. PI3K Pathway

Alterations involving genes within the PI3K (phosphatidylinositol 3-kinase) signaling pathway are regularly observed in PCa [103]. Upon phosphorylation and activation by activated Ras protein, PI3K phosphorylates PIP2 (phosphatidylinositol (4,5)-diphosphate) into PIP3 (phosphatidylinositol (3,4,5)-trisphosphate). This in turn leads to the tethering of Akt/PKB and Rho-GEFs to the plasma membrane. Akt/PKB subsequently phosphorylates and activates effector proteins such as FOXO and mTOR, while phosphorylating and inactivating effector proteins such as GSK-3β and Bad [104]. Ultimately, this results in increased cell growth, cell proliferation, cell motility, cell survival, and protein synthesis [105]. One key player in this signaling pathway is Pten (Phosphatase and Tensin Homolog), a protein phosphatase that reverses the actions of PI3K by converting PIP3 into PIP2. In human prostate cancer, mutations and inactivation of Pten are more common than amplifications and activation of PIK3CA, PIK3CB, PIK3R1, and AKT1 [106].

Several GEMMs associated with the activation or inactivation of the PI3K signaling pathway are present in the PCa literature. As a matter of fact, the AR and PI3K signaling pathways are the two most frequently altered in localized and metastatic PCa [107]. One Nkx3.1 knockout model, with hemizygous loss of the germline Pten allele, features hemizygous or homozygous loss of the Nkx3.1 gene [46,47], which is an androgen-regulated, homeobox gene [28]. The resulting phenotype of this model is HGPIN with invasive adenocarcinoma, most likely from increased Akt protein [46,47]. Invasive adenocarcinoma with metastasis, from increased PI3K signaling, is observed in a couple of different models, such as the NCoA2 and NSD2 knockout models. The NCoA2 model is characterized by the conditional gain of expression of the gene that codes for NCoA2 (Nuclear Receptor Coactivator 2) [81]. The NSD2 model features the conditional gain of expression of the gene that codes for NSD2 (Nuclear Receptor Binding SET Domain Protein 2) [82]. The Nkx3.1 model above is accompanied by the hemizygous loss of the germline Pten allele, whereas the NCoA2 and NSD2 models are accompanied by the homozygous loss of the conditional Pten allele. In addition to these models, the mpAkt model, which features gain of expression of Akt and Myc genes, demonstrates accelerated progression of mPIN to microinvasive carcinoma [43]. This model, like all of the models in this section, is characterized by PI3K pathway signaling activation [43]. Mice of this

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model also exhibit basement membrane disruption, stromal remodeling, and lymphocyte infiltration [43].

PCa knockout models associated with AR and PI3K signaling pathways are displayed in Figure 4.

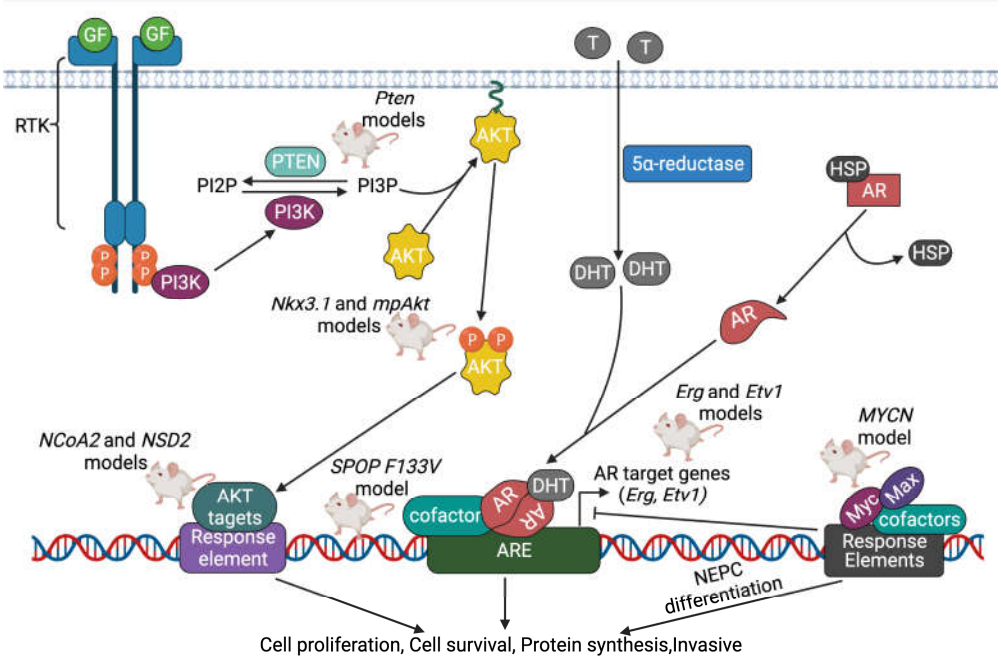

Figure 4. Summary of PCa knockout models associated with PI3K and AR signaling pathways. Several signaling pathways have been identified that play a role in the development of PCa. To explain the phenotypes of each KO model, researchers propose one or more signaling pathways that could be altered in the model that they developed. In the PI3K pathway, once activated by Ras protein, PI3K phosphorylates PIP2 into PIP3, leading to the tethering of Akt/PKB and Rho-GEFs to the plasma membrane. Pten reverses the actions of PI3K by converting PIP3 back into PIP2, and thus plays a key role in inhibiting cell growth and proliferation. In addition to the PI3K pathway, another oncogenic signaling pathway involves the nuclear receptor known as AR. In the presence of androgens such as testosterone and dihydrotestosterone, Heat Shock Protein (HSP) is released from AR, and AR binds the androgen in the cytoplasm. ARs translocate to the nucleus and undergo dimerization; the dimer then acts as a transcription factor by binding to androgen response elements (AREs). With recruitment of other cofactors, this again leads to increased cell proliferation, cell survival, and protein synthesis.

5.3. TP53 Pathway

TP53 (Tumor protein 53), commonly referred to as the guardian of the genome, is a tumor suppressor responsible for controlling processes such as DNA repair, cell cycle (growth) arrest, and apoptosis [108]. In humans, the TP53 protein is coded by the TP53 gene; in mice, however, the protein is known as TRP53 and is coded by the TRP53 gene. Signals from metabolic stress or genomic damage, including a lack of nucleotides, UV radiation, ionizing radiation, oncogene signaling, hypoxia, and blockage of transcription cause TP53 levels to rapidly accumulate from its normally low steady state concentration [104]. TP53 regulates whether the DNA will be repaired, or if the damaged cell will undergo apoptosis. By preventing cells with mutated DNA from proliferating, TP53 plays a significant role in prohibiting the development of tumors [108]. As a matter of fact, TP53 is the most commonly mutated gene in human cancer [104].

In undisturbed cells, TP53 binds to the promoter of the Mdm2 (Mouse double minutes 2) gene, and the encoded Mdm2 protein binds to TP53 itself and initiates TP53's ubiquitylation, export to cytoplasm, and degradation in the proteosome [104]. TP53 is protected from Mdm2 by being activated by the Ser/Thr-kinase Chk2; Chk2 is in turn

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activated by ATM, another Ser/Thr-kinase that receives signals from sensors of double-stranded DNA breaks [104]. TP53 is also protected from Mdm2 by being activated by the Ser/Thr-kinase Chk1; Chk1 is in turn activated by ATR, another Ser/Thr-kinase that receives signals from sensors of single-stranded DNA [104]. Likewise, ATM kinase can phosphorylate Mdm2, in a way that leads to its destabilization and thus promote increased levels of TP53 [104]. Another protein, ARF (p19ARF in mouse cells and p14ARF in human cells), can accumulate in the nucleolus and form stable complexes with Mdm2, and thus inactivate the TP53 antagonist [104].

The TRP53 mouse model features conditional loss of expression of the TRP53 gene, and consequently, invasive adenocarcinoma with metastasis is observed [83-85]. Cited signaling pathways include senescence bypass, Myc activation, and neuroendocrine differentiation [83-85]. In addition, DNA damage signaling and genomic instability have been proposed following the observation of invasive adenocarcinoma in Chk1 knockout mice [55]. The Chk1 model features hemizygous loss of the Chk1 gene [55] which, as described above, encodes a Ser/Thr-kinase that phosphorylates/activates TP53 and phosphorylates/inactivates Mdm2. The Phlpp1 knockout model is characterized by the hemizygous or homozygous loss of the Phlpp1 gene, and the resulting phenotype is invasive adenocarcinoma [54]. The proposed signaling pathway is p53-mediated senescence bypass [54]. Phlpp1 (PH domain and Leucine rich repeat Protein Phosphatase 1), together with Phlpp2, are protein phosphatases that regulate Akt/PKB [109], the kinase that is involved in the PI3K pathway in the section above. Akt/PKB is significant in the TP53 pathway as well because Akt/PKB phosphorylates and activates Mdm2 [104], which itself binds to TP53 and inactivates it by targeting it for degradation. The models featuring signaling pathways associated with TP53 above are also accompanied by loss of Pten function. Lastly, in the ACSS3 model, increased expression of ACSS3 (Acyl-CoA synthetase short-chain family member 3) was found to limit PCa progression [40]. ACSS3 overexpression significantly induced endoplasmic reticulum (ER) stress, which in turn was found to activate apoptosis [40], one of the pathways regulated by TP53.

PCa knockout models associated with TP53 and DNA repair signaling pathways are displayed in Figure 5.

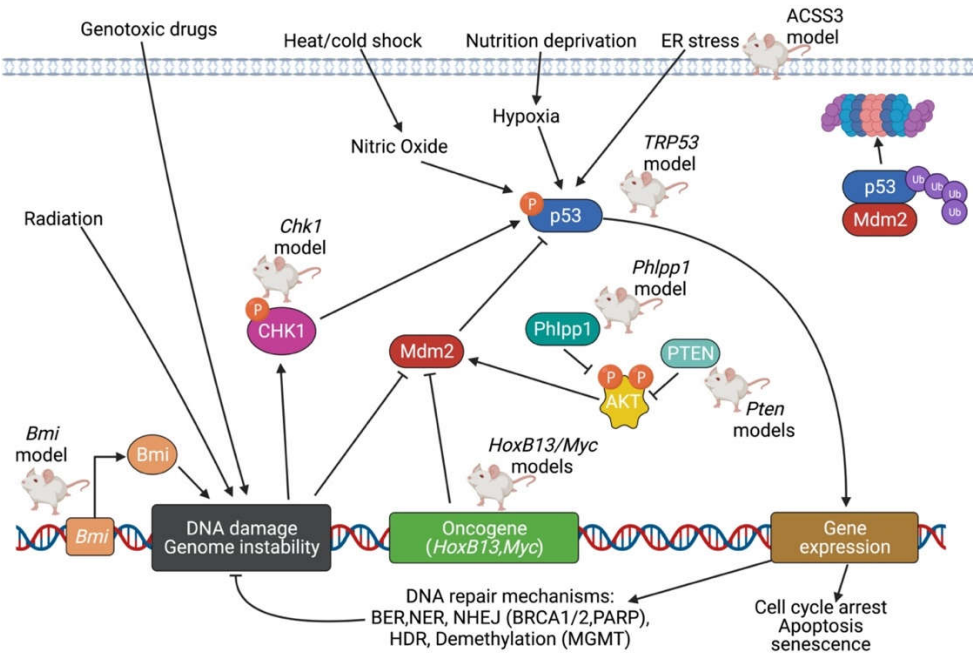

Figure 5. Summary of PCa knockout models associated with TP53 and DNA repair signaling pathways. In normal conditions, TP53 levels are at their low steady-state concentration. In the presence of metabolic stress or genomic damage – such as a lack of nucleotides, presence of UV radiation, ionizing radiation, oncogene signaling, hypoxia, or blockage of transcription – TP53 levels

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rapidly accumulate. As the guardian of the genome, TP53 regulates processes such as DNA repair, cell cycle arrest, and apoptosis; TP53 itself is activated by Chk1 and inhibited by Mdm2. The DNA repair stimulated by TP53 can itself be identified as its own signaling pathway. One component of the DNA repair pathway includes MGMT/AGT, which removes methyl and ethyl adducts from the O6 position of guanine. Excision pathways such as nucleotide-excision repair and base-excision repair can be stimulated in response to single-stranded DNA breaks. With the help of proteins such as BRCA1 and BRCA2, double-stranded DNA breaks can be repaired via either homology-directed repair or non-homologous end joining.

5.4. MYC Pathway

The oncoproteins of the Myc family, when expressed in a deregulated fashion, function as growth-promoting transcription factors in the cell nucleus [104]. More than 50% of human cancers overexpress either Myc (often termed c-Myc), or one of its two close cousins: N-Myc and L-Myc [110]. These proteins are encoded by the C-MYC, MYCN, and MYCL genes, respectively [110]. Myc family proteins all share a homologous motif at the C-terminus that consists of a basic DNA-binding domain, followed by amino acid sequences forming an α-helix, a loop, a second α-helix, and a Leucine zipper (BR/HLH/LZ motif) [110]. Myc, as well as other transcription factors with this BR/HLH/LZ motif, form homodimers or heterodimers with themselves [104]. The dimer can associate with specific regulatory DNA sequences called E-boxes (CACGTG), found on the promoters of target genes [104]. The association between Myc and its partner Max, for example, drives the expression of a large cohort of genes that favor cell growth and proliferation [110]. Myc/Max is able to induce expression of Cyclin D2 and CDK4, which promote advance through early G1 phase of the cell cycle, as well as Cul1 and Cks1, which degrade $p27^{Kip1}$, and thus, promote advance into the S-phase of the cell cycle [104]. Myc/Max also induces expression of the genes encoding the E2F transcription factor proteins, which are normally negatively regulated by pRb [104]. The activation of these target genes is made possible due to the recruitment of chromatin-modifying complexes such as GCN5, TIP60, TIP48, and TRRAP by Myc/Max [110].

One GEMM that features Myc, and one that has already been mentioned in the section describing the AR signaling pathway, is the MYCN model. In this model, repression of AR signaling is cited for causing invasive adenocarcinoma with NEPC, upon the conditional gain of expression of the MYCN gene [70]. As another proposed signaling pathway, induction of PRC2 (Polycomb repressive complex 2) [70], which is a protein complex containing histone methyltransferase activity [111], is included in this model [70]. It is interesting how increased expression of the oncogene MYCN results in invasive adenocarcinoma, but it is accompanied by decreased AR signaling. In addition to the Nkx3.1 model explained in the section describing the PI3K signaling pathway, a different Nkx3.1 model features enhanced Myc transcriptional activity [44]. This model is characterized by the conditional loss of expression of Nkx3.1, a phenotype of HGPIN with microinvasion, and is in the presence of Myc transgene activation that is driven by the CMV enhancer and β-actin promoter [44]. Lastly, the Braf V600E model, which is characterized by the gain of expression of the mutated Braf gene, features senescence bypass and Myc activation [93]. Invasive adenocarcinoma with metastasis is observed in this model [93]. Braf encodes a protein known as B-Raf, which is a member of the Raf kinase family of growth signal transduction protein kinases [112]. The MYCN model and Braf V600E model are also accompanied by loss of Pten function. Furthermore, in the RIPK2 model, receptor-interacting protein kinase 2 (RIPK2) has been found to stabilize c-Myc, and is proposed as a therapeutic target in PCa metastasis [97]. As illustrated by these three models, there is a large overlap in the Myc, AR, and PI3K signaling pathways – all of which are major oncogenic conduits leading to cell growth and proliferation.

5.5. Wnt Pathway

18% of a total 150 patients afflicted with mCRPC (metastatic castration-resistant prostate cancer) were found to harbor alterations in the Wnt signaling pathway in a

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genomic study [106]. The Wnt signaling pathway can be subclassified as canonical Wnt signaling or non-canonical Wnt signaling – both pathways involve Wnt growth factors that bind to a family of Frizzled receptors [113]. In canonical Wnt signaling, and in the absence of Wnt, a complex of Axin and APC allows GSK-3β (Glycogen Synthase Kinase-3β) to phosphorylate and target for destruction of β-catenin [113]. In the presence of Wnt, however, GSK-3β is inhibited, allowing β-catenin to avoid destruction, accumulate, and enter the nucleus [113]. There, β-catenin associates with a group of DNA-binding proteins termed Tcf/Lef, and such protein complexes enable expression of genes that favor proliferation and the stem cell state [104]. In individuals with mCRPC who harbored alterations in the Wnt signaling pathway, hotspot activating mutations in the gene that codes for β-catenin, CTNNB1, were observed [106]. Recurrent alterations were also seen in the TSG known as APC (adenomatous polyposis coli) [106], which plays a critical role in negatively regulating β-catenin levels. By promoting increased differentiation and decreased proliferation, APC prevents formation of an adenomatous polyp or tumor [104].

GEMMs associated with altered Wnt signaling have been relatively understudied compared to models based on other signaling pathways [114]. However, the role of the Wnt signaling pathway should not be underestimated, for it has been validated as a therapeutic target in metastatic prostate cancer [115]. In PBCre4 APCflox conditional knockout mice, PIN followed by local adenocarcinoma is observed [51]. These knockouts are based on a conditional loss of APC, and no distant metastases were displayed [51]. One LADY transgenic model features conditional gain of expression of β-catenin, in collaboration with the gain of expression of a viral oncogene, the SV40 large T-antigen, under the control of the large Probasin promoter [74]. This model displays adenocarcinoma with neuroendocrine differentiation by 18 weeks [74]. Furthermore, co-activation of ALK and N-Myc is able to transform mouse prostate basal stem cells into aggressive PCa with neuroendocrine differentiation [78]. These ALK/N-Myc tumors display activation of the Wnt/β-catenin signaling pathway. If ALK is inhibited, Wnt is also inhibited, leading to suppression of NEPC and neuroblastoma *in vitro*, and suppression of tumor growth and metastasis *in vivo* [78]. Lastly, in the MMP7 model, invasive adenocarcinoma through induction of epithelial-to-mesenchymal transition (EMT) is observed [64]. It was found that IL-17 induces Matrix Metalloproteinase 7 (MMP7) expression to release β-catenin from E-cadherin/β-catenin complex [64]. Released β-catenin was able to promote EMT, allowing tumor cells to break through the basement membrane and promote invasion.

PCa knockout models associated with MYC and Wnt signaling pathways are displayed in Figure 6.

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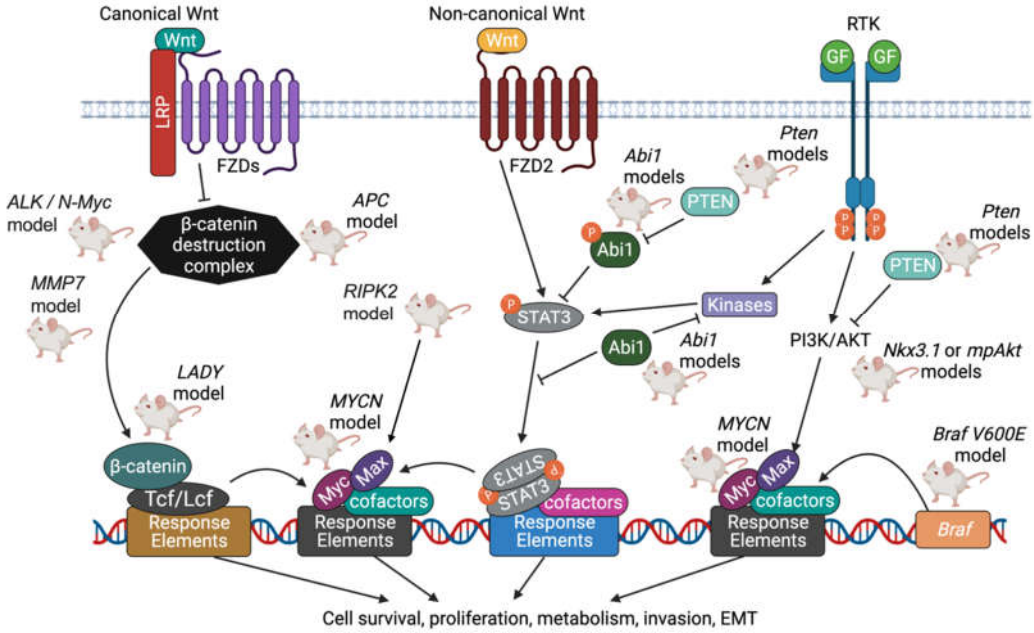

Figure 6. Summary of PCa knockout models associated with MYC and Wnt signaling pathways. Myc family oncoproteins, including c-Myc, N-Myc, and L-Myc, function as growth-promoting transcription factors when expressed in a deregulated fashion. Myc proteins form homodimers with themselves, or as heterodimers with other proteins that share similar structures, before associating with specific DNA response elements. Max, whose levels are increased through the actions of the PI3K pathway, is one protein that associates with Myc. Together, Myc and Max promote advance through the cell cycle by stimulating Cyclin D2/CDK4 and inhibiting $p27^{Kip1}$. In canonical Wnt signaling, Wnt binds to the Frizzled receptor family, and the β-catenin destruction complex (which includes Axin, APC, and GSK-3β) is inhibited. The levels of β-catenin accumulate, and β-catenin complexes with DNA binding proteins termed Tcf/Lef, which together enable expression of genes that favor cell survival and proliferation. In non-canonical Wnt signaling, STAT3 (signal transducer and activator of transcription 3) is activated in response to the binding of Wnt to Frizzled receptors. Proteins such as Abi1 inhibit STAT3 from entering the nucleus and acting as an oncogenic transcription factor [116].

5.6. DNA Repair Pathway

In response to the presence of nucleotides of abnormal chemical structure, an intricate DNA repair pathway will be mobilized to repair the damage and maintain integrity of the genome [104]. Many of the pathways of DNA repair machinery are downstream of and directly induced by TP53, since TP53 levels accumulate upon signals such as a lack of nucleotides, UV radiation, ionizing radiation, and blockage of transcription [104]. One component of the DNA repair pathway is MGMT (O6-methylguanine-DNA methyltransferase), or also known as AGT (O6-alkylguanine-DNA alkyltransferase). This enzyme removes methyl and ethyl adducts from the O6 position of guanine [117]; this is in fact the only system of removing alkylated bases in human cells [104]. Two very important systems associated with repair machinery for single stranded DNA breaks include BER (base excision repair) and NER (nucleotide excision repair). BER enzymes cleave the glycosylic bond linking the modified nitrogenous base to the deoxyribose sugar, and act in response to lesions that do not create structural distortions of the DNA double helix [118]. These lesions are due to endogenous sources, such as reactive oxygen species and depurination events [118]. NER enzymes, however, cleave the entire modified nucleotide from the DNA helix, and act in response to lesions that create large, helix-distorting alterations [119]. These lesions are due to exogenous sources, such as UV photons and chemical carcinogens [119].

The BRCA1 and BRCA2 proteins are perhaps the most well-known constituents of the DNA repair pathway. These proteins gather a group of other DNA repair proteins,

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such as RAD50/Mre11 and Rad51, into large physical complexes to repair double-stranded DNA breaks [104]. Two separate mechanisms of repairing dsDNA breaks are homology-directed repair (HDR) and non-homologous end joining (NHEJ) [104]. HDR involves the presence of sister chromatids and thus is more accurate than the more error-prone NHEJ [104].

Due to the association between the TP53 pathway and DNA repair pathway in the cell, knockout models featuring altered DNA repair pathways are often presented with genomic instability: an outcome frequently observed in TP53 models. As described in the TP53 section, the Chk1 knockout model is characterized by DNA damage signaling and genomic instability upon hemizygous loss of Chk1, and the resulting phenotype is invasive adenocarcinoma [55]. In the Bmi model, invasive adenocarcinoma is also documented [52]. Upon the gain of expression of Bmi, a polycomb ring finger oncogene [120], DNA damage signaling and genomic instability occur [52]. In the HoxB13/Myc model, which features conditional gain of expression of HoxB13 and Myc genes, invasive adenocarcinoma with metastasis is observed [92]. As with most models associated with altered TP53 and DNA repair pathways, the HoxB13/Myc model is also characterized by genomic instability [92]. HoxB13 encodes a transcription factor that is part of the homeobox gene family and functions as a tumor suppressor [121]. The G84E mutation in the HOXB13 gene has been firmly associated with an increased risk for familial PCa [122]. The Chk1, Bmi, and HoxB13/Myc models are also accompanied by loss of the Pten allele.

6. Conclusions

Our knowledge of PCa has greatly increased thanks to the diverse array of models that have been established over the past several decades. Following the development of the first immortalized cell lines, more sophisticated technologies such as gene targeting using embryonic stem cells, Cre-Lox recombination, and CRISPR/Cas9 gene editing allowed for a more comprehensive and accurate understanding of human pathophysiology. The models most commonly used today to study tumorigenesis, including 2D cultures, 3D organoids and spheroids, xenografts and allografts, and knockout mice, have their unique applications, advantages, and disadvantages. After choosing a model, the researcher must decide which gene(s) of interest to manipulate to define the pathway of tumor progression. The resulting phenotype – such as the stage of tumor progression and degree of metastasis – as well as the disrupted molecular signaling pathways elucidate the function of the studied gene in either promoting or inhibiting the progression of PCa. The signaling pathways most often identified in PCa models include AR, PI3K, TP53, Myc, Wnt, and DNA repair pathways. As novel models are being generated, bringing all the models together enables the researcher to identify clearer connections between the genes, stages of tumorigenesis, and signaling pathways linked with PCa. Moreover, these models provide a critical tool for drug sensitivity studies, an obligatory step in translational research.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used Conceptualization of the review, L.K.; literature resources and analysis, R.A., L.K.; writing—original draft preparation, R.A., A.H-J., L.K.; figure preparation, M.A.O., X. L., B.A.P-H. writing—review and editing, R.A., A.H-J., L.K., M.A.O.; clinical aspects; I.N., G.B., L.K.; funding acquisition, L.K. All authors have read and agreed to the published version of the manuscript.

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