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Targeting mRNA-coding genes in prostate cancer using CRISPR/Cas9 technology with a special focus on androgen receptor signaling

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Abstract

Background Prostate cancer is among prevalent cancers in men. Numerous strategies have been proposed to intervene with the important prostate cancer-related signaling pathways. Among the most promising strategies is CRISPR/Cas9 strategy. This strategy has been used to modify expression of a number of genes in prostate cancer cells.

Aims This review summarizes the most recent progresses in the application of CRISPR/Cas9 strategy in modification of prostate cancer-related phenotypes with an especial focus on pathways related to androgen receptor signaling.

Conclusion CRISPR/Cas9 technology has successfully targeted several genes in the prostate cancer cells. Moreover, the efficiency of this technique in reducing tumor burden has been tested in animal models of prostate cancer. Most of targeted genes have been related with the androgen receptor signaling. Targeted modulation of these genes have affected growth of castration-resistant prostate cancer. PI3K/AKT/mTOR signaling and immune response-related genes have been other targets that have been successfully modulated by CRISPR/Cas9 technology in prostate cancer. Based on the rapid translation of this technology into the clinical application, it is anticipated that novel treatments based on this technique change the outcome of this malignancy in future.

Keywords Prostate cancer, Androgen receptor, CRISPR/Cas9

Introduction

The CRISPR system is an innovative genome editing apparatus that is able to reform the outline of cancer research and treatment. Its capacity to accurately target and edit certain genetic mutations that initiate the growth and spread of cancer cells has offered new prospects for the design of more effective and personalized options for treatment of cancer. Historically, it was discovered in the prokaryotes. In 1987, Ishino et al. made a groundbreaking discovery that revolutionized the medical and biological sciences: the recognition of the prokaryotic immune response mechanism CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). While conducting their research on the iap enzyme and its role in converting the alkaline phosphatase isozymes in the periplasm

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of E. coli K-12, they came across a certain set of 29 nucleotide repeats located downstream of the iap gene. These repeats were interspersed with five 32-nucleotide non-repetitive sequences [1–3]. This discovery, sparked intense curiosity and laid the foundations for the subsequent impressive progress in CRISPR research.

In 1989, Francisco Mojica, a researcher from the University of Alicante, discovered an outstanding feature of the CRISPR system. Two years following the discovery of Ishino et al., Mojica discovered a strange structure with a roughly palindromic pattern. This repetitive structure comprised a section of about 30 bases alternating with a spacer of 36 bases [4]. Mojica also observed the same repeats in the intimately associated organism *H. volcanii*, highlighting the intriguing nature of these sequences. He called these structures Short Regular Spaced Repeats (SRSRs). But as the scientific community investigated their function, they were eventually renamed "clustered regularly interspaced palindromic repeats" (CRISPR) [5].

In 2005, the role of CRISPR loci in adaptive immunity was discovered independently by three groups of researchers: Bolotin et al.; Mojica et al. and Pourcel et al. [6]. Their joint observations revealed an intriguing pattern: the spacer sequences of CRISPR loci were not randomly arranged; instead, they appeared to be originated from phage DNA and conjugative plasmids. This discovery implied that the unique spacer regions actually serve as a kind of immune memory and provide protection against invading phage or plasmid DNA [6].

The theory that CRISPR contributed to the adaptive immunity was additionally confirmed by Barrangou et al. They artificially altered the spacer sequences of the CRISPR clusters of phage-resistant *S. thermophiles*. By deleting the spacer, the phage-resistant strains converted susceptible to phage infection again, demonstrating that CRISPR loci play a direct role in adaptive immunity [7].

At the same time, Horvath and his team conducted another experiment that provided further evidence for the CRISPR-adapted immune system notion. They integrated the new phage DNA into the CRISPR array and demonstrated effective protection against further phage attacks [8].

These primitive hypotheses provided intriguing clues about the potential mechanism of CRISPR-encoded defense against phage invasion. In addition, the findings of subsequent research showed that the CRISPR/Cas systems incorporate tiny fragments of foreign DNA into the CRISPR locus. In-depth transcriptional analysis of small RNAs revealed that the spacer sequences between CRISPR loci and certain repetitive sequences were transcribed as an elongated transcript and processed into small CRISPR-associated transcripts, named CRISRR RNA (crRNAs) [6].

Subsequently, Siksnys et al. cloned the complete CRISPR-Cas locus from *S. thermophiles* [9]. Their experiments confirmed the capacity of this system to deliver resistance against foreign plasmids [9]. They also explained how the RuvC domain of Cas9 cuts the non-complimentary DNA strand, while the HNH domain cuts the complementary strand. It was further shown that Cas9 can be trained to target a specific site by altering the crRNA sequence [10]. At the same time, another group found that trans-activating CRISPR RNA (tracrRNA) creates a duplex with crRNA and directs Cas9 to its targets [11]. In 2012, Jinek et al. made a significant scientific contribution with their groundbreaking research [12]. The main focus of their study was on the exact mechanism of Cas9 protein binding to the foreign DNA and its cleavage. They identified the essential components required for the CRISPR/Cas system to operate in genome editing. It turned out that Cas9, the mature crRNA, and the protospacer-adjacent motif (PAM) alone do not trigger the Cas9-mediated cleavage of plasmid DNA. Nevertheless, the inclusion of tracrRNA, a crRNA that attaches to the target DNA, together with magnesium resulted in successful cleavage reactions. Jinek and his colleagues conducted various experiments to understand the function of Cas9 and tracrRNA in the CRISPR/Cas system [12]. After understanding the process, they designed small guide RNAs (sgRNA) that resembled the tracrRNA-crRNA complex, and simplified the process of an adaptable system. Since Cas9 is an RNA-programmed enzyme, it permit targeting and cleavage of certain DNA sequences [12].

In 2013, another major breakthrough was accomplished. In fact, it was development of two orthologs of Cas9 from *S. thermophilus* and *S. pyogenes*, which were successfully applied to mouse and human cells [13]. During the same year, another group developed type II bacterial CRISPR/Cas system with modified gRNA in human cells, which opened up new opportunities for CRISPR technology [14].

The CRISPR/Cas9 system has been further modified and adapted for various types of genomic applications, including genome regulation [15]. This development illustrates the power and flexibility of the CRISPR/Cas9 system in a wide diversity of genome-related studies [15]. Figure 1 shows a schematic representation of different milestones in the application of CRISPR/Cas9 technology.

CRISPR/Cas structures and mechanisms

There are three major types of CRISPR/Cas systems described as Type I (Cas3), Type II (Cas9) and Type III (Cas 10) [16]. Type I Cas genes encode helicases having ATPase activity on single-stranded DNA [17]. Type II Cas genes encode a multiple-domain protein working for

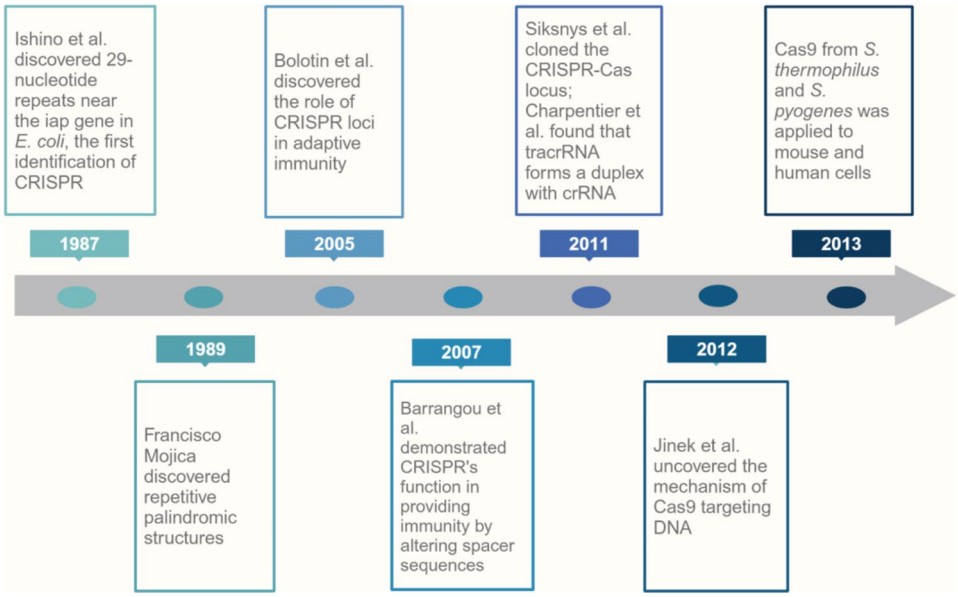

Fig. 1 Timeline of important events leading to application of CRISPR/Cas9 technology

the DNA cleavage and crRNA maturation [12]. In combination with Cas9, all CRISPR/Cas systems with Type II genes contain Cas1 and Cas2. Type III CRISPR/Cas genes encode Cas10, a multiple domain protein with palm domain [18]. In addition to Cas10 which is the active unit of this type of systems, other subunits exist in Type III loci, including a subunit that encodes Cas5 groups and numerous RAMP subunits for Cas7 [19]. While Cas9 is helpful for editing purposes, the method of cleavage of Cas3 makes it unsuitable for this purpose. In fact, Cas3 pinpoints its target sequence and starts single stranded DNA degradation in a way that can continue up to multiple kilobases [20]. Finally, Type III effector systems specifically spot and cleave RNA molecules [21].

The CRISPR/Cas systems exist on both chromosomal and plasmid DNA [22]. These systems are a portion of adaptive immune systems evolved by bacteria and archaea to guard themselves against invading phages and are made up of diverse CRISPR and Cas genes. The large Cas effector protein, which has one multidomain per protein, forms a complex with crRNA during the interference process [23].

CRISPRs contain a leader (which is adjacent to the first repeat of the CRISPR loci and is the promoter of CRISPR arrays), short direct repeats (which form hairpin configuration to stabilize the secondary structure of the RNA), and non-repetitive spacers (which capture foreign DNA sequences) [24, 25].

The CRISPR/Cas9 class II systems expressed by bacteria and archaea can hold a small section of the viral gene, a so-called spacer, in to the CRISPR array. When the same virus re-invades the cells, the bacteria can distinguish the virus from the spacer and lyse the virus by cutting its DNA. This process includes three phases, namely adaptation, expression and interference. When the foreign DNA enters the host cell, Cas1 and Cas2 proteins distinguish the PAM in the foreign DNA structure, and afterwards catch the DNA sequence neighboring the PAM as a protospacer [26]. Then, the Cas1/2 protein complex cuts the protospacer from the foreign DNA to make a spacer that is incorporated between two repeat sequences at the 5' end of the CRRSPR array [27, 28]. At the expression phase, CRISPR array is transcribed to create tracrRNA and crRNA precursor. The latter is trimmed into mature crRNA by the assistance of RNase III nuclease and Cas9. Then, repeats and tracrRNA make a double-stranded RNA (tracrRNA-crRNA) through pairing of complementary bases. This structure accumulates into the effector complex with the nuclease and Cas9 [29]. When the tracrRNA: crRNA duplex is processed, it can direct Cas9 to DNA sequences corresponding to the crRNA guide sequence [30]. In the interference phase, the Cas9 effector protein binds to the guide RNA before target recognition and cleavage. Afterwards, Cas9 protein is directed to the appropriate PAM sequence in a sequence-specific manner [31]. PAMs have a crucial role in the discrimination of self DNA from foreign DNA, since they are present only in the exogenous DNA, preventing the activation of the CRISPR mechanism for self-deletion. In fact, protospacer sequences inserted into the CRISPR locus are not cut since they are not adjacent to a PAM sequence [32].

In brief, pairing of crRNA with the complementary bases on the target strand creates an R-loop configuration that ultimately causes cutting of the target and non-target strands by Cas9, leading to flat-end cut at three

nucleotides upstream of the PAM [33]. This cleavage leads to formation of a double-strand break in the target sequence which in turn triggers two repair mechanisms: (1) non-homologous end joining (NHEJ) and (2) Homology directed repair (HDR). NHEJ pathway is an error-prone repair mechanism that repairs DNA double-strand break by inserting indels, thereby leading to diverse mutations in the open reading frames of the targets region [34, 35]. HDR is an innate DNA repair mechanism, which is triggered by double-strand DNA breaks [36]. This DNA repair mechanism could be used to repair the DNA double-strand breaks mediated by CRISPR [33, 37]. The DNA cleavage induced by sgRNA can be repaired by introducing a donor template into the target cells. The donor DNA harbors the intended insertions or modifications. The insertions are flanked with DNA segments that are homologous to the cleaved DNA. Therefore, the cells HDR mechanism inserts the desired gene into the targeted DNA segment by utilizing the donor template [14, 38].

CRISPR/Cas9 in cancer treatment

Although there has been considerable advancement in cancer treatment in recent decades, the continued mortality rate from this disease demonstrates the critical necessity for novel and more competent therapeutic modalities. CRISPR/Cas9-mediated genome editing is a potent research tool which provides vast promise in cancer therapeutic application, and it has been widely utilized in the research laboratories, due to its high specificity, efficiency, and accuracy, which have facilitated detection of the role of various oncogenes and tumor suppressors in the cancer cells [39–43]. The CRISPR/Cas9 tool has been also exploited to specifically target tumor-suppressors in order to suppress or diminish the tumorigenesis by reestablishing the functions of these genes [44]. It has also been used to recognize the heterogeneity of cancer cells and possible therapeutic targets for diverse cancer cells [45]. This technique has a potential to be used for repair of specific mutations, gene editing, knockdown of specific oncogenes, and engineered T cell immunotherapy [46]. In addition, CRISPR/Cas9 tool was used as a programmable base editing system to telomerase in order to demonstrate that base-editing of TERT rigorously affects cancer cell survival. In fact, haploinsufficiency of TERT resulted in telomere abrasion and growth retardation, showing the potential of TERT inactivation in cancer therapy [47]. The capacity of CRISPR/Cas9 system for editing of oncogenic mutations has also been confirmed [46]. CRISPR/Cas9 has also been applied in the clinical settings. In a phase I clinical trial, CRISPR/Cas9 PD-1-edited T cells were used in patients with advanced lung cancer. Results confirmed the feasibility and safety of this technique [48]. Here, we discuss the novel therapeutic aspects of this technology in the treatment of prostate cancer.

Prostate cancer

Prostate cancer is a very complicated disease with different genetic factors contributing to its pathogenesis [49]. Recent advancements in CRISPR technology offer a novel approach to targeting the specific genes associated with prostate cancer, leading to the development of new therapeutic strategies [50]. We discuss these studies in different sections of this article.

CRISPR-mediated modification of androgen receptor (AR) signaling

Due to the importance of AR in the pathogenesis prostate cancer, different methods were used to modulate this signaling pathway in prostate cancer. At the gene level, small interference RNA was used with great success [51, 52]. In addition, next-generation AR signaling inhibitors, bone-targeting substances, and poly(ADP-ribose) polymerase inhibitors have also been developed for such purpose [53]. CRISPR technology was also used by several groups as a tool for modification of this pathway (Table 1). For instance, Wei et al. employed the CRISPR/Cas method to effectively eliminate the *AR* gene in androgen- positive prostate cancer cells. Three distinct single sgRNAs were developed to target various regions of the *AR* gene. The investigators discovered the optimal sgRNA that effectively targeted the *AR* gene, leading to the inhibition of androgen-sensitive prostate cancer development. The AR sgRNA-guided CRISPR/Cas method induced targeted breaks in the *AR* gene, leading to cellular apoptosis and inhibition of the cell proliferation. The results showed that the CRISPR/Cas system might be an effective weapon for the treatment of this cancer [54].

Several other genes were found to be regulated by AR signaling. For instance, prostate-specific antigen (PSA) is among androgen-responsive genes [55]. This proto-oncogene is widely found in embryonic and prostate cancer cells, and is absent in normal prostate cells. A research team inspected the conditional transcriptional regulation of the PSA promoter and its application for the gene therapy. A CRISPR/dCas9-KRAB system was constructed using the PSA promoter to repress transcription of the *PSA* gene. This inhibition consequently resulted in the blockage of tumor growth and migration, and induction of apoptosis. Therefore, this study underlined the ability of the CRISPR-dCas9-KRAB system to control PSA expression as a therapeutic strategy for the prostate cancer gene therapy [56].

The steroid type II 5-alpha-reductase (SRD5α2) enzyme facilitates the conversion of testosterone to dihydrotestosterone, which are hormones associated with prostate cancer and benign prostatic hyperplasia.

Table 1 CRISPR-mediated modification of AR signaling in prostate cancer

Target gene	Study type	Cell line	Vector	Screening/verification	Experimental data	Ref
AR	In vitro	LNCaP	Lentiviral	PCR, CCK-8 assay, annexin V apoptosis assay	Reduced cell proliferation due to enhanced apoptosis	[54]
		LNCaP, R1-AD1	Plasmid	QPCR, sequencing, western blotting	High levels of tumor-specific AR variants and resistance to endocrine treatments in cell lines with AR gene rearrangements	[86]
		PacMet-UT1	Lentiviral	T7 endonuclease I assay, western blotting	Increased activity in the FGF and MAPK pathways	[87]
		C4-2, CWR22Rv1	Plasmid	Western blotting, qRT-PCR	Decreased cell proliferation	[88]
		LN-95	Plasmid	FACS, genotyping, western blotting, RNA-seq, IHC	Not necessarily leading to neuroendocrine differentiation	[89]
	In vitro, in vivo	LNCaP	Plasmid	IHC, RNA-Seq, qRT-PCR, luciferase assay, clonal and clonogenic assays, BrdU incorporation assays	Distinctive biological and tumorigenic characteristics, showing contrasting responses to enzalutamide as a therapeutic target	[90]
AR, NKX3-1	In vitro	LNCaP	Lentiviral	QPCR	Validation of the regulatory roles of specific enhancers in the expression of NKX3-1 and AR genes	[91]
AR, FABP5	In vitro	PC3-M, DU145, 22RV1	CRISPR/Cas9	Flow cytometry, western blotting, PCR, DNA sequencing, RNA sequencing	Inhibition of malignant cell characteristics, partly by disrupting the VEGF signaling pathway	[92]
AR-FL	In vitro	CWR22Rv1	Plasmid	Nucleofection, SURVEYOR assay, PCR and sequencing, FACS, western blotting, qPCR, RNA sequencing	AR variants drive cell growth and androgenic gene expression independently of FL-AR loss	[93]
AR-FL, AR-V7	In vitro	LNCaP-95	Plasmid	FACS, western blotting, genotyping, RNA sequencing, IHC	Identification of the necessity of AR-FL and AR-V7 in conferring resistance to abiraterone and enzalutamide	[94]
ERG	In vitro	Pten−/− R26-ERG organoids	Lentiviral	Histology, IHC, RNA-seq, ChIP-seq, ATAC-seq, proteomics, RIME	Significantly reduced AR-dependent gene expression	[95]
TMPRSS2-ERG	In vitro	Mouse prostate organoids	psCas9 plasmid	Puromycin selection, qPCR	Overexpression of ERG due to AR activity	[62]
GATA2	In vitro	22Rv1	Plasmid	Western blotting, qRT-PCR, single clone isolation, genotyping	Identification of GATA2 amplification's role in enhancing TGFβ1 and AR signaling pathways	[96]
GREB1	In vitro, in vivo	LNCaP/AR	Lentiviral	Single-cell cloning, flowcytometry	Restoration of enzalutamide sensitivity in cells with high AR output	[97]
TLE3	In vitro	LNCaPCWR-R1, 22Rv1, LAPC4	Lentiviral	Real-time live cell proliferation monitoring, colony formation assays, RNA-seq, ChIP-seq, ChIP-qPCR, western blotting, (IHC), qPCR	Increased resistance to AR inhibitors, including apalutamide and enzalutamide	[98]
DOT1L	In vitro	LNCaP, PC3	Lentiviral, plasmid	QRT-PCR, RNA-seq, Microarray, ChIP, PCR, LC–MS/MS	Reduced viability of AR-positive prostate cancer cells	[99]
SF3B2	In vitro	22Rv1, LNCaP95	Plasmid	Western blotting, PAR-CLIP	Identification of SF3B2 as a key factor in the expression of AR-V7	[100]
TGM2	In vitro	PC3	Plasmid	Sequencing, western blotting	Increased AR transcription and decreased MUC1 expression	[101]
LCMT1	In vitro	HAP1, LNCaP, VCaP, LNCaP-AR, LAPC4	lentiviral	Immunoblotting	Enhanced AR activity, promoting the growth of castration-resistant prostate cancer	[102]
PARP-1, PARP-2	In vitro, in vivo	LNCaP	Lentiviral, plasmid	ChIP assay, RNA-seq, ChIP-seq, cell viability, soft agar colony formation assay	Selective inhibition of PARP-2 disrupts its interaction with FOXA1, leading to reduced AR-mediated gene expression and inhibited growth of AR-positive prostate cancer	[103]

Table 1 (continued)

Target gene	Study type	Cell line	Vector	Screening/verification	Experimental data	Ref
IP6K2, XPO4, DRG1, PRKAB1, RP2	In vitro	C4, LNCaP, PC3, DU145, LAPC-4	Lentiviral, plasmid	Sanger sequencing, RT-qPCR, dose-response assays, AlamarBlue assay, quant-seq analysis	Altered response to enzalutamide following IP6K2 and XPO4 knockout due to deregulation of AR, mTORC1, and E2F signaling pathways	[104]
AREM1, AREM2	In vitro	C4-2B	CISPR/Cas9	Flow-sorting, qPCR, ChIP-qPCR, ChIP-seq	Significant decrease in AR mRNA levels	[105]
hnRNP A1	In vitro	CWR22Rv1	Plasmid	Puromycin selection, FACS, genomic PCR, western blotting	Increased expression of AR3	[106]
CAMKK2	In vitro	LNCaP, 22Rv1	Lentiviral, plasmid	Western blotting, immunofluorescence microscopy	Demonstrating that AR can utilize the CAMKK2-AMPK-ULK1 signaling pathway to stimulate prostate cancer by enhancing autophagy	[107]
		LNCaP	Plasmid	Western blotting, qRT-PCR	Decreased expression of two important lipogenic enzymes, acetyl-CoA carboxylase and fatty acid synthase	[108]
	In vitro, in vivo	C4-2	Lentiviral, plasmid	Western blotting	Impaired tumor growth	[109]
SOX2	In vitro	CWR22-R1	CRISPR/Cas9	Western blotting	Modified gene expression profiles, particularly in pathways associated with resistance to AR antagonists	[110]
YAP1	In vitro	LNCaP	Plasmid	Western blotting, qRT-PCR, immunofluorescence and microscopy	Revealing that androgen differentially regulates YAP1-dependent gene expression	[111]
G3BP1	In vitro	LNCaP, 22Rv1	Lentiviral, plasmid	Immunoblot, CellTiter96, xenograft studies, RNA-seq, qRT-PCR	Revealing a G3BP1-SPOP ubiquitin signaling axis that promotes PCa progression through activating AR signaling	[112]
EZH2	In vitro	16DCRPC	Plasmid	GeneArt genomic cleavage detection, FACS, western blotting, sanger sequencing	Establishing a collaborative role for AR and EZH2 in promoting drug resistance	[113]
	In vitro, in vivo	PC-3	Plasmid	SURVEYOR assay, western blotting, MTT assay, wound healing and proliferation assays, qRT-PCR, annexin V-PI assay	Decreased H3K27me3 levels and increased apoptosis	[114]
EZH2, SETD2	In vitro, in vivo	C4-2, LNCaP	PX330 plasmid	QRT-PCR	Demonstrating the role of the SETD2-EZH2 axis in linking metabolic and epigenetic signaling pathways to suppress prostate cancer metastasis	[115]
PSA	In vitro	LNCap, PC3, AT3B-1, DU145, RWPE1	Plasmid	Luciferase assay, qRT-PCR, MTT assay, colony formation assay, cell migration assay, transwell migration assay, annexin V-PI assay	Reduced cell proliferation and migration, enhanced apoptosis	[56]
Survivin	In vitro	PC3	Plasmid	Colony PCR, sequencing, qRT-PCR, XTT assay, annexin V-PE/7- AAD assay, CCK-8 assay	Induced apoptosis and downregulation of FBXO5 and RRM2	[59]
SPOP	In vitro	LNCaP,	Plasmid	Genomic DNA and cDNA sequencing	Elevated GLI3 protein levels, indicating decreased degradation	[116]
	In vitro, in vivo	DU145	Lentiviral	Western blotting, real-time quantitative PCR, IP, in vivo xenograft assay	Identifying SPOP as a tumor suppressor that promotes the ubiquitination and degradation of NANOG	[117]
SPOP, Caprin1	In vitro	293T, LNCaP, 22Rv1, PC-3, DU-145, C4-2	PX459 plasmid	Western blot, sanger sequencing, cell cycle distribution and cell death, qRTPCR, EdU incorporation assay, immunofluorescence, migration assay	Resistance to cell death induced by stress granule inducers (e.g., docetaxel, sodium arsenite, and H2O2)	[118]
SPOP, HIPK2	In vitro, in vivo	PC-3, DU145	PX459 plasmid	Western blot, sanger sequencing, CCK-8 assay, colony formation assay	High levels of genomic instability	[119]

Table 1 (continued)

Target gene	Study type	Cell line	Vector	Screening/verification	Experimental data	Ref
SPOP, SQSTM1/p62, ATG3, ATG5, ATG7, NFE2L2	In vitro	PC-3, DU145	PX459 plasmid	Western blotting, sanger sequencing	Increased autophagy and activation of Nrf2	[120]
FOXA1	In vitro	LNCaP	Plasmid	Western blotting, dot blotting	Altered expression of Casp-9, Bax, CCND1, CDK4, and fibronectin; no changes observed in Casp-3, Bcl-2, survivin, β-catenin, c-Myc, and GSK-3B; inhibition of CCND1 protein expression in LNCaP cells	[121]
		LNCaP, 22Rv1	Lentiviral, plasmid	Western blotting, cell proliferation assay, luciferase reporter assay, allele-specific ChIP-qPCR	Reduction in FOXA1 expression, leading to decreased cell growth	[122]
		LNCaP, 22RV1	Lentiviral, plasmid	Sanger sequencing, ChIP-seq	Reaffirming FOXA1 central role in mediating androgen receptor-driven oncogenesis	[123]
		LNCaP, LNCaP 42D, LNCaP 42 F	Lentiviral	Immunoblot, western blotting, immunohistochemistry	Inhibited cell proliferation	[124]
		LNCaP	Lentiviral	Puromycin selection, PCR, qRT-PCR, sanger sequencing	Upregulation of TGFB3, a gene encoding a ligand in the TGF-β pathway	[125]
	In vitro, in vivo	Mouse prostate organoids, Rosa26-Cas9 organoids	Lentiviral, plasmid	Western blotting, RNA-seq, ATAC-seq, ChIP-seq	Disruption of normal luminal epithelial differentiation programs	[126]
FOXA1, FOXP1, PTEN	In vivo	MEF cells from LSL-Cas9 mice, HEK293T	AAV-plasmid	Histochemical analysis, PCR	Induction of epithelial plasticity due to FOXA1 loss and increased cell proliferation due to FOXP1 loss	[127]
NANOG and NANOGP8	In vitro, in vivo	DU145	PX330 plasmid	PCR, genotyping, western blotting	Reduced malignant potential, diminished sphere formation, anchorage-independent growth, migration ability, and chemoresistance	[61]
SRD5α2	In vitro	DU145	A cationic liposome preparation carrying sgRNA on its surface	QRT-PCR	Discovery of a novel gene-editing approach targeting SRD5α2 to offer alternative treatments for prostate cancer without the adverse effects of current medications	[57]
CHD1	In vitro	22Rv1	CRISPR/Cas9	Immunoblotting, IHC, FISH, DNA sequencing	Improved response rate and extended efficacy during abiraterone treatment	[128]
	In vitro, in vivo	22Rv1, RWPE-1	Plasmid	FACS, SURVEYOR mutation assay, PCR, sanger sequencing	Increased vulnerability to DNA damage, reduced efficiency of error-free HR repair, and elevated reliance on error-prone NHEJ repair, leading to genomic instability	[129]
BRCA2	In vitro	LNCaP	Lentiviral	Western blotting	Heightened sensitivity to SPA, resulting in increased DNA damage and apoptosis	[130]
		LNCaP	Lentiviral	Sanger sequencing, qPCR	Elevated SRC phosphorylation and greater responsiveness to SRC inhibitors like dasatinib, bosutinib, and saracatinib	[131]
BRCA2, RB1	In vitro	LNCaP, 22RV1	Lentiviral	FISH, western blotting, qPCR, RNA sequencing	Induced epithelial-to-mesenchymal transition, leading to greater invasiveness and a more aggressive cancer phenotype	[132]
TP53, RB1	In vitro, in vivo	LNCaP	Lentiviral, plasmid	Western blotting	High proliferation rates, resistance to different therapies	[133]

Table 1 (continued)

Target gene	Study type	Cell line	Vector	Screening/verification	Experimental data	Ref
HOXB13	In vitro	22Rv1, DU145, LNCaP, C4-2, PC3, RWPE-1, VCaP, C4-2B	Plasmid, CRISPR/Cas9 constructs (GFP expressing)	Western blotting, qPCR, immunofluorescence studies, MTT assay, BrdU assay, wound scratch assay	Induction of apoptosis and strong suppression of tumor cell proliferation and migration	[134]
		CWR22Rv, LAPC4	Lentiviral, plasmid	Western blotting	Altered cell proliferation patterns	[135]
CTCF	In vitro	22Rv1	Lentiviral, plasmid	PCR, sanger sequencing, western blotting	Reorganization of CTCF looping and changes in H3K27ac loops at the MYC region	[136]
SMYD2	In vitro	CWR-R1ca	Plasmid	Western blotting, wound-healing assay, colony formation assay, transwell assay	Decreased viability, reduced migration and invasion capacity, and fewer colonies formed	[137]
SYNCRIP	In vitro	LNCaP/AR, CWR22Pc, MDA-PCa-2b	Lentiviral, plasmid	CellTiter-Glo, flowcytometry, western blotting	Increased APOBEC-driven mutagenesis	[138]
p54/nrb (NONO)	In vitro, in vivo	CWR22Rv1	Plasmid	Western blotting, qRT-PCR, FACS, genomic PCR	Lowered PCGEM1 expression, leading to reduced tumor growth	[139]
ARNTL	In vitro, in vivo	LNCaP, LNCaP-42D, LNCaP-ResA	Lentiviral, plasmid	Western blotting, puromycin selection, cell viability and xenograft studies	Inhibited growth of prostate cancer cells	[140]
(N-terminal domain of) PIP5K1α	In vitro, in vivo	LNCaP C4-2, LNCaP C4-2 SG	CRISPR/Cas9	Sequencing	Reduced ability of cancer cells to grow and migrate	[141]
NKX3.1	In vivo	C57BL6, Swiss-Webster, B6D2F1 mice	PX330 plasmid	Genotyping, histologic analysis, in situ TUNEL staining	Allelic loss of Nkx3.1 led to decreased Nkx3.1 protein level and increased proliferation	[64]

Suppression of SRD5α2 activity is regarded as a possible therapy for these disorders; however, current pharmacological treatments have certain side effects, and novel therapeutic modalities are desirable. Akbaba et al. established a cationic liposome preparation that encapsulates Cas9 protein and carries sgRNA on its surface. Such well-structured liposome exhibited favorable physicochemical characteristics, stability, low cytotoxicity, efficient cellular intake, and high gene knockout efficiency. The Lipo/Cas9 with sgRNA resulted in a 29.7% reduction in relative SRD5α2 mRNA expression. The research indicated mentioned liposomal formulation is a promising method for treating prostate cancer or BPH while avoiding the side effects associated with current medications [57].

Survivin is another molecule that plays a role in both AR-dependent and AR-independent drug resistance in prostate cancer. Its expression was up-regulated by androgen in both AR-positive LNCaP and AR-negative prostate cancer cells [58]. CRISPR-mediated silencing of Survivin induced apoptosis of prostate cancer cells [59].

NANOG is another factor that is involved in AR signaling. It can reprogram prostate cancer cells to castration resistance through suppressing the AR/FOXA1 signaling route [60]. In another study, Kaneda and colleagues demonstrated the role of NANOG and its pseudogenes in prostate cancer. Using the CRISPR/Cas9 technology, researchers obtained NANOG- and NANOGP8-knockout DU145 cells and examined their malignant features. Knockout of NANOG and NRFP8 significantly inhibited various malignant characteristics of these cells, namely sphere construction, anchorage-independent growth, migration ability, and chemoresistance. NANOG and NANOGP8 knockout did not suppress in vitro cell proliferation. However, the tumorigenic ability of cells was reduced significantly. It was revealed that both NANOG and NANOGP8 have an equivalent role in the aggressiveness of prostate cancer. This study highlighted the possibility of CRISPR-based targeting of NANOG and NANOG88 for the treatment of prostate cancer by inhibiting the malignant characteristics of cancer cells [61].

Another group achieved a great success by developing mice prostate models with the TMPRSS2-ERG gene fusion, a common genetic alteration in prostate cancer. Gene fusion was successfully induced by a CRISPR/Cas9-based technique, leading to the overexpression of ERG, a crucial factor in prostate development, in cancer cells. Treatment with the AR antagonist nilutamide led to the restoration of the fusion transcript expression to normal ERG levels. This novel model offered a helpful platform for studying the underlying processes of TMPRSS2-ERG fusion and exploring potential therapeutic interventions [62].

NKX3.1 is a homeodomain transcription factor that has androgen-responsive regions in its 3' untranslated region [63]. The suppressor gene NKX3.1 has a crucial role in prostate cancer by acting as a gatekeeper. NKX3.1 is haplo-insufficient, and pathogenic decrease in its protein level might be due to genetic loss, reduced transcription, and elevated protein degradation resulting from inflammatory reactions or PTEN loss. CRISPR/Cas9 method was used to introduce a missense mutation in murine Nkx3.1 to substitute alanine with serine at aminoacid 186, the region that is target for Dyrk1b phosphorylation. The $Nkx3.1^{S186A/-}$ mouse exhibited elevated Nkx3.1 protein levels, decreased prostate size, normal histology, decreased cell growth as well as enhanced DNA labeling. The findings demonstrated that modification of only Nkx3.1 can lead to longstanding control over precancerous alterations related to DNA damage in the prostate. This also displayed the feasibility of CRISPR-guided modulation of NKX3.1 as a therapeutic strategy for prostate cancer [64].

Figure 2 demonstrates a number of genes in the AR signaling that were targeted by CRISPR/Cas9 in prostate cancer.

CRISPR-mediated modification of PI3K/AKT/mTOR pathway

PI3K/AKT/mTOR pathway as one of the important pathways in the carcinogenesis of prostate cancer has been the focus of several CRISPR-mediated knockout studies in this field (Table 2). PI3K/AKT pathway is hyperactivated in nearly all advanced prostate cancers, mostly *via* PTEN loss [65, 66]. The serine/threonine protein kinase mTOR belongs to the PI3K/AKT/mTOR pathway and controls cell growth, proliferation, survival, autophagy, and metabolism [67]. This protein establishes two distinctive complexes, namely mTORC1 and mTORC2. Notably, DEPTOR is a common constituent of both complexes that directly binds to mTOR to inhibit the activity of both complexes [68]. DEPTOR expression was considerably reduced in prostate cancer samples, in correlation with disease progression. CRISPR-mediated depletion of DEPTOR enhanced proliferation, survival, migration, and invasive capacity of these cells. This intervention led to activation of cell proliferation and survival, and induction of an AKT-dependent epithelial–mesenchymal transition (EMT) and nuclear translocation of β-catenin leading to cell migration and invasive features. Most notably, Deptor knockout speeded prostate tumor growth in animal models through activation of mTOR signaling. Therefore, reactivation DEPTOR might be suggested as a therapeutic strategy for prostate cancer [69].

In another recent study, a flexible aptamer-liposome CRISPR/Cas9 chimera was developed for effective and targeted transfer of CRISPR/Cas9 into specific cells. The chimera had an RNA aptamer that precisely bound cells that expressed the prostate-specific membrane

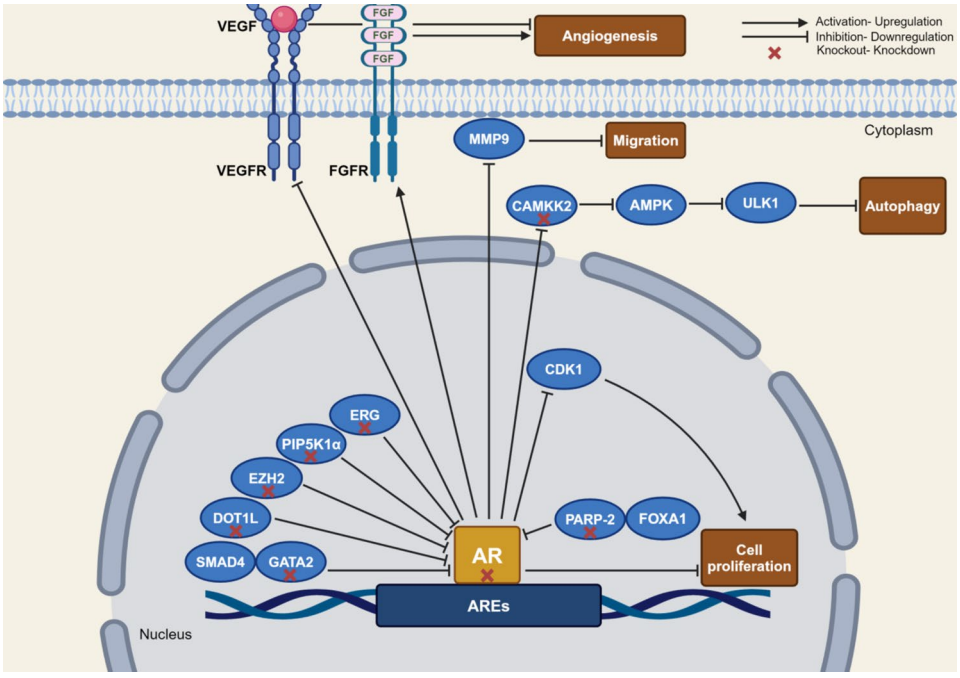

Fig. 2 An overview of different genes in the AR signaling targeted by CRISPR/Cas9 in prostate cancer, and consequences of gene editing on cell proliferation, autophagy, migration and angiogenesis. Figure was depicted using BioRender

antigen. Conjugating cationic liposomes with aptamers enabled directed delivery of therapeutic CRISPR/Cas9 molecules to tumor cells, resulting in significant gene silencing effects in vitro. Besides, in vivo assays demonstrated that prostate cancer regressed following gene silencing. This innovative approach offers a universal method for cell-type-specific transfer of CRISPR/Cas9, addressing an important challenge for the extensive therapeutic application of CRISPR/Cas9 and other nucleic acid agents [70].

In addition, CRISPR/Cas9 technology was employed to simultaneously modify several genes in mouse prostate cells. The researchers focused on investigating the function of the activating protein-1 (AP-1) transcription factor, which is composed of JUN and FOS subunits and has a crucial role in several types of cancer. They discovered that loss of either JunB or Fos, in conjunction with Pten loss, resulted in the progression of prostate cancer to an invasive stage. On the contrary, lack of Fos enhanced the expression of Jun and CRISPR knockout of Jun also reduced cell proliferation in this context. In brief, JunB and Fos exhibited tumor suppressor functioned by suppressing invasive disease. However, Jun functioned as an oncogene and increased cell proliferation. This demonstrated that AP-1 factors are involved in the different stages of progression of this type of cancer and displayed dual functions as tumor suppressor and oncogene in this process [71].

In another study, GPRC6A, a gene associated with prostate cancer, was investigated, focusing on a polymorphism known as "Y.K" situated in the third intracellular loop that evolved in most humans and replaced the ancestral RKLP. Notably, this variant is prevalent in all human-originated prostate cancer cell lines. The researchers created GPRC6A-deficient PC-3 cells using CRISPR/Cas9 technique and examined its consequences on cell proliferation and migration. Their findings demonstrated that these cellular responses were blocked by targeting GPRC6A in vitro. At the same time, in a xenograft mouse model, their evidence indicated that the proliferation of GPRC6A-deficient PC-3 cells and their resistance to osteocalcin-related progression of prostate cancer was reduced compared to control cells that expressed GPRC6A. The results suggested that GPRC6A might be a potential antagonist for treating prostate cancer, particularly in addressing racial disparities in the disease [72].

The androgen-responsive gene, NKX3.1 can also affect the activity of PI3K/AKT pathway. CRISPR-mediated deletion of exon 1 of this gene led to increased weight of prostate. Ki67 was densely stained in the epithelial cells of these animals, while p53 levels were suppressed in these cells. In addition, PI3K/AKT/mTOR pathway was inhibited in NKX3.1-knockout cells [73]. Figure 3 shows selected genes from PI3K/AKT/mTOR pathway that have been targeted by CRISPR/Cas9 in prostate cancer.

Table 2 CRISPR-mediated modification of PI3K/AKT/mTOR pathway in prostate cancer

Target gene	Study type	Cell line	Vector	Screening/verification	Experimental data	Ref
DEPTOR	In vitro	DU145, 22RV1	PX459 plasmid	Puromycin selection	Enhanced cell proliferation, survival, migration, and invasion, activated AKT-dependent EMT, and nuclear translocation of β-catenin	[69]
BLM	In vitro	PC-3	Plasmid	Immunohistochemistry, western blotting, T7 endonuclease I assay, PCR	Lower level of phosphorylated AKT (Ser473) and PRAS40 (Thr246)	[143]
ELOVL5	In vitro, in vivo	C4-2	Plasmid	Western blotting, qRT-PCR	Reduced AKT-mTOR signaling activity	[144]
SIRT5	In vitro	PC-3	Plasmid	Western blotting, FACS	Elevated IL-1β expression and increased PI3K/AKT/NF-κB signaling	[145]
	In vitro	PC-3	Plasmid	Western blotting, FACS, wound-healing assay, transwell assay, CCK-8 assay	Increased cell invasion capability	[146]
AKT 1/2	In vitro, in vivo	CWR22rv1	Lentiviral, plasmid	Puromycin screening, western blotting, sequencing	Suppressed metastasis	[147]
KDM1A (LSD1)	In vitro	22Rv1	Lentiviral	Puromycin screening, immunoblotting	Inhibited PI3K/AKT signaling	[148]
ATF3	In vitro	LNCaP, DU145, PC3	Plasmid	Western blotting, Surveyor mutation detection assay, qRT-PCR, zymography	Promoted AKT activation, resulting in enhanced cell proliferation	[149]
CKB	In vitro	PC3	PX458 plasmid	Sanger sequencing, western blotting, flow cytometry	Activation of AKT, driving EMT and cell migration	[150]
ARID4B	In vitro, in vivo	PC3, DU145	Plasmid	Western blotting, MTT assay	Identification of ARID4B as a key regulator in the PTEN-PI3K pathway	[151]
NKX3.1	In vivo	C57BL/6 mice	Cas9 protein and sgRNA	Western blotting, IF staining, IHC staining	Inhibition of the PI3K/AKT/mTOR pathway	[73]
PSMA	In vitro	TRAM,22RV1	Lentiviral, plasmid	Puromycin selection, sequencing, TIDE	Decreased PI3K-AKT signaling, with increased MAPK-ERK1/2 signaling	[152]
PPARG	In vitro, in vivo	PC3-M	Plasmid	Puromycin selection	Identification of a functional impact of PPARG on AKT3, associated with a more aggressive disease phenotype	[153]
PTEN	In vitro, in vivo	MSK-PCa1, MSK-PCa2, MSK-PCa3, MSK-PCa8, MSK-PCa11, MSK-PCa12, MSK-PCa15, MSK-PCa16, LNCaP, DU145, CWR22Pc, CWR22Pc-EP	Lentiviral	Sequencing	Effective pan-PI3K inhibition, with upregulation of IGF1R and promoted resistance	[154]
	In vitro	2924 V	PX459 plasmid	DNA sequencing, western blotting, MTT assay	Enhanced phosphorylation of RAC alpha serine/threonine protein kinase and increased cyclin D1 level	[155]
PTEN, Fos, Jun	In vivo, in vitro	BPH-1, PC-3, DU145, murine prostate epithelium	Lentiviral, plasmid, AAV	Validation of guide RNA efficiency, Histology, IHC, qRT-PCR, sanger sequencing	Loss of Fos contributing to disease progression through accelerated proliferation and invasiveness, partly via Jun activation	[156]
PTEN, α6-integrin, β4-integrin, plectin	In vitro, in vivo	RWPE1, DU145, PC3, 22Rv1, LNCaP, VCaP, JIMT-1	Lentiviral, plasmid	Western blotting, qRT-PCR, proliferation assay, soft agar assay, RNA-Seq	Activation of EGFR/PI3K/Akt and FAK/Src	[157]
WHSC1	In vitro, in vivo	PC3	Plasmid	Western blotting, qRT-PCR, ChIP	Condensed chromatin structure at the RAC1 gene locus, leading to reduced transcription	[158]

Table 2 (continued)

Target gene	Study type	Cell line	Vector	Screening/verification	Experimental data	Ref
Gia2	In vitro	PC3	Plasmid	Sanger sequencing, western blotting, flow cytometry	Impaired lamellipodia establishment at the leading edge of migrating cells and attenuation of TGFβ1 signaling	[159]
KDM5B	In vitro	LNCaP, C4–2B, PC3	Lentiviral, plasmid	QPCR, western blotting, IF staining,	Significant reduction in P110α and PIP3 levels, leading to decreased cell proliferation	[160]
PLK-1	In vitro	PC-3	Plasmid	Western blotting	Suppression of tumor growth	[161]
	In vitro, in vivo	LNCap, PC-3	Aptamer-liposome-CRISPR/Cas9 chimera	Apoptosis assay, MTT assay	Noticeable regression of prostate cancer	[70]
ATM	In vitro	DU145, 22Rv1, LNCaP	Lentiviral, plasmid	Immunoblotting, NGS	Altered DNA damage response signaling	[162]
		C4-2	Lentiviral, plasmid	Puromycin selection, western blotting	Increased sensitivity to Enzalutamide	[163]
		22Rv1	Plasmid	Immunostaining, sanger sequencing	Heightened sensitivity to ATR inhibition	[164]
MCL1	In vitro, in vivo	LNCaP	Plasmid	Western blotting	Significant sensitization of cancer cells to apoptosis induced by navitoclax, but not by venetoclax	[165]
GPRC5A	In vitro, in vivo	PC3, DU145	Guide-it™ CRISPR/Cas9 System	Real-Time RT-PCR, MTT and BrdU assays, cell cycle assay, RNA-seq	Decreased cell proliferation	[166]

CRISPR-mediated modification of immune-related genes

A group of other genes, namely those related to immune function has been the target of CRISPR-mediated editing in the prostate cancer (Table 3). Most of these genes were enriched in the NF-κB signaling pathway. TUBB4A is an over-expressed gene in human prostate cancer, whose expression is related with aggressive phenotype and poor patient survival. CRISPR-mediated TUBB4A knockout reduced cell growth and migration but induced DNA damage through elevating γH2AX and 53BP1 levels. Notably, TUBB4A knockout led to severe DNA damage, reduced NF-κB signaling response, and retardation of tumor growth and metastases. Mechanistically, TUBB4A knockout reduced MYH9-mediated GSK3β ubiquitination and degradation, resulting in inactivation of β-catenin signaling and its pertinent EMT [74].

In an attempt to find the mechanisms by which conversion of different FGFR isoforms participates in the progression prostate cancer, Wang et al. conducted a study. They reported that FGF promoted NF-κB signaling in prostate cancer cells and in association with FGFR1 expression. CRISPR/Casp-mediated of FGFR1 disruption retracted both FGF activity and NF-κB signals. Further experiments revealed that the role of FGFR1 in stimulation of NF-κB signaling is mediated by TGF β-activating kinase 1 (TAK1). In fact, FGFR1 promoted NF-κB signaling through decreasing TAK1 degradation and thus maintaining continued NF-κB activation. Collectively, this study revealed that FGFR1 promoted progression of prostate cancer through induction of inflammatory responses in the tumor microenvironment [75]. Figure 4 demonstrates selected targeted genes in prostate cancer participating to the immune responses.

CRISPR-mediated modification of other genes and signaling pathways

A recent work by Kypta et al. explored the potential of using CRISPR to activate the *DKK3* gene as a therapeutic modality for prostate cancer. Human DNA contains *DKK3*, a tumor-suppressor gene that suppresses prostate cancer progression and metastasis. Its expression is reduced by promoter methylation in prostate cancer. The researchers demonstrated that a DNA methyltransferase inhibitor had a hypomethylating effect on the DKK3 promoter, leading to elevated DKK3 level in prostate cancer cells. Moreover, by transcription from DKK3 using CRISPR-dCas9-VPR, the activity of TGF-β/Smad pathway was suppressed, thus proliferation and metastasis of prostate cancer cells were inhibited. Crucially, these findings open the door for the application of CRISPR-mediated DKK3 induction in prostate cancer treatment [76].

One notable endeavor focused on targeting lipocalin 2 (Lcn2/NGAL), a member of the lipocalin superfamily

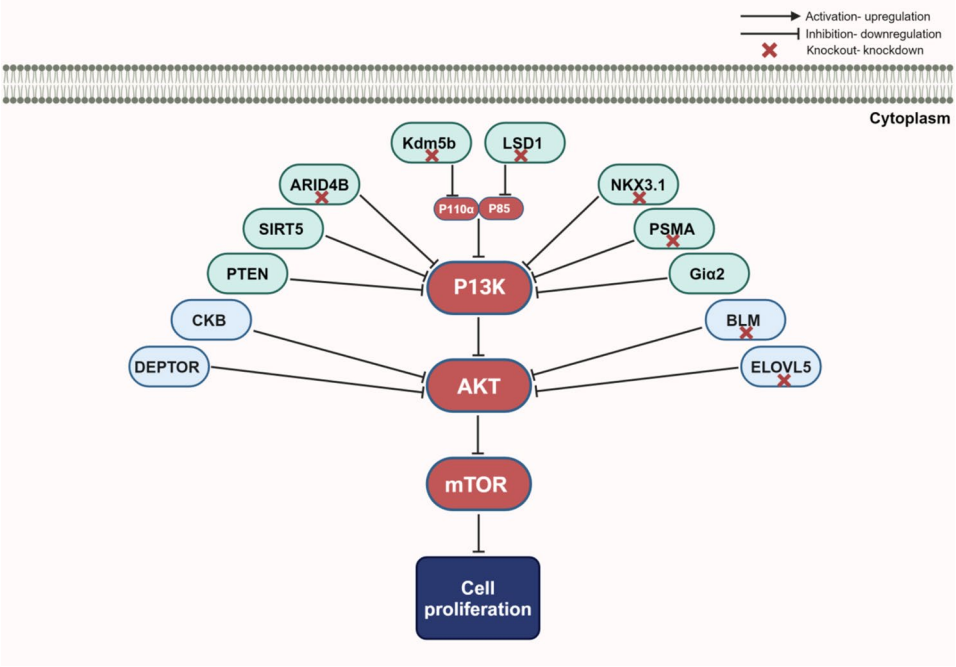

Fig. 3 Targeting PI3K/AKT/mTOR pathway by CRISPR/Cas9 in prostate cancer. Modification of expression of these targets has led to reduction of prostate cancer cell proliferation. Figure was depicted using BioRender

with various roles, to investigate its precise role in cancer development. This study utilized CRISPR/Cas9 technology to knockout Lcn2 expression in a metastatic and invasive prostate cancer cell line, PC3. This study aimed to evaluate the efficacy of combining Lcn2 knockdown with cisplatin, a widely used chemotherapeutic medication. The researchers co-transfected PC3 cells with the control CRISPR/Cas9 plasmid and the human Lcn2 CRISPR/Cas9-knockout plasmid or the HDR CRISPR/Cas9 plasmid. Stable cells were selected with puromycin. Then, the PC3 cells with Lcn2 knockout (Lcn2-KO) were treated with and without cisplatin, focusing on their proliferative ability, apoptosis rate, and migratory capabilities. Their study revealed that suppressing Lcn2 in PC3 cells repressed cell proliferation and enhanced the effectiveness of cisplatin. The significant decrease in expression of Lcn2 expression resulted in increased cisplatin induced apoptosis in PC3 cells. Moreover, cell migratory ability of PC3 cells decreased following Lcn2 knockout by CRISPR/Cas9 technology. The results demonstrated how Lcn2 can be both a reliable biomarker for prostate cancer diagnosis and prognosis in addition to a potential therapeutic target. The study also suggested that CRISPR-based technology to target Lcn2 may lead to improved disease outcomes for patients with extremely aggressive prostate cancer [77].

Based on recent advancements, researchers investigated the molecular biology of aberrant gene expression in prostate cancer cells. Yang et al. employed CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) techniques to control the transcription of ITGB5, TIMP1 and TMEM176B in prostate cancer cells. Various cellular experiments were conducted to define the involvement of these gens in prostate cancer, and it was discovered that their activation has an inhibitory effect on prostate cancer. Furthermore, these three genes were found to synergistically influence cancer cell proliferation, invasion and migratory capacity, highlighting their potential as therapeutic targets [78].

Another research team conducted a genome-wide CRISPR/Cas9 investigation on a mouse prostate cancer model to identify potential gene targets that might improve the effectiveness of docetaxel chemotherapy. Transcription elongation factor A-like 1 (TCEAL1) was identified as the most appropriate gene among the 17 candidate genes. Although the specific function of TCEAL1 is not yet completely clarified, it is supposed to regulate transcription in a promoter-dependent manner. Inhibition of TCEAL1 in several human prostate cancer cell lines increased effect of docetaxel, suggesting a potential target for combination therapy Investigators. Using gene culture analysis and flow cytometry, researchers further verified that loss of TCEAL1, in conjunction with docetaxel, alters the cell cycle profile. findings supported TCEAL1 as a potential option for enhancing the therapeutic effect of docetaxel [79].

Table 3 CRISPR-mediated modification of immune-related genes

Target gene	Study type	Cell line	Vector	Screening/verification	Experimental data	Ref
TUBB4A	In vitro, in vivo	DU145	PX458 plasmid	Western blotting, PCR, sanger sequencing, flow cytometry	Induced DNA damage, reduced NF-κB signaling response	[74]
FGFR1	In vitro, in vivo	DU145	Lentiviral	Puromycin selection, western blotting	Abolished both FGF activity and NF-κB signaling	[75]
ARID1A	In vitro	Myc-CaP	Lentiviral, plasmid	IHC, transwell assay	Unleashed CXCR2 ligand-mediated MDSC chemotaxis by silencing the enhancer of A20 deubiquitinase, a critical negative regulator of NF-κB signaling	[167]
RON	In vitro, in vivo	LMyc-CaP	PX458 plasmid	Western blotting, qRT-PCR	Demonstrating the role of RON in activating β-catenin and NF-κB	[168] [169]
IL30	In vitro, in vivo	PIN-SCs, DU145, PC3	PX458 plasmid, CRISPRMAX™ Cas9 transfection reagent	Real-time PCR, western blotting, ELISA	Significant downregulation of BCL2, NF-κB, STAT3, IGF1 and CXCL5 expression, upregulation of SOCS3	[170]
	In vitro, in vivo	PIN-SC	Lentiviral, CRISPR/Cas9	ELISA, western blotting	Decreased tumor onset, proliferation	[171]
SEPT5	In vitro, in vivo	RM1	Lentiviral, plasmid	Western blotting, real-time PCR	Inhibited cancer progression, increased CD8 + T cell infiltration, elevated expression of CCL5, CXCL5, CXCL9, CXCL10, and INFGR1	[172]
TLR3	In vitro, in vivo	DU145	Lentiviral, plasmid	Western blotting, DNA sequencing	Lower motility	[173]
IL-8/CXCL15	In vitro, in vivo	Myc-CaP	Plasmid	ELISA, PCR, sanger sequencing	Increased density of polyfunctional CD8 T cells, delay in the onset of castration resistance	[174]
LEDGF/p75	In vitro	HEp-2	PX458 plasmid	FACS, immunofluorescence assay	Identified the role of LEDGF/p75 in inducing inflammatory processes	[175]
NEK6	In vitro	DU-145	PX459 plasmid	Western blotting, PCR, sanger sequencing	Decreased clonogenic capacity, proliferation, cell viability, mitochondrial activity	[176]
RelB	In vitro, in vivo	RM-1	Plasmid	Immunofluorescence, luciferase reporter assay	Decreased PD-L1 expression, improved susceptibility to T cell immunity	[177]
ASIC1	In vitro, in vivo	22Rv1	Plasmid	Western blotting, real-time quantitative PCR	Reduced cell invasion, and decreased castration resistance	[178]
MAP4K4	In vitro	PC3	Lentiviral, plasmid	Western blotting, PCR, sanger sequencing,	Inhibition of metastatic PCa cells migration and clonogenic properties	[179]
CXCR2	In vitro, in vivo	C4-2B/MDVR	PX458 plasmid	FACS	Suppressed tumor growth	[180]
CXCR7	In vitro	LNCaP, C4-2B	Plasmid	RT-q-PCR, senescence assay, proximity ligation assay (PLA)	A complete stop in cell proliferation, disrupted EGFR signaling	[181]
	In vitro, in vivo	C4-2B	Lentiviral, plasmid	Genotyping, PCR, flow cytometry	Inhibition of tumor growth	[182]
		LNCaP, C4-2B	Lentiviral, plasmid	Cell proliferation, colony formation, cell invasion assays, FACS	Demonstrating CXCR7-mediated MAPK activation as a mechanism of resistance to second-generation antiandrogen therapy	[182]

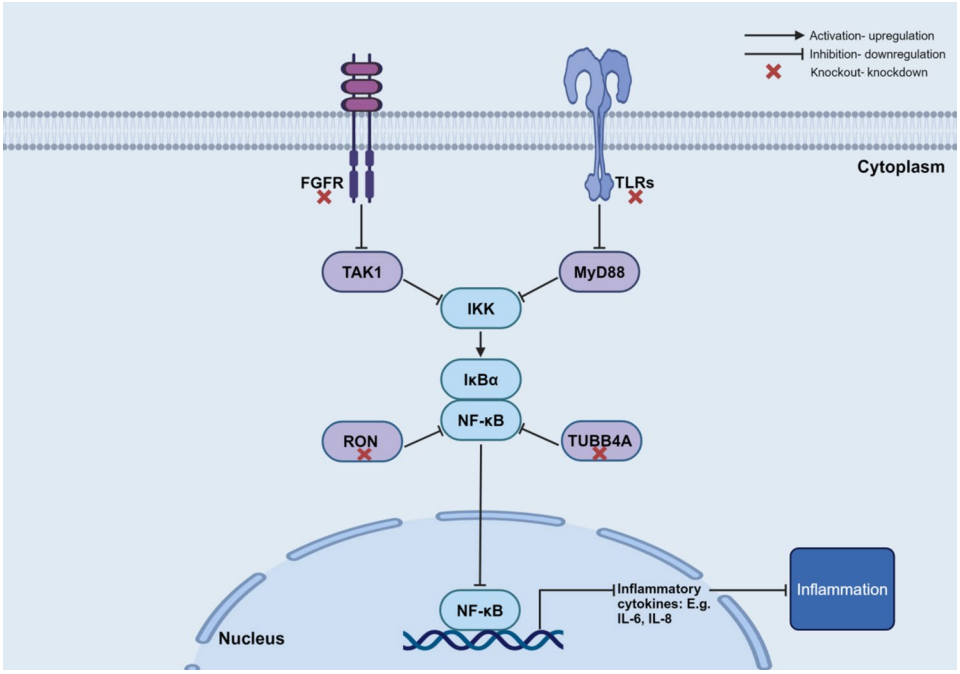

Fig. 4 Targeted genes by CRISPR/Cas9 in prostate cancer participating to the immune responses. Figure was depicted using BioRender

Discussion

Prostate cancer, a prevalent and deadly disease with limited treatment options, requires innovative approaches to address its progression and overcome therapy resistance. Recent advancements in CRISPR technology have revolutionized research in this field, resulting in a significant progress in the detection and treatment of prostate cancer. Moreover, application of CRISPR-based techniques for gene regulation has offered opportunities to investigate gene function in cancer cells. In fact, it has demonstrated the role of interaction between classical tumor suppressors and epigenetic factors in the pathogenesis of this type of cancer [80].

The recent projects utilizing CRISPR technology in prostate cancer research have also provided new perceptions about the diagnostic and therapeutic strategies for this disease. Actually, the capacity of CRISPR/Cas system to target RNA has provided novel approaches to tumor diagnostics. However, most of applied research in this field has used mice models of cancer, thus making translation of the obtained data to clinical application challenging.

Most notably, mentioned experiments have shown that CRISPR/Cas9-mediated suppression of a number of genes can enhance therapeutic effects of conventional chemotherapeutic agents, suggesting novel combinatory regimens for treatment of prostate cancer. This technique has also facilitated identification of molecular events that contribute to each phase of prostate cancer development and progression. In fact, genome-wide CRISPR activation and inhibition screening has shown several drivers and inhibitors of metastasis cascades in prostate cancer [81]. Thus, several potential molecular targets for combating diverse aspects of carcinogenesis have been uncovered using this technique. In vivo application of CRISPR technology has unveiled the function of several commonly mutated genes in prostate cancer and their differential effects in the promotion of tumor growth and regulation of cell fate and dissemination.

As a novel therapeutic option, chimeric antigen receptor (CAR) T cell therapies were found to be safely tolerated in patients with prostate cancer. Moreover, this treatment led to TCR repertoire diversity and modulation of the tumor immune niche in a group of patients [82]. Meanwhile, CRISPR/Cas9 technique offered an effective way for enhancement of the proliferation ability and perseverance of CAR-T cells in the body. This tool was used in CAR-T cells to produce a memory phenotype, decrease exhaustion, and find novel targets to enhance the anti-tumor ability [83].

The main obstacle to bringing CRISPR technology into the clinic is the delivery of CRISPR/Cas to prostate tumors. An optimal targeted delivery system should have a safe vehicle protecting RNA from nuclease degradation in the circulation; and a targeting moiety/ligand that specifically recognizes the receptor and efficiently direct cargo into a specific tissue or cell [70]. In fact, this strategy requires a highly specific targeting ligand that has high affinity to a cellular receptor [84]. Thus, recognition of specific receptors on prostate cancer cells which are not expressed on normal cells can enhance the efficiency

of this system. Among different delivery systems, the aptamer-liposome-CRISPR/Cas9 chimera has been proved to have a significant cell-type binding specificity, an extraordinary gene silencing impact in vitro, and a noticeable effect on in vivo regression of prostate tumors [70].

CRISPR therapy has experienced a truly extraordinary achievement, since it has been translated into the clinical application in just 11 years [85]. A number of clinical trials of CRISPR therapy are currently recruiting patients with hematological malignancies (NCT05885464) or solid tumors (NCT05795595). Thus, it is expected that this technology revolutionizes current therapy of cancer in near future. At this time, identification of appropriate targets for this system is a priority. In the context of prostate cancer research and therapy, this target should have an indispensible role in the carcinogenesis being dysregulated in a wide array of patients with different pathological features.

Author contributions

MT and FSM searched the literature and designed the figure. EM contributed in literature search. SG-F supervised the study and wrote the manuscript. All authors approved the final draft.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

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