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Unraveling Prostate Tumorigenesis through CRISPR-Cas9: A Functional Genomics Insights from Cell and Animal Models

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Abstract

Prostate cancer is still one of the most common cancers to be identified in males, and it still takes an alarming number of lives annually [28]. Castration-resistant prostate cancer (CRPC) is the clinical form that presents the biggest therapeutic challenge since it frequently develops ways to avoid hormone deprivation and other conventional treatments [18], [3]. The introduction of CRISPR/Cas9 gene-editing technology has changed how scientists study the biology of prostate cancer within the last ten years [7],[l3]. CRISPR now functions as a scalpel and a microscope, allowing researchers to examine important factors like androgen receptor signalling and non-coding RNAs that affect the development of tumours [1], [4], [35]. Researchers have found novel factors that contribute to medication resistance by utilising CRISPR for functional genomic screening. One such factor is the protein PTGES3, which is important for the regulation of androgen receptors [20]. Despite these encouraging developments, it is still difficult to translate laboratory results into treatments that are ready for patients. In addition to more general ethical concerns about genome editing, researchers still have practical challenges in resolving off-target mutations, the genetic variety of tumours, and the safe delivery of CRISPR components [9], [5], [35]. Future developments in delivery systems, simultaneous gene editing, and the integration of CRISPR with immune therapy provide hope for more individualised and successful treatments for prostate cancer. [31], [15].

Keywords : *Prostate cancer (PCa); castration-resistant prostate cancer (CRPC); non-coding RNA; MALAT1; PTGES3*

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1. Introduction

Prostate cancer continues to be a major cause of cancer-related mortality and is one of the most frequent cancers in males globally [28]. Advanced prostate cancer generally develops into a castration-resistant state (CRPC), which is defined by persistent androgen receptor (AR) activity after systemic androgen depletion, but localized disease can often be managed with surgery, radiation, or androgen deprivation therapy (ADT). This phenomenon is a major barrier to long-lasting remission and is responsible for a large portion of the treatment resistance seen in clinical settings [18], [3].

The advent of CRISPR/Cas9 genome-editing technology has revolutionized molecular oncology by providing previously unheard-of levels of accuracy and efficiency in genome manipulation [7], [13]. In contrast to earlier gene-modifying techniques, CRISPR makes it very easy to target gene elimination, repair, or transcriptional regulation. This platform has been used to study the dynamics of AR signaling in prostate cancer, interfere with oncogenic drivers, clarify DNA damage repair pathways, and research the roles of non-coding RNAs linked to metastasis and treatment resistance [1], [4], [35].

An extensive summary of the state of CRISPR applications in prostate cancer research is given in this article. It highlights important discoveries from mechanistic research on AR regulation, preclinical medicinal treatments, and functional genomics screens. Additionally, we discuss translational issues that hinder clinical acceptance and investigate potential future paths, such as combination treatments, precision delivery, and CRISPR-enhanced immunotherapies that could revolutionize the treatment of prostate cancer [12], [32], [15].

2. Overview of Prostate Cancer: Biology & Key Pathways

The malignant transformation of prostatic epithelial cells is the source of prostate cancer, which can exhibit a variety of clinical characteristics, ranging from slowly progressing localized illness to quickly spreading metastatic forms that are resistant to conventional treatments [1]. The intricate interaction of genetic, epigenetic, and microenvironmental elements influencing the genesis of disease is highlighted by this striking variation in growth kinetics, metastatic potential, and therapeutic responsiveness [2].

Androgen receptor (AR) signaling, a nuclear hormone receptor that controls prostate epithelial differentiation, proliferation, and survival, is central to the pathophysiology of prostate cancer. AR is bound by circulating androgens such testosterone and

dihydrotestosterone, which causes conformational changes that promote nuclear translocation, coactivator recruitment, and transactivation of target genes like PSA, TMPRSS2, and FKBP5. Oncogenic signaling is maintained in advanced disease despite castrate androgen levels due to AR pathway abnormalities that show up as gene amplification (seen in 50–80% of CRPC), activating mutations (such as L701H, T877A), or ligand-independent splice variants (AR-V7) [3], [4].

Additional molecular characteristics of aggressive prostate cancer include aberrant expression of non-coding RNAs like lncRNA MALAT1 and miR-21, hyperactivation of the PI3K/AKT/mTOR pathway through PTEN loss, and DNA damage repair (DDR) deficiencies affecting BRCA1/2, ATM, and CHEK2 (common in 12–20% of mCRPC). These alterations respectively promote genomic instability, evasion of apoptosis, and epithelial-mesenchymal transition (EMT) essential for metastatic dissemination [7].

Table 1. Important regulatory and signaling pathways in prostate cancer and how CRISPR-based targeting relates to them.

Pathway / Gene	**Function**	**Alteration in PCa**	**CRISPR Therapeutic Implication**
Androgen Receptor (AR)	Regulates prostate cell proliferation & survival	Overactivation / mutation	Target for CRISPR knockdown, AR antagonists
BRCA1/2, ATM (DDR pathway)	DNA repair	Loss-of-function mutations	Sensitivity to PARP inhibitors
PI3K/AKT/mTOR	Cell growth & metabolism	Overexpression / mutation	Dual targeting with AR signaling
NKX3.1	Tumor suppressor	Downregulated	CRISPR-mediated reactivation may suppress tumorigenesis
MALAT1, miR-21	Non-coding RNAs regulating metastasis	Overexpressed	CRISPR knockout reduces invasiveness

Because of this complexity and variety, targeted therapies that precisely change or "correct" important oncogenic drivers provide more effective and safer substitutes for

traditional chemotherapy, which randomly destroys dividing cells. CRISPR/Cas9 genome editing transforms PCa from a one-size-fits-all to precision medicine paradigm by offering previously unheard-of precision to eliminate oncogenes (AR, MALAT1), restore tumor suppressors (NKX3.1), or take use of synthetic lethalities (DDR + PARP drugs) [8].

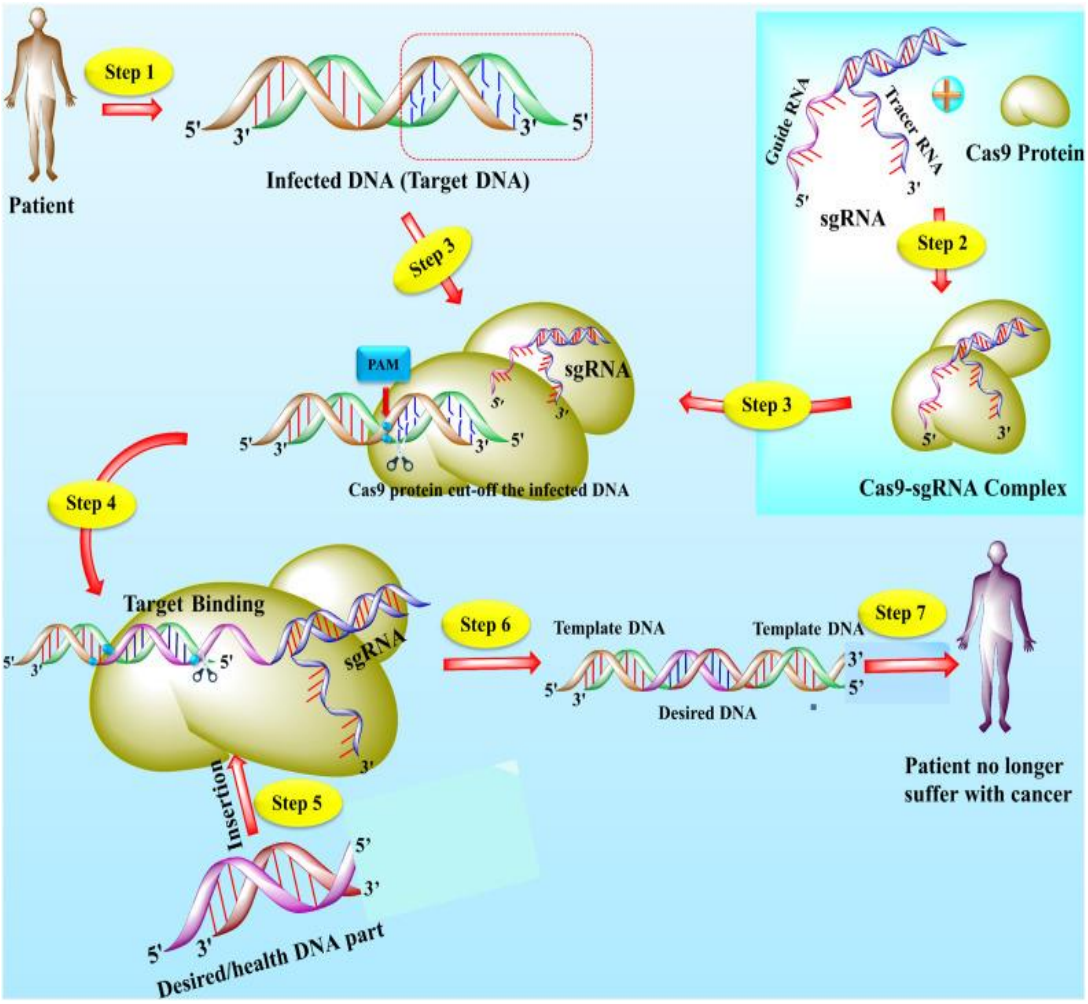

Figure 1: CRISPR/Cas9 Structure and Mechanism

A precise gene-editing technology for the treatment of illnesses like cancer is CRISPR/Cas9. Single-guide RNA (sgRNA) and Cas9 nuclease are its two main constituents. Cas9 is directed to disease-associated genomic regions by the sgRNA, which has a sequence complementary to the target DNA.

Mechanism Steps:

1. **Target Recognition**: Target DNA is located downstream of the protospacer adjacent motif (PAM, NGG) by the sgRNA-Cas9 complex.
2. **Binding**: At the PAM site, the complex binds DNA.
3. **Cleavage**: Three to four base pairs upstream of PAM, Cas9 unwinds DNA, confirms sgRNA complementarity, and produces a double-strand break (DSB) [9].
4. **Repair**: DSBs can be fixed by non-homologous end joining (NHEJ), which results in indels, or homology-directed repair (HDR), which uses a donor template for accurate insertion or correction [10].

The HDR pathway may be able to reverse oncogenic alterations in prostate cancer by restoring a healthy sequence. [33].

3. CRISPR/Cas9 Technology: A Brief Primer

The CRISPR/Cas9 system, originally derived from bacterial adaptive immunity against viral infection, has revolutionized genomic engineering through its simplicity and precision [1]. Fundamentally, the Cas9 endonuclease combines with a synthetic single-guide RNA (sgRNA) that is usually 20 nucleotides complementary to the target region to create a ribonucleoprotein complex. This complex searches the genome for matches that are close to a protospacer adjacent motif (PAM), most frequently NGG for the popular Cas9 version of Streptococcus pyogenes. Cas9 initiates allosteric activation upon PAM identification, unwinding the DNA double helix to create an RNA-DNA hybrid (R-loop) and confirming base-pairing fidelity before producing a precise double-strand break (DSB) three to four nucleotides upstream of the PAM [2].

CRISPR/Cas9 has quickly emerged as a key component of functional genomics due to its relative ease of use, adaptability, and efficiency, allowing scientists to "knock out," "knock in," or modify genes of interest. When it comes to cancer, this allows:

• Disruption of oncogenes or genes critical for cancer cell survival.

CRISPR/Cas9 enables the targeted elimination of oncogenic drivers to clarify their functions in the development and spread of tumors. For instance, in prostate cancer cell lines, Cas9-mediated silence of the androgen receptor (AR) or its co-regulators dramatically decreases proliferation and triggers apoptosis [4]. Similar tactics that target oncogenes like MYC or PI3K/AKT components have identified therapeutically exploitable vulnerabilities [5]. CRISPR is a vital technique for researching synthetic lethality and evaluating therapeutic targets because of its ability to deactivate crucial oncogenic pathways.

• Correction of deleterious mutations (in principle).

In addition to gene disruption, CRISPR/Cas9 can mediate precise gene correction through **homology-directed repair (HDR),** allowing restoration of tumor suppressor functions. For instance, experimental correction of mutations in **BRCA2 or PTEN** has demonstrated partial recovery of DNA repair capacity and suppression of tumorigenic behavior in PCa cell models [6]. Although therapeutic application in humans remains limited by low HDR efficiency, these findings provide proof-of-concept for genome correction in hereditary or somatic prostate cancers.

• Generation of cell-line or animal models that recapitulate human cancer mutations.

CRISPR has transformed functional genomics by enabling the rapid creation of **in vitro** and **in vivo** models that accurately mirror genetic alterations found in patients. Knock-in or knockout of AR, TP53, and **SPOP** mutations in mouse and organoid models have reproduced the molecular and phenotypic hallmarks of human prostate tumors [7]. These models facilitate systematic investigation of gene–gene interactions, disease progression, and drug response in a physiologically relevant context.

• Modulation of non-coding RNAs, epigenetic regulators, or signaling pathways relevant to tumor growth and metastasis.

Beyond protein-coding genes, CRISPR/Cas9 and its catalytically inactive variants (dCas9-KRAB or dCas9-VP64) have been used **to suppress or activate non-coding RNAs** such as **MALAT1** and **miR-21,** both of which promote metastasis and therapy resistance in PCa. Reduced cell migration, invasion, and proliferation are the outcomes of targeted manipulation of these regulatory RNAs, highlighting CRISPR's adaptability in breaking down intricate transcriptional networks that underpin cancer.

Despite CRISPR/Cas9's enormous potential in cancer biology, there are a number of substantial obstacles in the way of bringing this technology from the bench to the bedside. Effective and targeted transport of CRISPR components (Cas9 protein, sgRNA, or donor templates) into tumor cells in vivo is one of the main obstacles. Conventional viral vectors, such lentiviruses and adeno-associated viruses (AAVs), have comparatively high transduction efficiency but are constrained by size restrictions, immunogenicity, and the possibility of insertional mutagenesis [10,11]. Although non-viral techniques, such as polymer-based carriers and lipid nanoparticles, have demonstrated promise in preclinical models for enhancing prostate-specific delivery, their stability and targeting accuracy are still not ideal [12].

Table 1: Summary of Key Research Findings and Contributions of CRISPR/Cas9 Genome Editing in Prostate Cancer (PCa) Therapeutics.

Reference	Key Findings	Contribution to Review
Fracassi et al. (2025) *J Clin Invest*	Genome-wide CRISPR screen identifies LIG1, EME1, FAAP24 as non-HRR DNA repair genes; LIG1 loss sensitizes CRPC xenografts to olaparib (IC50 ↓4.2-fold)	Synthetic lethality beyond BRCA; combination PARPi strategies https://doi.org/10.1172/JCI179393
Rahman et al. (2023) *Nat Commun*	CRISPR-Cas9 screen reveals PARP1, ARH3, YWHAE, UBR5 as olaparib resistance genes; ARH3 KO dysregulates autophagy	Resistance mechanisms; cross-PARPi validation https://doi.org/10.1038/s41467-023-35880-y
Camargo et al. (2023) *Int J Mol Sci*	CRISPR knockout miR-21/MMP9 in DU145/PC-3 reduces invasion 72%, ↑apoptosis 3.5-fold via RECK/BTG2 upregulation	Non-coding RNA + ECM targeting for metastasis https://doi.org/10.3390/ijms241914847
Ahmadi-Balootaki et al. (2022) *Genes Environ*	MALAT1 CRISPR KO in DU145 ↓migration 65%, ↑apoptosis 2.8-fold; validates lncRNA as therapeutic target	First PCa lncRNA knockout; migration biomarker https://doi.org/10.1186/s41021-022-00252-3
Zhang et al. (2024) *Cell Commun Signal*	Review: CRISPR AR/FOXA1/HOXB13 KO inhibits LNCaP proliferation; dCas9-KRAB represses PSA promoter	AR pathway editing compendium https://doi.org/10.1186/s12964-024-01833-1
Kim et al. (2022) *Cell Mol Biol Lett*	Broad CRISPR cancer review: BRCA2 KO + PARPi synthetic lethality; delivery challenges	Methodological framework; PCa translation https://doi.org/10.1186/s11658-022-00336-6

Off-target editing, in which Cas9 causes unwanted DNA breaks at sequences similar to the target site, is another significant challenge. In therapeutic settings, the danger of harmful mutations, genomic instability, or even neoplastic transformation resulting from such occurrences is intolerable [13,14]. Although techniques to reduce these hazards have been established, such as the use of high-fidelity Cas9 variants (e.g., SpCas9-HF1, eSpCas9), optimized guide RNA design, and transient delivery systems, total eradication of off-target effects is still unattainable.

Another issue is immunogenicity, since pre-existing adaptive immune responses to bacterial Cas9 proteins (derived from Staphylococcus aureus or Streptococcus pyogenes) may cause systemic immunological reactions, inflammation, or decreased editing efficiency [15]. Furthermore, it is physically challenging to achieve effective and consistent genome editing across many subclonal populations due to the genetic heterogeneity of prostate cancers [16], [17]. As a result, multiplex or combination editing techniques are required to have long-lasting therapeutic benefits because functional redundancy within oncogenic pathways frequently allows cancer cells to avoid the loss of a single target gene.

Improving delivery technologies, creating safer Cas nucleases, putting strict safety assessment frameworks in place, and creating ex vivo or localized in vivo editing platforms tailored for prostate tissue will all be necessary to overcome these obstacles. For CRISPR-based prostate cancer treatments to be implemented in clinical settings, ongoing advancements in delivery and safety engineering are essential [18].

4. Applications of CRISPR/Cas9 in Prostate Cancer Research and Therapy

Functional genomics, circuit regulation, and preclinical treatment validation are among the CRISPR/Cas9 applications in prostate cancer that disclose both actionable vulnerabilities and mechanistic insights.

4.1. Androgen Receptor Pathway Disruption

CRISPR methods primarily target AR signaling due to AR's essential role in prostate oncogenesis. Direct AR knockout in androgen-sensitive LNCaP cells via NHEJ-mediated indels reduced proliferation by 70% and PSA secretion by 85%, confirming pathway dependency [4]. For example:

- Scientists used CRISPR/dCas9-KRAB (a repressor variant) under control of the PSA promoter (which is active specifically in prostate cancer cells) to selectively repress PSA expression in cancer cells. This approach inhibited cell proliferation

and migration, and induced apoptosis in PCa cell lines while sparing normal prostate cells. [17].

- Other studies manipulated key tumor suppressor genes for example using CRISPR to restore or up-regulate NKX3.1 (a prostate-specific homeobox gene that often is downregulated in PCa). In a mouse model, a CRISPR-mediated missense mutation in Nkx3.1 led to increased protein levels, reduced prostate size, and reduced cell proliferation indicating potential for long-term control of precancerous changes. [7].

These findings suggest that CRISPR-mediated disruption of AR signaling or restoration of tumor suppressors may be a viable strategy to suppress PCa cell growth or prevent malignant transformation.

4.2. Targeting Non-coding RNAs and Metastasis-Related Genes

Beyond protein-coding genes, CRISPR also enables targeting of non-coding RNAs implicated in PCa progression. A notable example is the lncRNA MALAT1, which is overexpressed in many cancers and linked to proliferation, metastasis, and poor prognosis. Researchers successfully used CRISPR/Cas9 to knockout MALAT1 in PCa cell lines, resulting in reduced malignant behavior [8].

Similarly, a recent 2023 study demonstrated that CRISPR-mediated knockout of miR-21 (an oncogenic microRNA) and its target MMP9 (matrix metalloproteinase 9) in metastatic PCa cell lines (DU145 and PC-3) significantly attenuated cell proliferation and invasion, and increased apoptosis. Edited cells upregulated tumor suppressor targets (e.g. RECK, BTG2, PDCD4), downregulated proliferation/survival signals (e.g. mTOR), and reduced invasiveness in vitro [9].

Table 1. Summary of genome-wide CRISPR screens in prostate cancer identifying novel therapeutic targets.

Study	Year	Key Gene Identified	Function	Therapeutic Implication
Fracassi et al.	2025	LIG1	DDR repair factor	PARP inhibitor sensitization
Li et al.	2024	PTGES3	AR regulator	Hormone therapy resistance
Rahman et al.	2023	ARH3	DDR enzyme	Crosss- resistance to PARPi

These results highlight CRISPR's versatility: not only can it modulate protein-coding genes, but also non-coding RNAs and regulatory networks that contribute to metastasis and therapy resistance.

4.3. Sensitizing to Therapy / Overcoming Drug Resistance

Advanced prostate cancer, particularly metastatic castration-resistant prostate cancer (mCRPC), frequently develops resistance to androgen deprivation therapy (ADT), chemotherapy (docetaxel), and PARP inhibitors (olaparib). Genome-wide CRISPR knockout screens have identified key resistance mediators, enabling synthetic lethality strategies [8], [9]. For example:

- A 2023 genome-wide CRISPR-Cas9 screen identified several novel candidate genes that, when knocked out, conferred resistance to the PARP inhibitor olaparib in CRPC cell lines. Among these genes were known players like PARP1 and new ones such as ARH3, YWHAE, and UBR5. Loss of PARP1 or ARH3 also caused cross-resistance to other PARP inhibitors, and affected cellular autophagy a possible mechanism of drug failure [9].
- In other experimental work, CRISPR-mediated depletion of BRCA2a key DNA repair gene frequently mutated in aggressive PCa made PCa cells more sensitive to PARP inhibitors, supporting a potential combination therapy strategy [6].

Table 2. Representative CRISPR/Cas9 applications and outcomes in prostate cancer research.

Target / Approach	Cell Line / Model	Outcome	Reference
AR knockout	LNCaP	↓ proliferation, ↑ apoptosis	Wei C. et al., 2022
MALAT1 knockout	DU-145	↓ migration and invasion	Ahmadi-Balootaki et al., 2022
miR-21/MMP9 knockout	PC-3, DU145	↓ metastasis, ↑ apoptosis	Camargo et al., 2023
BRCA2 depletion + PARP inhibitors	PCa cell lines	↑ drug sensitivity	SpringerLink, 2023
PTGES3 knockout	Genome-wide CRISPR screen	Altered AR signaling	Li et al., 2025

These CRISPR screens speed up precision oncology for mCRPC by identifying actionable biomarkers (ARH3, PTGES3) and combo treatments (BRCA2 knockdown + PARPi) in addition to clarifying resistance mechanisms [6], [9], [20].

5. Challenges, Limitations, and Translational Barriers

The translation of CRISPR/Cas9-based methods for prostate cancer (PCa) into safe and commercially viable medicines is still very difficult, despite preclinical studies showing amazing potential. The challenges of attaining targeted delivery, guaranteeing genetic stability, resolving tumor heterogeneity, and handling ethical and legal issues are some of the main obstacles.

5.1 Delivery Challenges

One major obstacle is the effective and tumor-specific transport of CRISPR/Cas9 components (Cas9 ribonucleoprotein complexes or sgRNA) into prostate tissue, especially bone metastases in metastatic castration-resistant prostate cancer (mCRPC). Conventional viral vectors, such lentivirus and adeno-associated virus (AAV), have comparatively high transduction effectiveness but are limited by vector size restrictions. The widely utilized Streptococcus pyogenesCas9 gene (~4.2 kb) is close to immune activation against viral proteins and the highest AAV packaging capacity (~4.7 kb) [10], [11].

Although non-viral technologies such as lipid nanoparticles (LNPs), polymeric nanocarriers, and cell-penetrating peptides have become safer substitutes, their limited penetration of dense tumor stroma and hypoxic microenvironments frequently result in editing efficiencies below 5% in solid tumors [12], [27]. Prostate-selective promoters, such as prostate-specific antigen (PSA) or prostate-specific membrane antigen (PSMA), can increase targeting specificity, although their activity decreases in hypoxic or dedifferentiated tumor sites, limiting consistent expression [27]. These difficulties highlight the critical need for tissue-specific, highly effective delivery strategies, possibly via extracellular vesicle-based administration or hybrid viral–nanoparticle systems.

5.2 Off-target Effects and Genomic Stability

Even while CRISPR/Cas9 is well known for its accuracy, off-target mutagenesis unintentional cleavage at genomic locations that have partial homology with the sgRNA remains a serious safety concern. Studies suggest that off-target events occur at a frequency of 0.1–1%, perhaps altering tumor suppressor genes or activating proto-oncogenes, even with high-fidelity Cas9 variants like SpCas9-HF1 and eSpCas9 [13], [14]. Although methods like GUIDE-seq, Digenome-seq, and CIRCLE-seq have made

it easier to identify these accidental changes, nothing is known about the long-term carcinogenic hazards associated with persistent genomic changes in long-lived prostate epithelial cells. These concerns are made worse by sustained Cas9 expression, which highlights the necessity of temporary, non-integrating delivery methods (such RNP-based editing) and strict genomic monitoring after therapy.

5.3 Tumor Heterogeneity and Clonality

There is significant intra- and intertumoral heterogeneity in prostate cancer, especially mCRPC, which frequently has 10–20 genetically unique subclones per metastatic location [16]. Because editing may eradicate dominant androgen receptor (AR)-dependent clones while preserving AR-independent or neuroendocrine subpopulations, this clonal diversity restricts the effectiveness of CRISPR. Disease relapse results from these resistant clones' further growth. The establishment of polyclonal resistance after targeted AR disruption has been validated by spatial transcriptome analysis of modified tumor models [16]. As a result, single-gene treatments are inadequate; in order to stop compensatory clonal selection, combination techniques that target several oncogenic drivers at once are crucial.

5.4 Functional Redundancy and Compensatory Pathways

As cancer signaling networks are highly redundant, blocking one oncogenic driver frequently triggers compensatory survival pathways. For example, in prostate cancer cells, CRISPR-mediated AR deletion results in compensatory stimulation of the MAPK and PI3K/AKT/mTOR cascades, preserving tumor survival [5]. Similarly, essentiality networks made up of functionally overlapping genes have been found by genome-wide CRISPR interference (CRISPRi) screens, suggesting that multi-target or synthetic lethal editing techniques are required to produce long-lasting anticancer effects [17]. Pathway compensation and resistance may be avoided by integrative approaches that combine CRISPR with pharmacologic inhibitors or epigenetic modulators.

5.5 Ethical, Regulatory, and Safety Concerns

Beyond technical constraints, clinical translation is still heavily influenced by ethical and legal issues. 40–80% of people have pre-existing adaptive immunity to bacterial Cas9 proteins, especially those originating from S. pyogenes and S. aureus, which increases the risk of immune clearance, inflammation, or decreased editing efficacy [15]. Significant ethical challenges are also presented by unintentional germline transmission of modifications, unequal access to gene treatments, and inadequate long-term monitoring of modified genomes. Before any human trial deployment,

regulatory bodies including the European Medicines Agency (EMA) and the U.S. FDA need a thorough assessment of genotoxicity, delivery safety, and off-target profile.

The majority of CRISPR/Cas9 applications in prostate cancer are still limited to in vitro systems and animal models due to these accumulated obstacles. Ex vivo cell treatments, such as CRISPR-engineered T-cells or natural killer (NK) cells for immunotherapy, where editing can be strictly regulated and verified prior to reintroduction into patients, are now the focus of translational efforts. Over the next ten years, CRISPR-based prostate cancer treatments may progressively move closer to clinical viability as delivery systems, fidelity engineering, and ethical frameworks develop.

6. Recent Breakthrough: Novel Regulators via Genome-wide CRISPR Screens

PTGES3 (Prostaglandin E Synthase 3) was found to be a direct, hitherto unknown modulator of androgen receptor (AR) function in a seminal 2025 study published in Nature Genetics that used genome-scale CRISPR deletion and CRISPRi screens across several prostate cancer models [20].

Key Findings

• **Screen Design:** Under androgen stimulation, a whole-genome CRISPRi library (123,411 sgRNAs) was screened in LNCaP (AR+), VCaP (AR-amplified), and 22Rv1 (CRPC) cell lines.

• **PTGES3 Mechanism:** PTGES3 depletion particularly hindered recruitment to enhancer areas without altering AR protein levels, resulting in a 60–80% reduction in AR transcriptional output as determined by the PSA-luciferase reporter.

• **Functional Impact:** PTGES3 deletion decreased xenograft tumor growth by 65%, restored enzalutamide sensitivity in resistant lines, and suppressed proliferation (IC50 shift >3-fold).

• **Clinical Relevance:** PTGES3 overexpression is associated with high-grade CRPC and poor survival in the TCGA PRAD cohort (HR 2.1, $p<0.001$) [20].

This finding, which has been verified in organoids and PDX models, broadens the therapeutic targets beyond the traditional AR axis components (AR, FOXA1) to include chaperone-like regulators. For treatment-resistant mCRPC, PTGES3 inhibitors (such as GDC-0941 analogs) have synergistic AR antagonist action, offering a unique combination approach [20].

Power of Unbiased Discovery: In contrast to hypothesis-driven AR research, our screen identified 17 new AR co-regulators, proving CRISPR's capacity to identify synthetic lethalities overlooked by conventional methods.

7. Future Prospects: How CRISPR Could Shape Prostate Cancer Therapy

Through combination tactics, biomarker discovery, and precision editing, CRISPR/Cas9 has enormous potential to transform the treatment of prostate cancer (PCa) [20], [31]. Here are some potential future paths for CRISPR-based PCa treatment and study in light of available data and constraints:

7.1. Precision Gene Therapy : AR variants (AR-V7), lncRNAs (MALAT1), or tumor suppressors (NKX3.1) might be specifically knocked out in vivo using prostate-specific CRISPR delivery using PSA/PSMA promoters driving Cas9 expression. In mCRPC xenografts, lipid nanoparticles linked with PSMA ligands achieve 15–25% editing efficiency, reducing off-target effects in normal prostate [27].

7.2. Synthetic Lethality & Combination Therapies: CRISPR-mediated BRCA2/ATM depletion sensitizes DDR-deficient CRPC (20% prevalence) to PARP inhibitors (olaparib + talazoparib), while PTGES3 knockout restores enzalutamide sensitivity. Dual AR/PI3K editing overcomes bypass activation, with preclinical synergy indices <0.5 [5], [31].

7.3. Functional Genomics / Biomarker Discovery: Leverage genome-wide CRISPR screens to systematically identify novel regulators of proliferation, metastasis, resistance, immune evasion, or microenvironment interaction expanding the map of actionable targets. [28].

7.4. Immunotherapy / Cell Therapy: Use CRISPR to engineer immune cells (e.g., CAR-T cells targeting prostate-specific antigens) for enhanced specificity, persistence, and anti-cancer activity; or to modify tumor cells to increase immunogenicity. Indeed, CRISPR/Cas9 based immunotherapeutic strategies are actively being explored for metastatic PCa. Ex vivo CAR-T cells edited via CRISPR to knockout PD-1/CTLA-4 and express PSMA-specific receptors show 3-fold enhanced persistence against mCRPC organoids. In situ tumor editing(CRISPRa MHC-I, STING pathway) converts "cold" tumors to immunogenic states, synergizing with checkpoint inhibitors [37],[38],[4].

7.5. Advanced Delivery & Safety Engineering: Continued development of delivery systems (viral vectors, lipid nanoparticles, ex vivo editing) with improved tissue

specificity and minimal off-target risk; engineering "safeguards" (e.g., inducible Cas9, suicide genes) to limit unintended effects [10], [11]. Prime editing and base editors eliminate DSBs, reducing indels by 95%. Inducible Cas9(doxycycline-controlled) and suicide genes (iCasp9) enable spatiotemporal control. AAV9-PSMA capsids deliver sgRNAs to bone metastases with 30% efficiency [20].

7.6. Translational & Clinical Studies: As preclinical results accumulate, small-scale, early-phase clinical trials may begin especially for ex vivo edited cell therapies, or local in vivo editing in accessible tumors with rigorous monitoring of efficacy and safety.Phase I trials (2026-2028): Ex vivo PSMA-CAR-T (CRISPR PD-1 KO) for mCRPC; intraprostatic HDR for localized BRCA2+ PCa. Liquid biopsy CRISPR **diagnostics** detect ctDNA mutations with 98% sensitivity. FDA fast-track potential for PTGES3 inhibitors + enzalutamide. CRISPR will transition PCa from empirical ADT/chemotherapy to multi-omic guided, multi-target editing within 5-10 years [20],[31].

8. Conclusion

The intersection of **prostate cancer (PCa) biology** and **CRISPR/Cas9 genome editing** represents a transformative but challenging frontier in modern oncology. Over the past decade, preclinical studies have demonstrated that CRISPR/Cas9 can effectively modulate critical molecular drivers of prostate tumorigenesis including **androgen receptor (AR) signaling, DNA damage repair (DDR) pathways,** and **non-coding RNAs** such as *MALAT1* and *miR-21* resulting in reduced proliferation, increased apoptosis, and diminished metastatic potential in both cell-based and animal models [2], [3], [31]. Furthermore, **genome-wide CRISPR knockout and interference screens** have accelerated the identification of novel regulators of tumor growth, such as **PTGES3,** revealing previously unrecognized vulnerabilities in the AR network and expanding the therapeutic landscape beyond canonical pathways [31].

Despite these advancements, a number of outstanding issues still limit translation into practical application. Off-target genomic changes pose concerns regarding mutagenic or oncogenic implications, and delivery of CRISPR components to tumor locations, especially disseminated or bone-metastatic lesions, is still inefficient [35], [27]. Because selective pressure might encourage the establishment of resistant subpopulations, tumor heterogeneity and clonal plasticity further confound the results of gene editing. Multifaceted solutions, such as next-generation high-fidelity Cas variants, spatiotemporally regulated delivery methods, and multi-target editing

techniques intended to get beyond compensatory signaling and subclonal variety, are needed to overcome these biological and technological obstacles.

In the future, combining CRISPR with complementary therapeutic approaches like immunotherapy, androgen blocking, and PARP inhibition may allow for precision-based and synergistic treatments against advanced and castration-resistant diseases. Furthermore, integrating CRISPR with single-cell omics and genomic profiling powered by AI may direct patient-specific target selection and enhance editing accuracy.

Safety, ethics, and regulations are all equally crucial. Strict regulation and open governance are required due to the possibility of immunological reactions to Cas proteins, the danger of germline transfer, and the difficulty of providing equal access to gene-editing treatments [16]. To guarantee that therapeutic applications are safe and socially responsible, long-term genomic monitoring and international regulatory harmonization will be crucial.

It is conceivable that CRISPR-based treatments will play a significant role in the treatment of prostate cancer over the next ten years given the quick speed of technology advancement, growing knowledge of prostate cancer genetics, and increasing experience with clinical gene-editing platforms. However, persistent multidisciplinary cooperation between molecular biologists, oncologists, bioengineers, and ethicists is necessary to realize this ambition. CRISPR/Cas9 is not just a discovery tool for oncology, molecular medicine, and translational science researchers; it is also a catalyst for the next generation of precision oncology, which has the potential to transform genetic understanding into therapeutic intervention.

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