# Role of CRISPR Technology in Prostate Cancer

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## Abstract

Prostate cancer ranks at the top of the maximum communal forms of cancer in males. Prostate tumors develop gradually in the prostate gland and may not affect the prostate gland much, but are localized. Basic and applied research on beneficial gene modification has been at the lead of public scientific discourse for decades. Genome editing using applications like Cas9-based technology can be used to treat human diseases according along with ongoing clinical trials. Gene Therapy research and clinical trials emphasize prostate cancer research and treatment. Gene therapy is a propitious treatment possibility for prostate cancer due to some factors, involving anatomical considerations that allow direct tumor sampling and injections, provide preclinical immune-competent models, and recognize tumor-related antigens that may serve as targets for an induced immune system. "Chimeric Antigen Receptor" (CAR) T cell therapies and novel technologies utilizing CRISPR-Cas9-assisted technologies, such as vaccine-based immune stimulating treatments, are very promising and are now being researched in both the lab and the clinic. Future research holds enormous potential for the establishment of gene therapy in prostate cancer treatment, even though laboratory and preclinical advancements have not yet produced oncologically relevant outcomes in the clinic

**Keywords:** Oncology Research, CRISPR Technology, Global Health Challenge, CRISPR-Cas9, Gene therapy.

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## Introduction

Every year, there are 1.6 million cases of prostate cancer (PCa) and 367,000 deaths due to the disease. PCa may be a frequent kind of cancer in the U.S.A and the 2nd worst killer of men from cancer (Mabjeesh et al., 2002). PCA in men can be treated majorly by androgen deprivation therapy (ADT) because androgens are important elements that encourage the growth of PCa cells (Mucci, 2018). The first line of treatment for this disorder is "Androgen Deprivation Therapy" (ADT), even though the condition typically advances to a more destructive form of the disease called "Castrate Resistant Prostatic Adenocarcinoma" (CRPC). To develop new prostate cancer treatments, there is an vital requirement to comprehend the molecular pathophysiology of CRPC and find fresh pharmacological targets (Huang et al., 2005).

### 2. Characteristics Associated with Prostate Cancer

Adenocarcinomas, which make up the majority of prostate tumors, have a lot in common with other common epithelial malignancies, including breast and colon cancer. The following are some crucial elements of prostate cancer that are important for examining the disease mechanisms.

### 2.1. Aging

Prostate cancer is closely linked to the aging process, with advanced age being the most critical risk factor for its development. While earlystage lesions, such as "Prostatic Intraepithelial Neoplasia" (PIN), can appear in men as young as their twenties and become increasingly common by their fifties, invasive prostate cancer typically becomes clinically detectable between the ages of 60 and 70. Notably, precursor lesions are far more prevalent, affecting approximately one in three men, compared to invasive carcinoma, which develops in only one in nine men (Bechis et al., 2011). This disparity highlights the distinction between the early initiation of cellular changes and the less frequent progression to invasive disease, which is heavily influenced by aging.

### 2.2. Environmental and Nutritional Factors

Epidemiologically, prostate cancer is a global problem but it is more prevalent in western countries as compared to Asia. Dietary habits,

environmental and personal patterns may be a major reason of this disparity as (Oczkowski et al., 2021; Yahya et al., 2021). Different investigations revelaed that processed food like meat, milk and other items can also have impact on microbiota which ultimately imposed strong effects on prostate gland leading to cancer so pattern and choice food consumption is a highlighting concern (Matsushita et al., 2020).

**2.3. Genomic Factor**

Genetic affects are responsible for prostate cancer upto 10-20%. The early development of disease is mostly linked to genetic aspects. Various genetic aspects like BRCA1, BRCA2, HEK2 and PALB2 are recognized as causing agents of prostate cancer. Ovarian cancer and Breast cancer is also linked with these genetic aspects. Furthermore, mutations in genes like "PMS2, MLH1, MSH2, and MSH6", linked to Lynch syndrome have been concerned in prostate cancer risk. While rare, germline alterations in high-risk genes can significantly influence treatment decisions and screening strategies in affected individuals (Abate-Shen et al., 2000; American Cancer Society, 2023; MedlinePlus Genetics, 2025).

Additionally, alterations in the "HOXB13" gene have been associated with early-onset prostate cancer, highlighting its role in familial cases. Despite advancements in identifying genetic risk factors, the molecular basis for the link between hereditary P.Ca and other cancers, such as breast cancer, remains unclear. Current research aims to uncover additional genetic variations that may contribute to prostate cancer susceptibility and improve clinical management

**2.4. Effect of Steroids**

Signaling of Steroid receptors is critical in all phases of prostate cancer development. In particular, there is a typical age-related decline in the proportion of androgens versus estrogens in men, which may be a contributive aspect in the instigation of prostate cancer (Bosland, 2000). Recent studies throw light on a major symptom of PCa that is alterations in androgen independency (Soronen et al., 2004).

**3. Molecular Mechanisms of Prostate Cancer**

Various variations in molecular mechanisms like genetic, signalling pathways and epigenetic variations lead to PCa. Alterations in TP53 gene and PTEN gene along with changes in oncogens i.e. ERG also leads to PCa. More oftenly observed alterations include combination of TMPRSS2 and ERG which causes aberrant transcriptional activity leading to PCa (Abate-Shen & Shen, 2010; Testa et al., 2019). Castration therapy is distributed by reduction of PTEN that promote cell multiplication and division by P13K/mTOR pathway (Taylor et al., 2010). Androgen receptor is crucial for development of PCa. Structural changes are observed in AR, when androgen such as testosterone and dihydrotestosterone bind to AR,like protein movement into nucleus from cytoplasm and also initiates transcription that control cell growth and reproduction (Ho & Dehm, 2017).

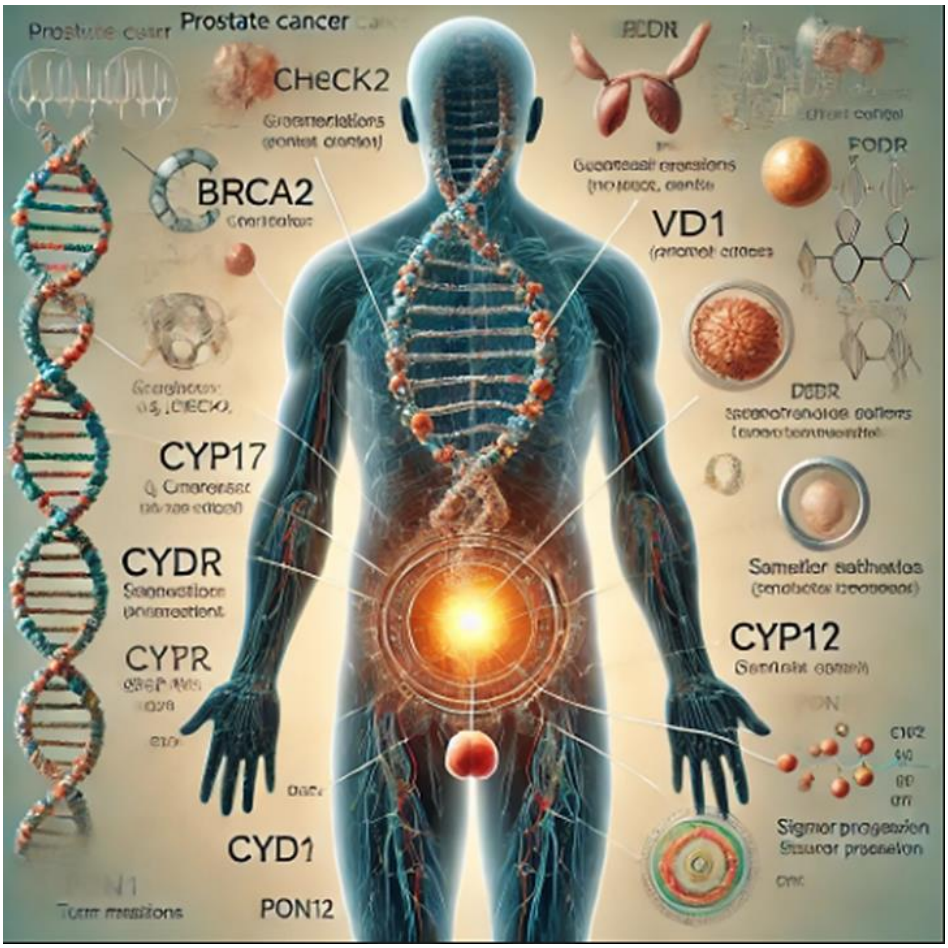


**Fig. 1:** Molecular Mechanism of Prostate Cancer

In PCa, gene methylation and histone alteration are essential genetic changes. These changes lead to oncogen ignition and may inhibit tumor suppressor genes (Figure 1). For instance, hypermethylation of PTEN is associated with malignant disease profile. Micro RNAs, including miR-379, has been found to play a crucial role in regulating metastasis in PCa. During metastasis, PCa cells often undergo epithelial cell into the mesenchymal transition, a process characterized by loss of cell polarity and adhesion properties. This process is frequently associated with down regulation of E-cadherin, a key molecule involved in cell adhesion. Due to this cancer cells penetrate extracellular matrices and also travel to bone marrow stroma. Matrix metalloproteinases (MMPs) are proteolytic enzymes which promote the metastatic dissemination. These studies about molecular mechanisms gives knowledge related to onset of disease, advancement of disease and resistant property of disease. Developing research including epigenetics, microRNA and immune checkpoint inhibitors offers advanced precision therapies of targeted disease (Ho & Dehm, 2017).

Histone modification and methylation of genes are critical epigenetic changes observed in PCa. These alterations can silence tumor suppressor genes or activate oncogenes. For example, hypermethylation of PTEN has been linked to aggressive disease phenotypes. Additionally, microRNAs such as miR-379 have been shown to regulate metastasis by modulating cell adhesion properties, providing potential therapeutic targets. PCa metastasis often involves E.M.T where cells lose polarity and adhesion properties mediated by E-cadherin downregulation. This enables cancer cells to invade extracellular matrices and migrate to distant sites like bone marrow stroma. Proteolytic enzymes such as matrix metalloproteinases (MMPs) also play a role by degrading barriers for metastatic spread. Understanding these molecular mechanisms provides insights into prostate cancer initiation, progression, and resistance to treatment. Emerging research on epigenetics, microRNAs, and immune checkpoint inhibitors offers promising avenues for developing targeted therapies.

## 4. Genes Related to Prostate Cancer

### 4.1. GSTP1

Prostate cancer frequently involves alterations to the glutathione S-transferase pi gene (GSTP1), and one typical mechanism that inhibits its interpretation is excessive methylation of its promoter region. An early event in prostate carcinogenesis, this epigenetic alteration is seen in approximately 6% of proliferative inflammatory atrophy (PIA) samples, 70-75% of prostatic intraepithelial neoplaxia (PIN) cases, and 92.95% of prostate cancer cases (Nakayama et al., 2004).The detoxifying enzymes encoded by GSTP1 catalyze the conjugation of glutathione with toxic electrophilic chemicals, which can be generated internally or come from outside the body (Hayes & Pulford, 1995).

For cells to be protected from carcinogenic substances, this protective function isessential. Hypermethylation-induced decrease of GSTP1 expression highlights its tumor suppressive role by increasing oxidative DNA damage and promoting cancer cell survival under extended oxidative stress (Lin et al., 2009). Hypermethylated GSTP1 has been identified as a potentially useful prostate cancer diagnostic sign. The possibility for non-invasive diagnosis was demonstrated by the effective detection of GSTP1 hypermethylation in bodily fluids using techniques such methylation-specific PCR (Jerónimo et al., 2002). Additionally, studies show that GSTP1 silencing is associated with the evolution of the disease from healthy tissue to malignant prostate cancer morphologies and preneoplastic lesions (PIN) (Yegnasubramanian et al., 2008).

Apart from methylation-induced silence, GSTP1 polymorphisms also affect the risk of prostate cancer. Variants such as Ala 114 Val and Ile 105 Val have been linked to changes in enzyme activity and regulatory effects on pathways including MAP K-JNK signaling, which affects the mechanisms of DNA repair, apoptosis, and proliferation in prostate cancer cells (Wang et al., 2012). But by totally suppressing GSTP1 expression in the later phases of the illness, promoter hypermethylation negates these polymorphic effects (Nakayama et al., 2004). All things considered, GSTP1 contributes significantly to prostate carcinogenesis through both genetic and epigenetic modifications. In addition to aiding in the growth of tumors, its early silencing through hypermethylation is a useful biomarker for prostate cancer risk assessment and early detection. (Yegnasubramanian et al, 2008; Lin et al., 2009)

### 4.2 NKX 3.1

The most likely factor for the frequently reported 8p deletion in prostate cancer is the “Human homeobox gene NKX3.1”, which is located at 8p21. Although, the deletion of the gene is probed in prostate cancer, the residual allele has not yet been found to have any mutations. However, it has been demonstrated that advanced tumour stage and hormone-refractory illness are related to the decrease of NKX3.1 expression. The likelihood of haploinsufficiency as a method to remove the tumour suppressive effect of NKX3.1 has been raised due to the nonexistence of alterations in the remaining allele of NKX3.1. Moreover, “NKX3.1þ” (heterozygous) / and “PTENþ/” compound mutant mice develop prostate cancer (Abate-Shen & Shen, 2010).

### 4.3 PTEN

The PTEN tumour suppressor gene is situated at 10q23, a location where cytogenetics, LOH, and CGH have frequently detected deletions in prostate cancer. Dual specificity phosphatase, which is encoded by the EN gene, controls important signal transduction pathways.

Phosphatidylinositol 3,4,5-trisphosphate is the primary target of its primary lipid phosphatase activity (PIP-3). PTEN downregulates the Akt/PKB signaling pathway, which encourages cell survival and opposes apoptosis, by dephosphorylating PIP-3. Molecular mechanisms of prostate cancer (Abate-Shen & Shen, 2010).

### 4.4. TP53

A crucial cycle regulator i.e. Tumor Protein TP53 gene which shifts G1 To S phase in cell cycle when mutated ultimately leads to malignancies. When DNA damage is present, TP53 can either cause apoptosis or stop the cell cycle to allow for DNA repair (Wyman & Kanaar, 2006).

### 4.5. AR

The relationship between androgens and prostate cancer development has been understood for more than 50 years. The androgen signaling pathways are reactivated throughout the advancement of hormone-refractory prostate cancer, according to more recent cDNA

microarray studies. A particular receptor mediates the action of androgen i.e the androgen receptor (AR), triggers in the target cells (AR). Analysis of genetic AR changes in prostate cancer is thus natural (Abate-Shen & Shen, 2010).

**5. Pros of CRISPR/Cas9 Technology**

In molecular biology, genome editing accurate and effective change is attained by causing breakage of double-stranded DNA, which often involves the attachment of a specific DNA-binding domain sequence to a non-specific binding domain, thus activating the DNA repair mechanism (Wyman & Kanaar, 2006). Recent research have highlighted the importance of nucleases enzymes as they are not only effective for diseases like sickle cell anaemia but also for cancer. Traditionally, two discrete genome editing methods, i.e., "Transcription activator-like effector nucleases (TALENs)" and "zinc-finger nucleases (ZFNs)" have been used for DNA repair by targeting the desired gene. The specificity and affinity of the nucleases are the only factors that affect these procedures. (Qu et al., 2013; Hazafa et al., 2020)

Inactivated Cas-9 (d-Cas9) can be used to act on the epigenome by repressing the enzymatic action of HNH domains without disturbing the sequence. "CRISPR/Cas9" has various other varieties which can be implemented in other cases. Similar to this, another study found that HeLa cells' endogenous genes, such as CXCR-4 and CD-71, were strongly suppressed by CRISPR-I. (Gilbert et al., 2013). As per literature survey, it is confirmed that advance technology i.e CRISPR/Cas9 is more 80% more effective as compared to other methods for editing human pluripotent stem cells.

**6. Methodology of CRISPR Screen**

**6.1. Library**

You can browse the pooled libraries for KO screens on Add gene, including the Ge CKO, H1/H2, Brunello, and TKOCRISPR-KO libraries. These libraries contain almost 4 to 10 sgRNAs and almost 17,000 genes. The activation screen libraries for CRISPRa and SAM and the repression screen libraries for CRISPRi are also shared. Particularly interesting investigations can benefit from custom libraries (Tsujino et al., 2021)

**6.2. Packaging**

In a pooled screen using the CRISPR/Cas9 technology, the first step is to make a library of damaged cells by using lentiviral infection of an sgRNA library. Transfecting a sgRNA library into the proper host cells, such as the highly capable HEK 293FT cells, results in the creation of viruses. By estimating virus titer, a low (0.3) multiplicity of infection (MOI) can be ensured, which prevents uncertainty in the event that the host cells absorb multiple sgRNAs, targeting various genes within each cell / his stage has a drawback that should be noted: some PCa models, such as NCI-H660 and VCAP cells, make it difficult to apply tools using lentiviral methods (Tsujino et al., 2021).

**6.3. Viability-Based Screens**

Finding genes that affect cellular fitness is the basic strategy used by researchers. The idea behind negative selection screening is that as the screening process goes on, gene disruptions that lower fitness will become less common or disappear completely. Negative selection screens are widely used in cancer biology to identify tumor cell dependencies linked to certain mutations, copy number variations, and expression patterns, among other things. The simplest negative selection screening technique for determining critical growth genes entails long-term, continuous cell culture. Through the use of these screening methods, researchers have been able to identify the precise genes required for the growth and survival of specific cancer cell lines (Smith et al., 2021). A different negative selection screening method in specific genetic background cell lines identifies synthetic lethal interactions through the dual suppression of genes that leads to decreased cell viability. Researchers found synthetic interactions which will allow targeted therapy against cancer cells because drugs will function solely on cells with particular alterations and provide a fresh method for cancer treatment. The positive selection screen operates by identifying enriched cells over time instead of eliminating unwanted cells like the negative selection screen does. These screens have helped to find perturbations that provide resistance against small molecules and different conditions, including pathogen infections. During positive selection screening, the majority of the population is eliminated while the surviving perturbations become enriched by more than 100-fold. Through one screening process, researchers can obtain both positively selected and negatively selected phenotypic results. A viability-based knockout screen in cancer cells helps identify which oncogenes become depleted and which tumor suppressors become enriched. Intermediate dosages of small molecules often result in the identification of both sensitization genes and resistance genes (Tsujino et al., 2021)

**6.4. Marker Selection Screen**

Expression of of molecule is directly related with genetic factors so selection screen is used to identify those markers & mutation is used for determining the phenotype that affect the marker protein rather than cell survival. In this type of screening, the correspondent can be genetically altered by replacing the fluorescent marker with a gene coding sequence. Finally, fluorescence-activated cell sorting permits the identification of upstream expression regulators by isolating cells with sgRNAs that target genes affecting marker expression (Bock et al., 2022).

**6.5. Analysis (Algorithms)**

Polymerase Chain Reaction is used for the extraction of DNA and to analyze the gene causing the defect. Next-generation sequencing technology is used to evaluate the areas encoding sgRNAs using large-scale parallel sequencing. Several algorithms, such as Model-based Analysis of Genome-wide CRISPR/Cas9, CRISPR AnalyzeR , Bayesian Analysis of Gene EssentiaLity (BAGEL), and Platform-independent Analysis of Pooled Screens using Python (PinAPL-P), can be used to analyze the variations in sgRNA abundance between control and phenotypic samples (Butz & Patócs, 2019).

### 6.6. Authentication

Through screen analysis, a prioritized list of putative genes associated with the trait is found. To find out which genes affect the phenotype and how much, validation is necessary. Introducing single guide RNAs (sgRNAs) that target the gene of interest and determining if the predicted phenotype is consistently replicated is one of the most dependable validation techniques. As long as many sgRNAs targeting the same genomic region provide the same phenotypic results, recent improvements in sgRNA specificity eliminate the need to validate target binding (Smith et al., 2021).

However, when required, methods like Western blotting, RT-qPCR, and genomic PCR can be used to evaluate functional alterations in the targeted gene. It is important to recognize that off-target effects can still occur even after on-target activity has been confirmed. For ongoing phenotypic validation, it is therefore advised to use numerous sgRNAs per gene, particularly those with the highest on-target activity. Rescue experiments can also be used to ascertain whether a characteristic is due to a genetic component. These research seek to determine if CRISPR/Cas9-edited cells return to their wild-type condition when candidate gene expression is restored to normal levels (Johnson & Lee, 2022).

## 7. CRISPR Screens in Prostate Cancer

There are many types of screen.Below mentioned are some noticeable screening strategies till date

### 7.1. Detection of Potential Target

In the beginning of 2014, genome editing technology got significant revolution and Ist screen for “CRISPR” Knockout was published. The Ist genome-wide CRISPR-KO screen in prostate cancer (PCa) cells was later reported by Fei et al. (2017). This study utilized the GeCKO v2 library for a viability-based screen in LNCaP cells and employed custom-designed MAGeCK and MAGeCK-VISPR algorithms to identify heterogeneous nuclear ribonucleoprotein L (HNRNPL) as a critical gene essential for PCa growth. The study revealed that HNRNPL directly regulates its RNA targets, including those encoding androgen receptor (AR), either through back-spliced circular RNA formation or linear alternative splicing. Notably, PCa exhibits abnormal expression of both HNRNPL and its RNA targets, emphasizing their potential therapeutic significance. The authors suggested that HNRNPL could serve as a viable therapeutic target in PCa treatment (Fei et al., 2017).

Similarly, a CRISPR-KO screen using a nuclear protein single-guide RNA (sgRNA) sub-pool library demonstrated that heterozygous deletion of chromosome 17p, observed in up to 63% of metastatic PCa cases, creates a selective dependency on ring-box 1 (RBX1) (Lei et al., 2021). Furthermore, the combined suppression of RNA polymerase II (RNAP2) and RBX1 significantly inhibited castration-resistant prostate cancer (CRPC) growth, thereby enhancing the therapeutic efficacy of RNAP2 inhibitors, such as α-amanitin-conjugated anti-EpCAM antibodies (Lei et al., 2021).

Shalem et al. (2014) performed a detailed experiment in which 33 cancer cell lines and loss of genetic function was analyzed. Expanding on these findings, Yoshiyama et al. (2021) analyzed data from CRISPR-KO screens in PCa cell lines obtained from the Cancer Dependency Map (DepMap), a public repository for CRISPR-KO screenings. They identified a context-specific vulnerability in histone demethylase JMJD1C, particularly in AR-negative cells. Their findings demonstrated that JMJD1C depletion selectively suppresses the growth of AR-negative PCa cells by activating the tumor necrosis factor alpha (TNF-α) network. Consequently, they proposed JMJD1C inhibition as a potential therapeutic strategy for patients with AR-negative PCa who no longer respond to AR inhibitors (Yoshiyama et al., 2021).

Additional research acknowledged “Kinesin Family Member 4A” (KIF4A) and WD Repeat Domain 62 (WDR62) as significant contributors to PCa phenotypes in both in vitro and in vivo models. Based on clinical data, these genes were nominated as PCa driver genes (Jiang et al., 2021). Additionally, a CRISPR-based E3 ligase screening approach in DsRed/EGFP-PDK1 reporter HKT cells conducted by Jiang et al. (2021) identified the Cullin3SPOP E3 ligase as a regulator of PDK1 ubiquitination and degradation. Notably, approximately 15% of PCa cases exhibit SPOP mutations. Mechanistically, the study demonstrated that SPOP recognizes PDK1 through CK1/GSK3β-mediated phosphorylation and degron-dependent binding. Mutations within PDK1's binding region impair this recognition and ubiquitination process, leading to elevated PDK1 protein levels, enhanced AKT kinase activity, and increased tumor malignancy. These findings suggest that targeting the PDK1-AKT pathway could serve as a promising therapeutic approach for PCa (Jiang et al., 2021).

### 7.2. Resistance Mechanisms and Synthetic Lethality Targets

Most patients who get ADT for their locally progressed disease have remission. Although most patients who get ADT experience near-certain remissions lasting 1-2 years, CRPC causes cancer cells to progress resistance. The success of medications like abiraterone and enzalutamide demonstrates the critical role AR signaling plays in CRPC. Unfortunately, patients acquire treatment resistance and always end up dying from the illness. Regulators of AR inhibitor sensitivity has been identified using a genome-wide CRISPR screen. It’s interesting to note that TLE3 deletion increases the expression of the glucocorticoid receptor (GR), which is consistent with the interaction of TLE3 and AR at the GR locus. Their findings provide new insights into the regulation of the GR locus in the context of AR inhibition in PCa cells, identifying TLE3 as a key regulator of GR-mediated resistance to AR inhibitors (Setton et al., 2021).

The researchers then used a similar strategy to find kinases whose inhibition could increase the effectiveness of enzalutamide in PCa cells in an effort to find genes associated with resistance and potential medication combinations that could overcome enzalutamide resistance. It was demonstrated that in the main activating region of “BRAF” gene, PCA cell hides mutation by suppression, or downstream, MAPK or increased activity of other genes. These findings suggest that the co-inhibition of MAPK and AR pathways in BRAF-mutated PCa may hold therapeutic potential (Qu et al., 2013).

## Conclusion

Since “CRISPR screenings” have been conducted across multiple cancer lineages in hundreds of cell lines, including PCa, data portals like

DepMap serve as valuable resources for this research. As noted earlier, analyzing data from other tumors may provide valuable insights for PCa and vice versa. Further studies in PCa patients are needed to examine the nto examineear future to investigate new targets identified in screens presented in this paper. Advancements in genomic research, such as CRISPR screening, will enhance our understanding of the molecular characteristics of CRPC and be translated into clinical practice through increased molecular testing for therapeutic selection and clinical trial eligibility.

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