ISTANBUL MEDENIYET UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS

Investigation of The Tumor Suppressor Role and Molecular Mechanism of miR-27a-5p in Prostate Cancer

Master's Thesis

Ceyda Nur Zaim

August 2024

ISTANBUL MEDENIYET UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS

Investigation of The Tumor Suppressor Role and Molecular Mechanism of miR-27a-5p in Prostate Cancer

Master's Thesis

Ceyda Nur Zaim

Supervisor

Assoc. Prof. Dr. Nagehan Ersoy Tunalı

August 2024

THESIS JURY APPROVAL

This master thesis titled “Investigation of The Tumor Suppressor Role and Molecular Mechanism of miR-27a-5p in Prostate Cancer” written by Ceyda Nur ZAİM at the Department of Molecular Biology and Genetics was accepted by our jury.

JURY MEMBERS **SIGNATURE**

Supervisor:

Assoc. Prof. Dr. Nagehan ERSOY TUNALI

İstanbul Medeniyet University

Other Jury Members:

Prof. Dr. Asuman DEMİROĞLU ZERGEROĞLU

Gebze Technical University

Asst. Prof. Dr. Ümmühan DEMİR

İstanbul Medeniyet University

Date of Defense: 28/ 08 / 2024

STATEMENTS

Style and Reference Manual Statement

Having reviewed this thesis written under my supervision, I confirm that it has been written in accordance with American Psychological Association Manual of Style and used its reference format consistently throughout the entire text.

Signature

Assoc. Prof. Dr. Nagehan ERSOY TUNALI

Declaration of Originality

I hereby declare that all information in this dissertation has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conducts, I have fully cited and referenced all material and results that are not original to this work.

Signature

Ceyda Nur ZAİM

GENİŞ ÖZET

Prostat kanserinde miR-27a-5p'nin tümör baskılayıcı rolünün ve moleküler mekanizmasının araştırılması

ZAİM, Ceyda Nur

Yüksek Lisans Tezi, Moleküler Biyoloji ve Genetik Anabilim Dalı

Danışman: Doç. Dr. Nagehan ERSOY TUNALI

Ağustos 2024

Prostat kanseri, ana patogenezi androjen reseptörü (AR) sinyal yolağındaki değişimler olan ikinci en yaygın malignite türüdür. AR yolağının ve AR ilişkili yolakların baskılanması temel tedavi yaklaşımı olarak benimsenmiştir. Daha etkili tedavi yöntemlerinin hastaya özgün olarak geliştirilebilmesi için, AR'yi ve ilişkili yolaklarını düzenleyen mekanizmaların tam olarak belirlenmesi gerekmektedir. Kanserdeki düzenleyici fonksiyonlarından dolayı miRNA'lar umut vadeden terapötik hedeflerdir. miR-27a-5p ise tümörlerde proliferasyon, anjiyogenez, apoptoz, invazyon, ve migrasyon gibi pek çok hücresel süreçlerde önemli roller oynadığı bulunmuş olan bir miRNA olarak belirlenmiştir. Ancak, bu miRNA'nın fonksiyonları karmaşıktır ve miR-27a-5p tarafından hedeflenen genlerin açığa çıkartılması gerekmektedir. Bu tez çalışmasında, miR-27a-5p'nin prostat kanserindeki tümör baskılayıcı rolü, moleküler mekanizması ile birlikte araştırılmıştır. İlk olarak, miR-27a-5p tarafından hedeflenme potansiyeli olan genler *in silico* olarak belirlenmiş ve en yüksek ihtimale sahip hedef genler seçilerek, bu genler ile miR-27a-5p arasındaki moleküler ilişki *in vivo* ve *in vitro* olarak incelenmiştir. miR-27a-5p'nin en yüksek ihtimalli hedef genleri olarak CCL5/CCR5 kemokin sinyal yolağının üyeleri olan CCR5, STAT3 ve BCL-2 genleri belirlenmiştir . miR-27a-5p ifade seviyesi ve CCR5, BCL-2 ve AR ifade seviyeleri arasında, *in vivo* prostat tümörlerinde ters korelasyon bulunmuştur. DU145 ve LNCaP hücre hatlarında miR-27a-5p seviyesi arttırıldığında STAT3, BCL-2 ve AR mRNA seviyelerinde azalma tespit edilmiş olup, bu sonuç da miR-27a-5p'nin bu onkogenik sinyal yolaklarını negatif yönde düzenlediğini doğrular niteliktedir. Ancak, miR-27a-5p ifade seviyesi arttırıldığında CCR5 mRNA seviyesinde anlamlı bir azalma gözlemlenmemiştir. Bunlara ek olarak, miR-27a-5p'nin

onkogenik hücresel süreçlerdeki rolü de belirlenerek, miR-27a-5p'nin DU145 ve LNCaP hücrelerinde proliferasyon, migrasyon ve invazyonu baskıladığını ve bu hücrelerde apoptozu desteklediği de gösterilmiştir. Bu sonuçlar miR-27a-5p'nin prostat kanserindeki tümör baskılayıcı rolünün altını çizmektedir. Diğer taraftan, AR ve STAT3 genleri açısından negatif olan PC3 prostat kanseri hücre hattında miR-27a-5p'nin anti-proliferatif özelliği gözlemlenmemiştir. AR-pozitif olan ve BCL-2 ifade seviyesi DU145 hücrelerine göre daha fazla olan LNCaP hücrelerinin, AR-negatif olan DU145 hücrelerine göre, miR-27a-5p seviyesindeki artışa daha fazla apoptotik cevap verdiği gösterilmiştir. Bu sonuçlar, miR-27a-5p'nin tümör baskılayıcı rolünün duruma bağlı olduğunu göstermektedir. Sonuç olarak, miR-27a-5p, fazlaca ifadeleri bulunan onkogenik yolakları negatif düzenleyerek prostat kaserinde tümör baskılayıcı olarak hareket etmektedir. Bu tez çalışması kapsamında miR-27a-5p'nin STAT3, BCL-2 ve AR mRNA'larını negatif olarak düzenleyerek, prostat kanseri hücrelerinde proliferayon, migrasyon, invazyon ve hücresel sağ kalımı baskıladığı gösterilmiştir. Elde edilen veriler miR-27a-5p'nin moleküler mekanizmasını aydınlatmakla kalmayıp, aynı zamanda prostat kanserinde bu miRNA'nın terapötik potansiyelini de ortaya koymaktadır.

Anahtar Kelimeler: Prostat kanseri, miRNA, AR, CCL5/CCR5 kemokin yolağı, STAT3, BCL-2

ABSTRACT

Investigation of The Tumor Suppressor Role and Molecular Mechanism of miR-27a-5p in Prostate Cancer

ZAİM, Ceyda Nur

Master's Thesis, Department of Molecular Biology and Genetics

Supervisor: Assoc. Prof.Dr. Nagehan ERSOY TUNALI

August 2024

Prostate cancer is the second-most prevalent malignancy with its fundamental pathogenesis involving alterations in AR signaling. Suppression of AR and its related pathways constitutes the primary treatment approach. The mechanisms that regulate AR directly or AR-related pathways are required to be fully elucidated to develop more effective treatment approaches in a patient-specific way. miRNAs are highly promising therapeutic candidates owing to their regulatory functions in cancer. miR-27a-5p has been found to have vital roles in many cellular processes of tumors, including proliferation, angiogenesis, apoptosis, invasion, and migration. However, its functions are complex, and the genes that are targeted by miR-27a-5p remain to be identified. In this study, tumor suppressive function of miR-27a-5p with its molecular mechanism in prostate cancer was investigated. First, a list of potential target genes were identified *in silico* and then the most probable targets of miR-27a-5p were selected for further *in vivo* and *in vitro* investigation of the correlation between these genes and miR-27a-5p. CCL5/CCR5 chemokine signaling, involving CCR5, STAT3, and BCL-2 genes were identified as the most probable targets of miR-27a-5p. An inverse correlation between miR-27a-5p expression and the expressions of CCR5, BCL-2, and AR was determined in *in vivo* prostate cancer tumors. When miR-27a-5p is overexpressed in DU145 and LNCaP cell lines, expressions of STAT3, BCL-2, and AR mRNAs were found to be reduced, confirming that miR-27a-5p negatively regulates these oncogenic pathways. However, the expression level of CCR5 was not decreased with miR-27a-5p overexpression. The role of miR-27a-5p in oncogenic cellular processes was also revealed, where miR-27a-

5p overexpression decreased the proliferation, migration, and invasion while promoting apoptosis of DU145 and LNCaP cells, highlighting its tumor suppressor function in prostate cancer. On the other hand, miR-27a-5p was found to have no anti-proliferative effect in the PC3 cell line, which is STAT3- and AR-negative. It was also shown that apoptotic response to miR-27a-5p overexpression was greater in AR-positive LNCaP cells that have greater expression of BCL-2 than AR-negative DU145 cells. These results show that the tumor-suppressor function of miR-27a-5p is context-dependent. In conclusion, miR-27a-5p acts as a tumor suppressor miRNA in prostate cancer by negatively regulating upregulated oncogenic signaling pathways. In the framework of this thesis, We demonstrated that miR-27a-5p suppresses proliferation, migration, invasion, and cell survival of prostate cancer cells through negatively regulating STAT3, BCL-2, and AR. This study sheds light on the molecular mechanism of miR-27a-5p, as well as highlights the therapeutic potential of miR-27a-5p in prostate cancer.

Keywords: Prostate cancer, miRNA, AR, CCL5/CCR5 chemokine signaling, STAT3, BCL-2

ACKNOWLEDGEMENTS

I would like to highlight my sincerest thanks to my supervisor, Assoc. Prof. Dr. Nagehan ERSOY TUNALI for her encouraging guidance, and mind-expanding support during this journey. She is a precious mentor who shares her experiences and knowledge, providing novel insights with great motivation. It has been a great honor to be a member of her team.

Priceless thanks to the NET-Lab team, especially Zeynep Dilhan ÜN who makes this journey joyful and easier for me, and Derya AKYÜZ ŞENGÜN, and BİLTAM members, Dr. Gülşen GÜNEL, and Selçuk Kaan HACIOSMANOĞLU.

A heartfelt thanks to my dear family, my mother Esra SEZEROĞLU, my sister Edo, and my brother Semo. I always feel their endless support.

I would like to thank Asst. Prof. Dr. Ümmühan DEMİR for kindly providing me with some experimental materials.

I would also like to thank to members of Bezmialem Vakıf University Experimental Application and Research Center, especially to Experimental Animals Lab for their helps in the generation of *in vivo* model.

I would like to thank Tübitak for selecting me for the TUBITAK 2210-A National Graduate Scholarship Programme during my master's study.

Ceyda Nur ZAİM

TABLE OF CONTENTS

LIST OF SYMBOLS

<: less

° C: degrees centrigrate

µg: microgram

µl: microliter

µM: micromolar

ng/µl: nanogram per microliter

LIST OF ABBREVIATIONS

ADT: Androgen deprivation therapy

AF-1: Activation function-1

AF-2: Activation function-2

APEX1: Apyrimidinic endodeoxyribonuclease 1

AR: Androgen receptor

ARE: Androgen response elements

ARV: Androgen receptor variant

bHLHLZ: Basic helix-loop-helix leucine zipper

CCL5: C-C chemokine ligand 5

CCR5: C-C chemokine receptor 5

cDNA: Complementary DNA

CE: Cryptic exon

CRPC: Castration-resistant prostate cancer

DBD: DNA-binding domain

DHT: 5α-dihydrotestosterone

DNA: Deoxyribonucleic acid

DNMT3A: Methyltransferase 3A DNA

DRE: Digital rectal examination

ESCC: Esophageal squamous cell carcinoma

GCO: Global Cancer Observatory

GEMM: Genetically engineered mouse

GnRH: Gonadotropin-releasing hormone

GnRH: Gonadotropin-releasing hormone

GPCR: G protein-coupled receptors

HCC: Hepatocellular carcinoma

HIV: Human immunodeficiency virus

HNSCC: Head and neck squamous cell carcinoma

LBD: Ligand-binding domain

LNRH: Luteinizing-hormone-releasing hormone

MAPK: Mitogen-activated protein kinase

mCRPC: Metastatic castration-resistant prostate cancer

mHNPC: Metastatic hormone-naïve prostate cancer

miRNA: micro RNA

MOMP: Mitochondrial outer membrane permeabilization

mRNA: Messenger RNA

NES: Nuclear export signal

NFH_2O: Nuclease-free water

NLS: Nuclear localization signal

nmCRPC: Non-metastatic castration-resistant prostate cancer

NSCLC: Non-small cell lung cancer

NTD: N-terminal transactivation domain

PCa: Prostate cancer

PHB: Prohibitin

PHLPP: PH domain Leucine-rich repeat protein phosphatase

PIN: Prostate intraepithelial neoplasia

PIP2: Phosphatidylinositol 4,5 bisphosphate

PIP3: Phosphatidylinositol 3,4,5 trisphosphate

pri-miRNA: Primary-micro RNA

PSA: Prostate-specific antigen

PSMA: Prostate-specific membrane antigen

qRT-PCR: Quantitative real-time polymerase chain reaction

RANTES: Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted

RNA: Ribonucleic acid

SFRP: Secreted frizzled-related protein

TME: Tumor microenvironment

TRAMP: Transgenic adenocarcinoma of the mouse prostate

UTR: Untranslated region

WHO: World Health Organization

XPO5: Exportin 5

ZFAS1: ZNFX1 anti-sense RNA1

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

1.1.Prostate Cancer

Prostate cancer (PCa) is the second most prevalent type of cancer with 1,466,480 new cases and 396,792 deaths among adult men worldwide in 2022, according to the Global Cancer Observatory (GCO) (*Global Cancer Observatory,* n.d.). The risk factors for developing PCa include age, ethnicity, genetic predisposition, obesity, and environmental factors. PCa, which affects men aged 45-60 years, is diagnosed by prostate-specific antigen (PSA) test, digital rectal examination (DRE), and biopsy. DRE, which gives clues on any abnormalities, is used to determine the size of the prostate gland. Nevertheless, defining blood PSA levels is the main diagnostic approach for PCa screening in the clinic (Ferlay et al., 2010). PSA is a glycoprotein secreted from the prostate gland and its function is to liquefy semen. Even though it is mainly found in the semen, it can also be found in the bloodstream at deficient concentrations (<1 ng/mL) in healthy men (Collin, 2013). The cut-off point for the PSA level is identified to be 4 ng/mL; patients with PSA levels greater than 4 ng/mL have approximately 25% chance of developing prostate cancer, in which case, further testing, including biopsy, is required (Sekhoacha et al., 2022).

The healthy human prostate is comprised of three types of cells: luminal cells that express high levels of androgen receptor (AR) and PSA; basal cells that have low expression of AR; and a rare group of neuroendocrine cells. During the progression of PCa, the number of luminal cells with high AR expression increases, and basal cells are lost. Prostate cancer is characterized by the transformation of the normal prostate gland epithelial cells into the malignant form in three stages (Figure 1, A).

The initiation of disease development, stated as prostate intraepithelial neoplasia (PIN), is identified by the progressive loss of basal cells, leading to hyperplasia of luminal cells (Figure 1, B). This stage is regarded as the precancerous state. Further progression of the disease is defined by the complete loss of basal cells and the expansion in the luminal cell compartment. This stage is identified as adenocarcinoma androgen-dependent and is further subdivided into two stages, adenocarcinoma latent and clinical. At this

adenocarcinoma androgen-dependent stage, since the proliferation of tumor cells is androgen-dependent, the growth of the tumor can be suppressed by androgen deprivation. The final stage of PCa progression, adenocarcinoma androgen-independent, is the gain of resistance to androgen-deprivation therapy through altered molecular mechanisms, where the growth of tumors is no longer dependent on AR. This stage is also stated as castration-resistant prostate cancer (CRPC) and is characterized by metastasis and aggressiveness (Testa et al., 2019).

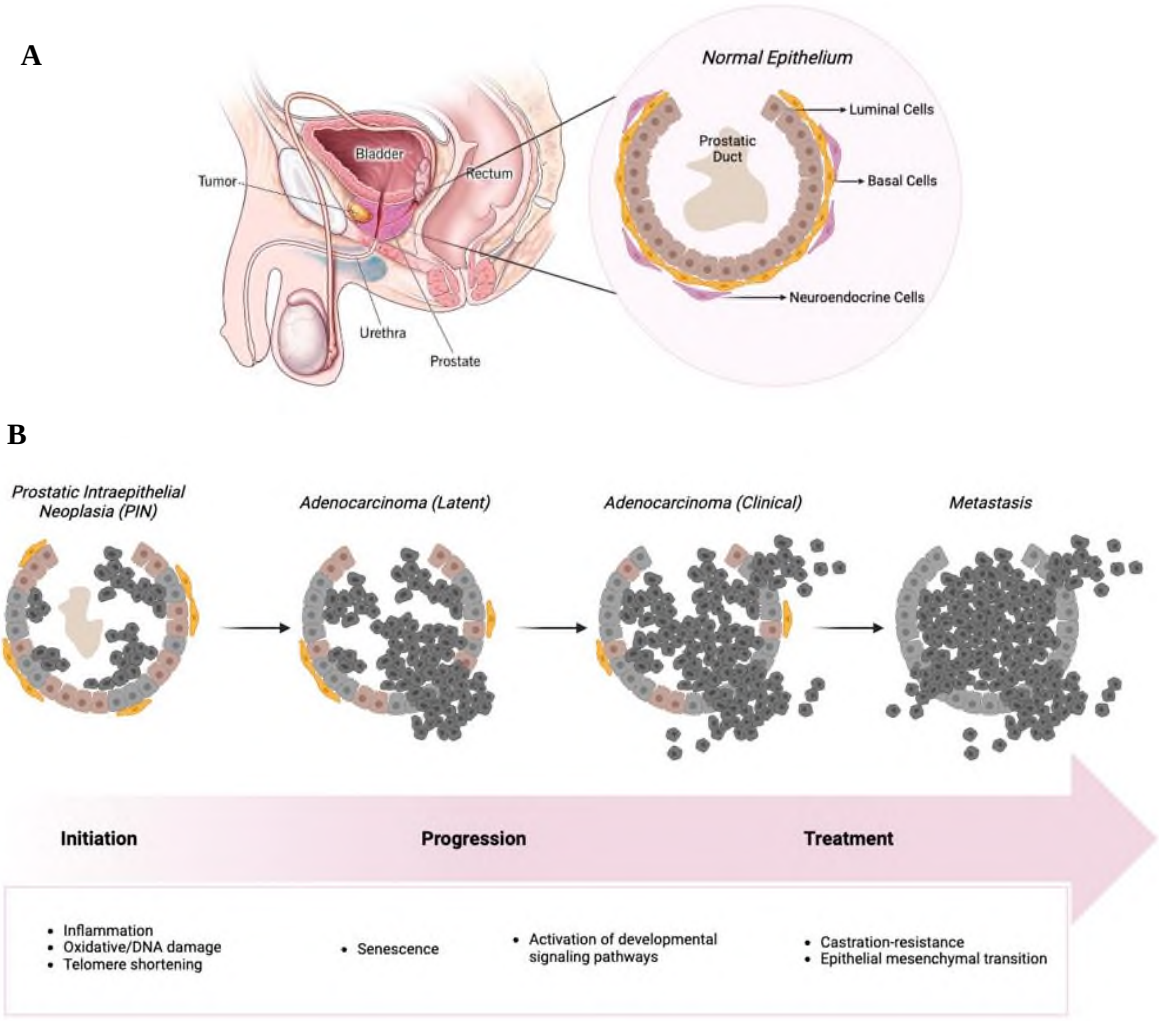
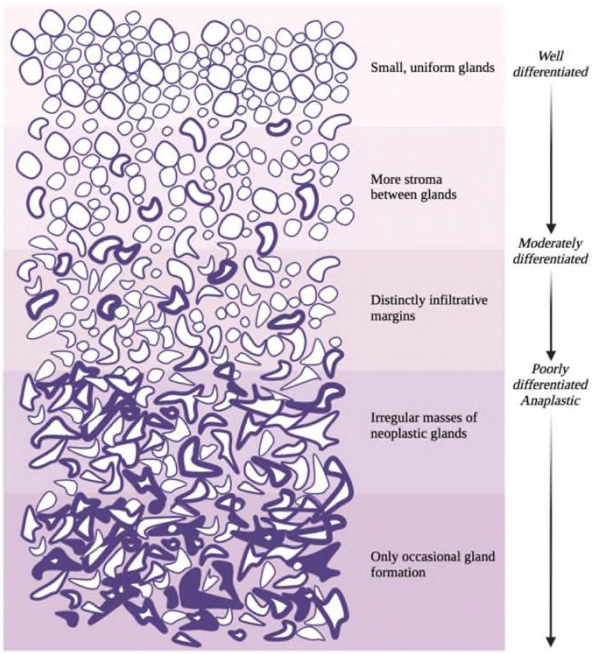


Figure 1. A. Normal prostate; B. Prostate cancer initiation and development. (Created with BioRender.com)

In the clinic, there is a unique scoring system accepted by the World Health Organization (WHO) in 2014, termed the Gleason score, which represents the histopathological evaluation of prostate tumors (Epstein, 2018). This scoring system was developed by Dr. Donald Gleason in the 1960s and assesses the similarity of prostatic tissue and normal prostate glands, in which the lowest grade, Grade 1, is related to normality, whereas the highest grade, Grade 5, corresponds to a lack of normal cells and glands and the existence of increased numbers of abnormal cells (Gleason, 1992; Mellinger, 1977). The Gleason scores are represented as the sum of two grades (3+4, 5+5, etc.), corresponding to the grades of two predominant cancer cell populations. The association between Gleason score, grade, and morphology of the cells are given in Table 1 for clear understanding.

Table 1. Gleason scoring system used in the clinic. (Created with BioRender.com)

Gleason score	*Grade*	*Cell morphology*
3+3=6	1	Cells and glands are of variable size and shape, looking similar to normal prostate tissue, and glandular architecture is conserved.
3+4=7	2	Cells and glands are highly similar to the Grade 1 tissue. The cells are likely to proliferate slowly.
4+3=7	3	Cells and glands are highly different from the normal tissue and cell proliferation is at a moderate rate.
4+4=8	4	Glands are fused and poorly formed, and cells look abnormal.
5+5=10	5	Glandular structures are absent, filled with sheets of abnormal cells and neoplastic zones.



1.2. Castration-Resistant Prostate Cancer

Treatment options for the localized PCa are determined by considering the recurrence risk. In the early stages, if the recurrence risk is clinically evaluated to be low, the main treatment options are radiation-based therapy or radical prostatectomy. However, if the patient has intermediate or high recurrence risk, androgen deprivation therapy (ADT), combined with radiation therapy is the treatment option. ADT is based on AR antagonists and surgical or medical castration. Despite the good response to ADT at first, which is indicated by reduced serum PSA levels, most patients eventually develop resistance to ADT, generating an advanced stage of the disease termed CRPC (Agoulnik & Weigel, 2006; Mohler et al., 2004). This stage of the disease is also termed hormone-refractory prostate cancer or androgen-independent prostate cancer since the growth of the tumor is no longer dependent on the androgens or AR. The evolution pattern of the disease can be briefly explained as follows: If there is no distant metastasis and the patient has not yet received ADT, the stage is termed localized prostate cancer. When cancer recurs after ADT in this patient, the stage is then termed non-metastatic castration-resistant prostate cancer (nmCRPC). On the other hand, if there is distant metastasis and the patient has not yet received ADT, the stage is referred to as metastatic hormone-naïve prostate cancer (mHNPC). Subsequently, when cancer relapses after ADT in this patient, it is then termed metastatic castration-resistant prostate cancer (mCRPC) (Mateo et al., 2019). The main purpose of ADT is to decrease serum testosterone levels under 50 ng/dL, which is referred to as castrate level, eliminating ligands of AR, thus inhibiting its activation and subsequent growth suppression (Harris et al., 2009). Therefore, the diagnosis of castration resistance is made when the disease progresses at the castrate level, which is detected by increased serum PSA level.

Despite CRPC tumors having been shown to activate alternative growth-promoting pathways, such as PI3K/Akt/mTOR, MAPK, JAK/STAT, and NF-**κ**B (Figure 2), to bypass the requirement for AR signaling (*see Section 1.3*), and despite the absence of testosterone; AR remains critical for signaling in CRPC (Culig et al., 1994). AR signaling is re-activated through either altered steroid metabolism, AR point mutations, changes in co-regulator proteins, or the generation of alternative splice variants of AR. The main and crucial modification in the alternative splice variants of AR is that they lack the regulatory

C-terminal hormone binding domain, promoting AR-supported growth regardless of the presence of androgens (Shafi et al., 2013). Further, steroid metabolism is altered in a way that suppressed testosterone synthesis is regained by *de novo* testosterone synthesis from cholesterol to sustain CRPC. Normally, 5α-dihydrotestosterone (DHT), which is the ligand of AR, is converted from testosterone, generated from gonadotropin-releasing hormone (GnRH) by the 5α-reductase isoforms, S5A1 and S5A2, through the classical androgen biosynthesis pathway at the tissue level (Harris et al., 2009). However, alternative androgen biosynthesis pathways and enzymes are activated due to ADT (Figure 2). For instance, CRPC tumors show higher levels of AKR1C3, which is an enzyme that converts 5α-Androstenedione, produced by the classical androgen biosynthesis pathway to DHT (Mitsiades et al., 2012). CYP17A1 is another enzyme found upregulated in CRPC and it is also involved in testosterone synthesis from cholesterol (Cai et al., 2011).

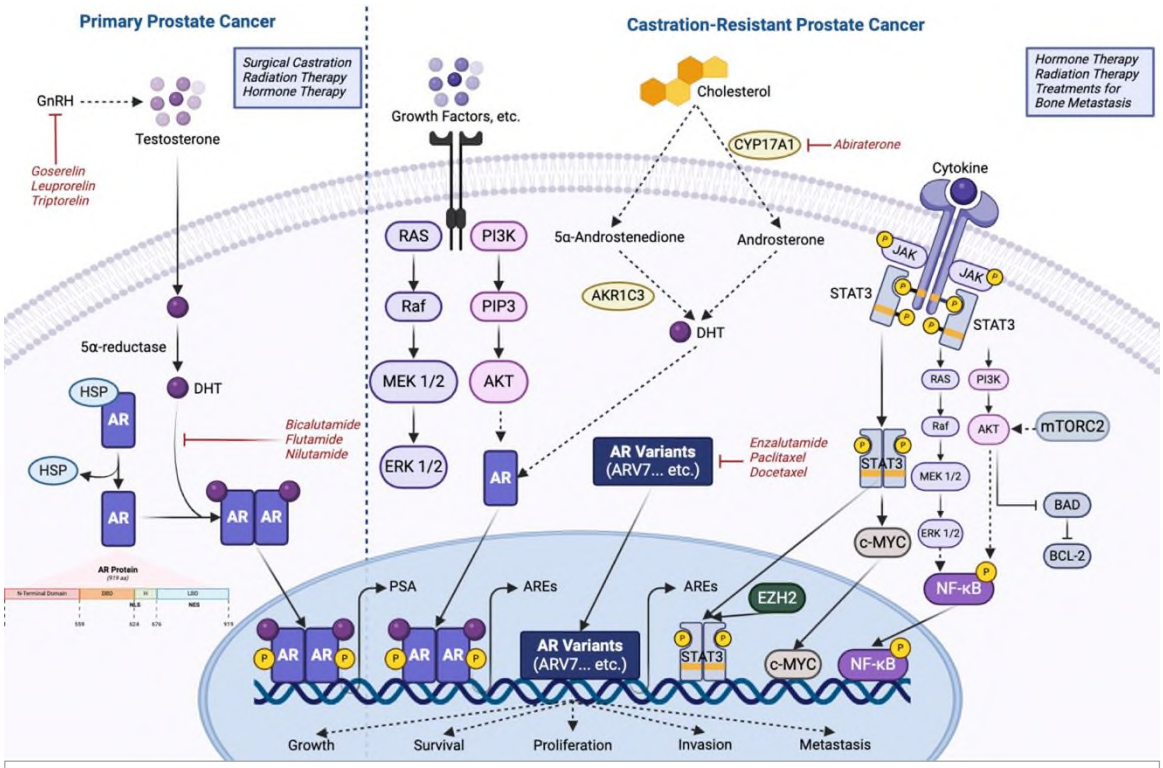


Figure 2. Molecular mechanisms involved in primary PCa and CRPC. (Created with BioRender.com)

1.3. Molecular Aspects of Prostate Cancer

As cancer is a disease of chaos, molecular mechanisms within a single cancer cell are also highly complicated, yet they need to be resolved. There are many receptors, proteins, enzymes, and miRNAs dysregulated in PCa, which results in either initiation, development or progression of the disease. These are all associated with growth, proliferation, invasion, migration, and apoptosis. Therefore, extensive knowledge on these growth-promoting or apoptosis-suppressor pathways would provide a better perspective to develop novel treatment and diagnosis options. The normal transcriptional network in prostate cells is disrupted during the initiation and progression of the PCa. As a frequent early event of PCa development, the NKX3-1 homeobox gene, which has a function in normal differentiation of the prostatic epithelium, is lost (Abate-Shen et al., 2008). At the same time, the TMPRSS2-ERG gene fusion and FOXA1 mutations determine significant molecular subtypes of the disease. Formation of the oncogene TMPRSS2-ERG fusion, which results in aberrant expression of the ETS family of transcription factors genes (ERG), presents in approximately 70% of PCa patients (Abeshouse et al., 2015; Kumar-Sinha et al., 2015; Tong, 2021; van Dessel et al., 2019). Overexpressed ERG binds to and interacts with the crucial transcription factors in PCa, such as AR, FOXA1, and HOXB13, which triggers the activation of Wnt and Notch signaling pathways in primary PCa. ERG-positive PCa tumors that also have PTEN loss are demonstrated to have an over-activated PI3K signaling pathway, leading to enhanced proliferation and invasion (Stelloo et al., 2018; Testa et al., 2019).

1.3.1. AR Receptor Signaling Pathway

The main pathogenesis of PCa is the aberrant activation of AR. AR, which is a member of the nuclear receptor superfamily, is a steroid receptor that functions as a transcription factor. The human AR gene is composed of eight canonical exons; exon 1 encodes for N-terminal transactivation domain (NTD); exons 2 and 3 encode for the DNA-binding domain (DBD); exons 4, 5, 6, 7, and 8 encode for hinge region and ligand-binding domain (LBD) with nuclear localization signal (NLS) and nuclear export signal (NES) (Gelmann,

2016). The gene coding for AR is located on the X Chromosome (q11.2). The AR gene is composed of more than 90 kb and codes for a protein that consists of 919 amino acids (Aurilio et al., 2020). The AR protein of 100 kDa has three functional domains (Figure 4); a highly conserved DBD, an LBD, exhibiting transcriptional activation function-2 (AF-2), and a less conserved NTD. In the cytoplasm, AR is bound with chaperons and heat-shock proteins (e.g. HSP-90/70/56) (Tan et al., 2015). The ligands of AR are DHT and testosterone. Upon ligand binding, AR disassociates from the other proteins, translocates into the nucleus, dimerizes, and binds to androgen response elements (ARE), located in the enhancer and promoter regions of AR target genes. Two zinc finger domains present in the DBD of AR are responsible for conferring specificity of DNA binding. A region in NTD, termed transcriptional activation function-1 (AF-1), is responsible for transcriptional activity, leading to the activation of AREs, including those associated with PSA and genes involved in cell proliferation and evasion of apoptosis (Dai et al., 2017).

An aberrant increase in the expression of AR is widespread in almost all primary and metastatic PCa. AR signaling remains active to provide survival of PCa cells through either full-length AR or AR variants, regardless of the stage or grade of PCa. Therefore, the main and crucial pathway to be suppressed is AR signaling and almost all current therapeutics are targeting AR. The basic working principle of androgen ADT depends on the anti-androgens that bind to the C-terminal region of LBD, inhibiting the activation of AR. At first, tumor growth can be successfully suppressed through ADT, but then, the cells become resistant to androgen deprivation by developing AR variants (ARV). These splice variants (e.g., AR-V7) lack LBD (Figure 3, A-B), which makes them constitutively active regardless of ligand binding, inducing an androgen-independent growth, termed CRPC (R. Hu et al., 2009).

AR-Vs can be classified into four groups according to their ability for nuclear localization. The first group of variants (e.g., AR-23) are ligand stimulated in a similar attitude to full-length AR (AR-FL). The second group, which is constitutively active variants, is the most studied variants of AR due to their continuous activation regardless of ligand binding. These variants (e.g., AR-V3, 4, 7, 12) lack DBD and exon 4, which is encoding for a part of the NLS, leading to the induction of the expression of AREs (Chan et al., 2012; R. Hu et al., 2009b). There are also conditionally active variants (e.g., AR-

45, AR-V1,9), inactive variants (e.g., AR-V13, 14, AR8), and variants whose functions are unknown such as AR-V2, 5, 6, 8, 10, 11 (Figure 3, A) (Abeshouse et al., 2015; C. Lu & Luo, 2013; Robinson et al., 2015).

The key mechanism of the expression of AR-Vs is the alternative RNA splicing, introducing cryptic exons (CE). AR-V7, the constitutively active variant, is one of the most crucial variants in CRPC. It has been shown that AR-V7 increases in the LNCaP95 cell when androgen is depleted (R. Hu et al., 2012). Likewise, AR-V7 was determined to be induced 4 days after castration in a xenograft model of VCaP (Yu et al., 2014).

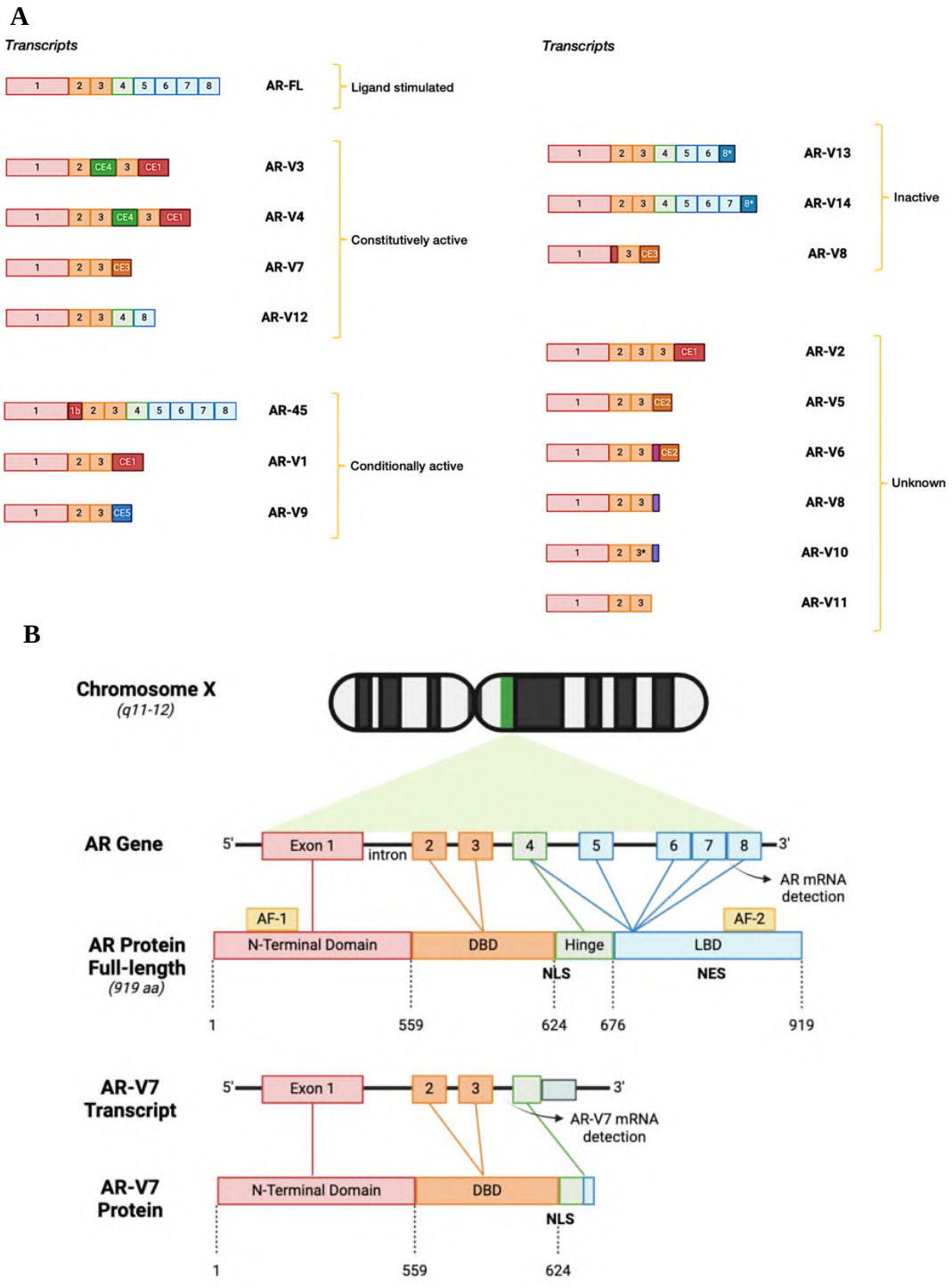


Figure 3. A. AR variants; B. Transcription and translation of AR and AR-V7. (Created with BioRender.com)

1.3.2. PTEN and PI3K/Akt/mTOR Signaling

PI3K/Akt signaling is activated in almost all cancer types, including PCa. PI3K/Akt pathway regulates many cellular processes such as proliferation, migration, apoptosis, and cell survival by inducing numerous downstream signaling molecules. These downstream signaling molecules, shown in Figure 4, include NF-**κ**B which is known to drive carcinogenesis by regulating gene expression of pro-inflammatory genes, and anti-apoptotic genes. Akt is known to phosphorylate BAD, which activates BCL-2, preventing apoptosis. Another important downstream effector of PI3K/Akt signaling is mTOR which promotes cell survival and regulates cell cycle. In addition, Akt regulates MYC activity by suppressing GSK3, which is a downstream protein of MYC, a well-studied oncogene. FOXO transcription factors are also downstream targets of PI3K/Akt signaling, and Akt dysregulates FOXO activity to prevent the expression of a group of genes, such as p27, which is a cell cycle inhibitor, further promoting the proliferation. PI3K/Akt signaling has been shown to be upregulated in PCa.

PTEN is a well-studied negative regulator of the oncogenic PI3K/Akt/mTOR pathway by converting phosphatidylinositol 3,4,5 trisphosphate (PIP3) back to phosphatidylinositol 4,5 bisphosphate (PIP2), preventing Akt activation, thereby acting as a tumor suppressor gene. PTEN gene mutation is frequently seen as an early event in PCa in which the loss of it was shown to be associated with recurrence, metastasis, and castration resistance. Diminished PTEN function and upregulation of the PI3K pathway are present in approximately 70% of late-stage PCa tumors (Mulholland et al., 2011). Lee et al. showed in a genetically engineered mouse model of PCa that PTEN loss worked together with TMPRSS-ERG fusion, which is the most frequent genomic alteration observed in PCa cases (S. H. Lee et al., 2015; Z. Wang et al., 2017). In addition, a crosstalk between AR and PI3K/Akt signaling was demonstrated, in which androgen depletion or knockdown in these murine PCa models was found to result in increased levels of active phosphorylated Akt. Therefore, the PI3K pathway becomes crucial in the progression to CRPC after ADT.

Another negative regulatory element of the PI3K/Akt pathway is the PH domain Leucine-rich repeat protein phosphatase (PHLPP) family, which consists of PHLPP1 and

PHLPP2. PHLPP suppress PIK3K/Akt signaling by dephosphorylating Akt and they have been considered as tumor suppressors in breast cancer, ovarian cancer, hepatocellular carcinomas, and PCa (Newton & Trotman, 2014). In a feedback loop, the mTORC1 complex, which is activated by Akt, decreases the protein levels of PHLPP1 and PHLPP2, promoting Akt activation (J. Liu et al., 2011). A large-scale genomic study revealed that both PHLPP1 and PHLPP2 were deleted in PCa patients. Furthermore, the same study concluded that PHLPP2 is more frequently deleted than PTEN or PHLPP1 and the frequency of deletion was further increased in mPCa tissues (Taylor et al., 2010).

The contribution of PI3K/Akt signaling to the progression of prostate cancer is clear in the literature that 40% of primary and 70% of metastatic prostate cancer patients have genetic alterations that promote the activation of the PI3K pathway (Carver et al., 2011; Taylor et al., 2010). For instance, PTEN mutations were found in approximately 17% of primary prostate cancers and 50% of mCRPC (Robinson et al., 2015b). Moreover, downstream elements of PI3K signaling also promote the disease. FOXO1, which is inactivated by Akt and functions as a tumor suppressor by regulating DNA damage repair, cell death, and cell cycle, was found to be downregulated in PCa patients (Abeshouse et al., 2015; Robinson et al., 2015; Dong et al., 2006;Yang et al., 2005). FOXO1 does not only regulate cellular events in PCa but also can bind directly to the AR to inhibit the transcriptional activity of either full-length AR or other AR variants (Bohrer et al., 2013; Mediwala et al., 2013). Nevertheless, AR is regulated by PI3K/Akt signaling in a more complex way, besides through FOXO1. There is a feedback loop between AR and Akt, in which Akt might phosphorylate AR, inhibiting its transactivation (Carver et al., 2011; Lin et al., 2001). On the other hand, Akt signaling is activated by AR inhibition through decreasing the expression of PHLPP (Carver et al., 2011; Mulholland et al., 2011). Since these two pathways, PI3K and AR, are the major mediators of PCa and inactivation of one of them leads to activation of the other one, combinational treatment approaches would be more effective. A preclinical study demonstrated that when AZD5363, a PI3K inhibitor, is combined with Enzalutamide, Enzalutamide resistance is delayed (Toren et al., 2015).

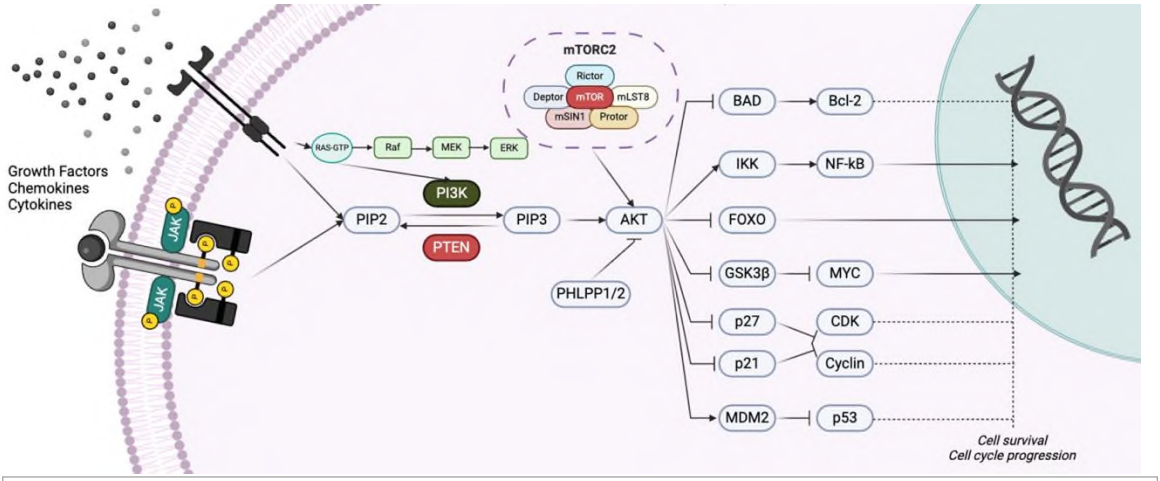


Figure 4. PI3K/Akt signaling pathway. (Created with BioRender.com)

1.3.3. BCL-2 Family Proteins

BCL-2 family proteins, which control mitochondrial (intrinsic) apoptosis, are categorized into three groups, depending on their functions: (1) anti-apoptotic proteins, including BCL-2, BCL-X_L, BCL-W, MCL-1, AND BFL-1/A1; (2) pro-apoptotic pore-forming proteins, including BAX, BAK, BOK; (3) pro-apoptotic BH3-only proteins, including BAD, BID, BIK, BIM, BMF, HRK, NOXA, PUMA. Each protein member of the BCL-2 family contains one of four BH domains, a BH3 domain, that is responsible for the interactions between these proteins. The multi-BH domain proteins are a group of anti-apoptotic and pore-forming proteins and contain all four BH domains that function as receptors for BH3 domains of other BCL-2 family members. The transcription factors STATs, NF-**κ**B, and ETS can induce the expression of BCL-2 family genes (Grad et al., 2000). BCL-2 proteins exert their functions of cell death control by regulating mitochondrial outer membrane permeabilization (MOMP), resulting in the release of intermembrane space proteins (e.g. SMAC, and cytochrome *c*), then caspases are activated and finally, the cell undergoes apoptosis (Figure 5) (Kale et al., 2017).

BCL-2 family proteins are of interest in cancer research, including PCa, due to their regulatory roles in apoptosis. The anti-apoptotic BCL-2 protein is a downstream member

of the PI3K/Akt signaling pathway. The pro-apoptotic BCL-2 associated agonist of cell death (BAD) protein, which suppresses BCL-2, is negatively regulated by Akt. Activation of PI3K/Akt suppresses BAD by phosphorylation, liberating anti-apoptotic BCL-2. RAS/ERK signaling is another signaling pathway that can control BCL-2 family proteins. RAS/ERK signaling can directly phosphorylate BAD and suppress pro-apoptotic BIM to inhibit mitochondrial apoptosis (Kinkade et al., 2008). BCL-2 was found to be frequently overexpressed in PCa and is considered as an adverse prognostic factor correlated with aggressive disease progression, high Gleason score, metastasis, and therapy resistance (Pilling et al., 2019; Soliman et al., 2021). BCL-2 is critical for the survival of androgen-independent PCa, as well as androgen-dependent PCa. Tamaki et al. revealed that a combination of BCL-2 family inhibitors with docetaxel, a therapeutic agent used in the treatment of metastatic PCa, demonstrated potent anti-tumor activity in a xenograft mouse model generated with PC3 cells which are known to exhibit lower sensitivity to docetaxel compared to other PCa cell lines (Tamaki et al., 2014). In a more recent study, it has been shown that, inhibition of Akt sensitized CRPC cells to Enzalutamide, another therapeutic widely used in the clinic to target AR signaling, including ARVs. This occurred through the activation of BAD that led to the downregulation of BCL-2 and increased apoptosis (Pilling et al., 2019). Further, the BCL-2 protein is elevated during the progression of androgen-independent PCa growth and is crucial for the transition to androgen-independent growth (Y. Lin et al., 2007; Soliman et al., 2021).

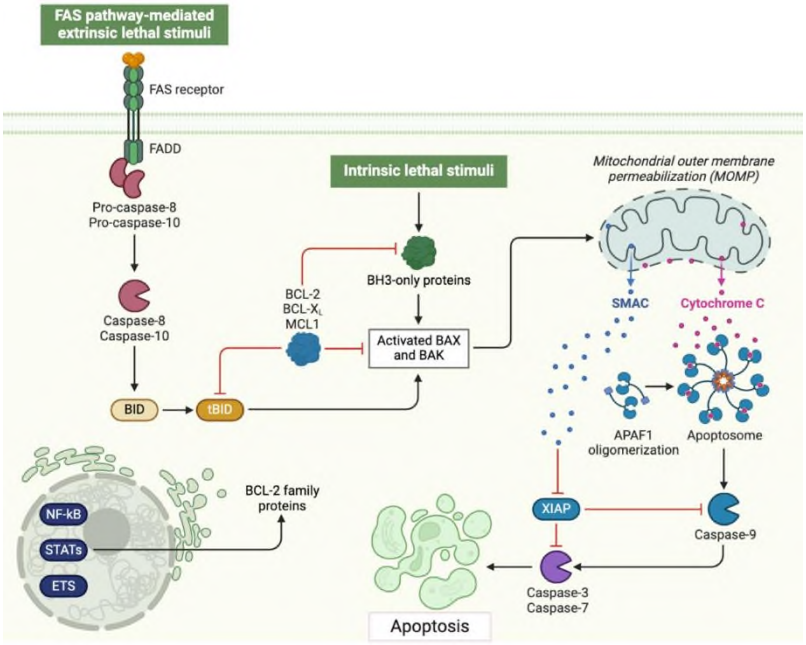


Figure 5. Apoptosis regulation by BCL-2 family proteins. (Created with BioRender.com)

1.3.4. MAPK Signaling Cascades: RAS/ERK

Mitogen-activated protein kinase (MAPK) signaling has been acknowledged for its roles in an extensive variety of cellular processes that are keys in promoting oncogenesis. MAPKs are protein kinases that use their own serine/threonine residues or those of their substrates to activate or inhibit their targets by phosphorylation (Johnson & Lapadat, 2002; Peti & Page, 2013). A MAPK cascade is activated upon a stimulus and MAP3K at the upstream phosphorylates a MAP2K, which in turn activates a downstream MAPK. There are three most notable MAPK pathways in mammalian cells; RAS/ERK, JNK1/2/3, and p38 (Figure 6). Growth factors activate RAS/ERK, inflammatory cytokines activate JNK1/2/3, and stress p38 signaling might be activated by stress stimuli through receptor tyrosine kinases (RTK) (Soares-Silva et al., 2016). Each three of the MAPK signaling cascades is well-known in the research of cancer as tumor drivers, however, the RAS/RAF/MAPK signaling cascade is the most important signaling among them owing to its crucial function in the growth and survival of tumor cells.

The extracellular signal-regulated kinases ERK1 and ERK2 isoforms are ubiquitous serine-threonine kinases, whose activation is initiated through the binding of growth factors to an RTK and occurs by sequential phosphorylation. This binding activates a small G-protein RAS, which then activates RAF (MAP3K), and further MEK1/2 (MAP2K) is phosphorylated by RAF for ERK1/2 (MAPK) activation. Once ERK1/2 is activated, it is translocated into the nucleus to regulate various transcription factors, for instance, oncogenes c-FOS, c-Jun, NF-**κ**B, and c-Myc; and Ets1 which have roles in the regulation of angiogenesis (Y. Guo et al., 2020). ERK might also phosphorylate a group of cytoplasmic proteins, including Raf-1 and MEK, resulting in a negative feedback regulation of RAS/ERK signaling. Deregulation of the RAS/ERK signaling cascade is frequently identified in many cancer types since it is directly involved in cell cycle regulation, cell differentiation, proliferation, apoptosis, and tissue formation (Rubinfeld & Seger, 2005). Various types of cancer, containing ovarian, colon, breast, and lung, have been shown to have increased ERK activation (Bhartiya & Singh, 2015; Rao & Herr, 2017; Q. Tang et al., 2017).

Regarding PCa, it has been demonstrated that elevated RAF expressions were correlated with advanced PCa, metastasis, increased Gleason score, poor prognosis, and androgen-independent growth (Carey et al., 2007; Keller et al., 2004; Mulholland et al., 2012; Nickols et al., 2019). In line with this, PCa patients who underwent ADT were demonstrated to express high levels of phosphorylated ERK for tumor cell survival (Karantanos et al., 2013). mCRPC patients have been shown to have increased phosphorylation of ERK1/2 and depending on this, there is an ongoing Phase II clinical trial (NCT02881242), in which mCRPC patients will be treated with MEK1/2 inhibitor Trametinib, which is an approved therapeutic for melanoma (Mulholland et al., 2012; Nickols et al., 2019; Taylor et al., 2010). LNCaP, PC3, and DU145 cell lines, which are the most widely used cells *in vitro*, express decreased inhibitors of RAF (McCubrey et al., 2007). Berriguete et al. suggested that PCa cell proliferation was triggered by ERK pathway activation through IL-6, which is a pro-inflammatory cytokine found to be upregulated in PCa tumors (Rodríguez-Berriguete et al., 2010). Further, there is also a regulatory correlation between AR and ERK, in which activated ERK enhances the transcriptional activity of AR by phosphorylation of AR itself and its coregulators, giving rise to PSA secretion (Grossmann et al., 2001; Liao et al., 2013; Peterziel et al., 1999). ERK was also demonstrated to induce the phosphorylation of BCL-2 family proteins, including BAD, BIM, and BCL-2, regulating apoptosis directly (Thakur et al., 2009). In addition, it was determined that apoptosis was inhibited by BAD phosphorylation and BIM suppression via activated ERK signaling (Chao & Clément, 2006; Kinkade et al., 2008; Sastry et al., 2006; Zoubeidi et al., 2010).

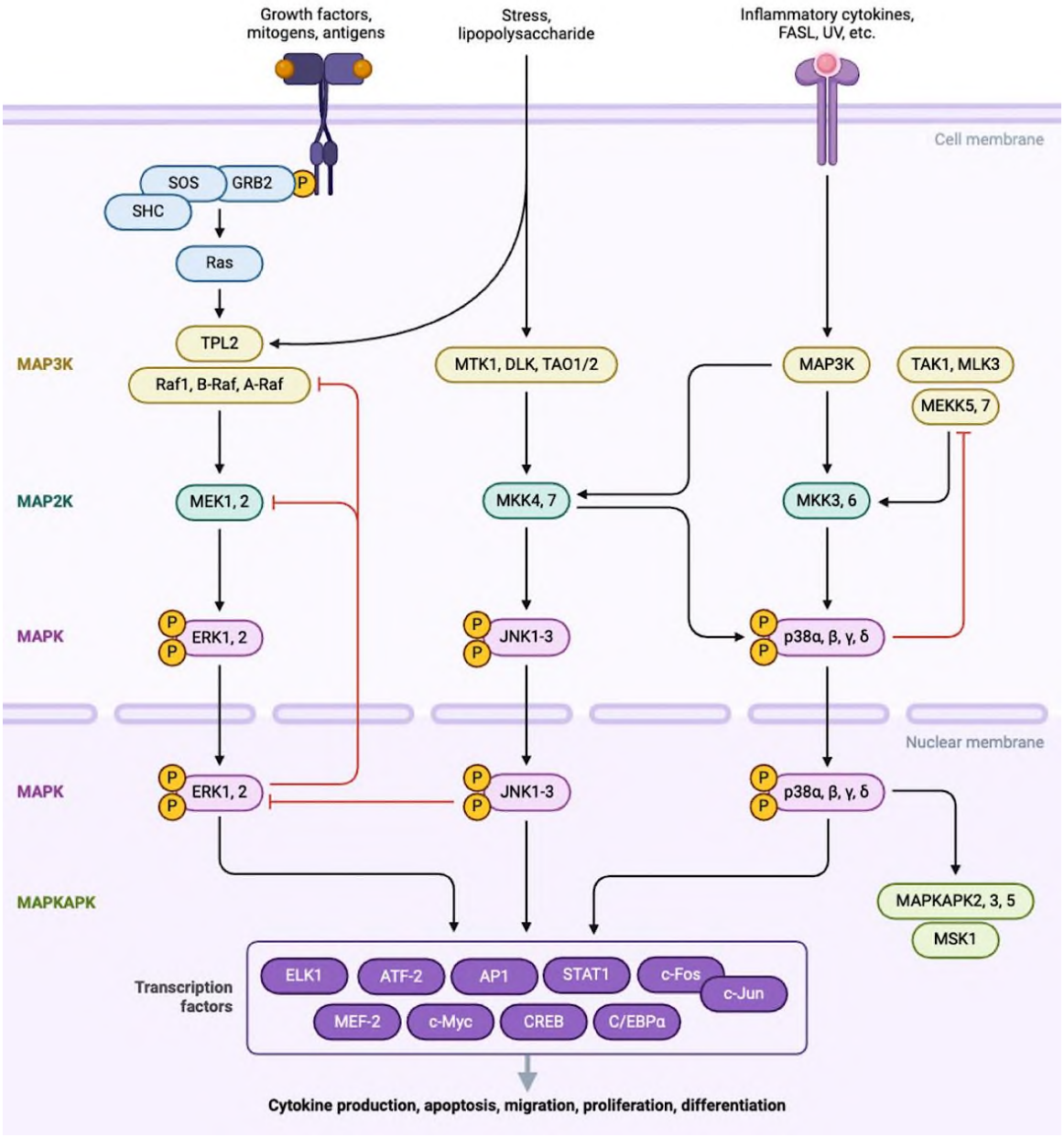


Figure 6. MAPK signaling pathways. (Created with BioRender.com)

1.3.5. MYC Family: c-Myc

The MYC family of proto-oncogenes includes three paralogs, N-Myc, L-Myc, and c-Myc and it is one of the most commonly altered and the most widely investigated gene family in various cancer types. c-Myc is the most abundantly expressed member, while others have moderate expression in cells (Madden et al., 2021). All three paralogs of the MYC family act as transcription factors and they are members of the basic helix-loop-helix leucine zipper (bHLHLZ) DNA-binding protein superfamily (Beaulieu et al., 2020; Carroll et al., 2018; Dang, 2016). Approximately 15% of the human genome is considered to be regulated by MYC family proteins (Dang et al., 2006; Duffy et al., 2021; Fernandez et al., 2003; Zeller et al., 2006). MYC proteins have traditionally been regarded as undruggable; however, it is a fact that they are the key drivers of cancer, with MYC gene expression found to be altered in up to 70% of cancers (Dang, 2012; Meyer & Penn, 2008). MYC proteins induce the expression of genes involved in angiogenesis, cell cycle, immune response, cell growth, DNA repair, differentiation, and apoptosis (Carroll et al., 2018; Dang, 2016; Duffy et al., 2021; González-Prieto et al., 2020).

PCa tumors demonstrate frequent overexpression of MYC oncogene and MYC expression levels, together with PTEN and Ki67 expressions were demonstrated to be more accurate predictors of progression-free survival compared to clinical evaluation (Antonarakis et al., 2012). MYC overexpression has also been shown to be a driver of PCa, in which MYC overexpression in prostate epithelial cells causes the development of PIN (Iwata et al., 2010). Furthermore, 89% of PCa patients display c-Myc amplification (Castro et al., 2015). It has been proposed that MAPK and PI3K/Akt signaling, which are determined to be highly correlated with PCa progression and poor prognosis, are upstream regulators of c-Myc (Figure 7), collaborating to enhance the expression of c-Myc, in advanced PCa (E. Li et al., 2017; J. Wang et al., 2012). The expression of EZH2 histone methyltransferase, which promotes castration resistance, was shown to be increased in c-Myc-overexpressed prostate tumors. This increase led to a decrease in the function of the tumor suppressor Interferon gamma receptor 1, which contributes to a crucial role in preventing cell survival (Wee et al., 2014). Moreover, c-Myc was

determined to antagonize AR transcriptional activity, deregulating AR-induced gene expression (Barfeld et al., 2017). Finally, Coleman et al. identified that BET protein inhibitors had potential anti-CRPC functions through c-Myc suppression; BET proteins are co-activators of AR-induced gene expression (Coleman et al., 2019).

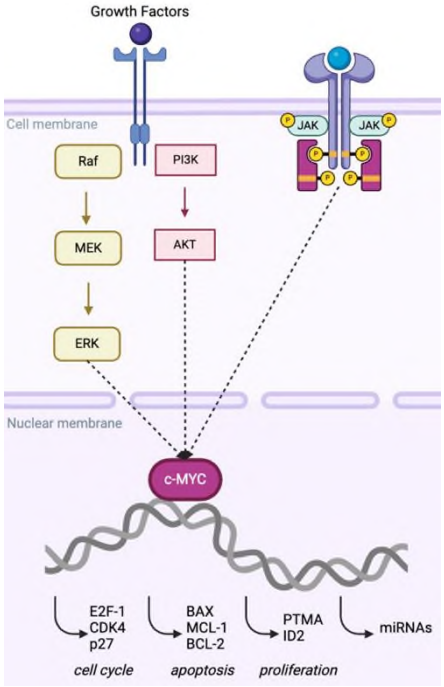


Figure 7. Regulation of c-myc. (Created with BioRender.com)

1.4. Treatment of Localized and Advanced Prostate Cancer

The treatment path for patients with PCa is determined by clinical evaluation of the stage and estimation of the recurrence risk through the prognostic factors, serum PSA levels, age, urinary function, biopsy, and Gleason's score (Figure 8). Active surveillance, which involves monitoring rather than active treatment, is appropriate for patients diagnosed with low-risk cancer (Choo et al., 2002; van den Bergh et al., 2009). Despite the favorable aspects of active surveillance, such as maintaining life quality and engaging in normal activities, there is a likelihood of missed opportunities for complete recovery, as well as the development of advanced cancer and metastasis. The first-line standard treatment options for stage I-III PCa are prostatectomy and radiotherapy. Prostatectomy involves surgically removing the prostate glands or some parts of it, seminal vesicles, and in some

cases lymph nodes as well (Mellman et al., 2011). Radical prostatectomy is a treatment approache for PCa patients with a life expectancy of more than 10 years, and many patients respond well to it, reducing the risk of recurrence for over five years (Sekhoacha et al., 2022). Another benefit of prostatectomy is that it provides a clearer understanding of the stage and recurrence risk of the disease, enabling early detection of relapse indicated by an increase in the serum PSA levels previously undetectable. Alternatively, radiotherapy, which is widely used to treat many types of cancers, is also an effective choice for PCa treatment. This treatment preference is made based on the patient's eligibility for prostatectomy; if the patient is not appropriate for surgical procedures, prostate cancer cells are targeted and destroyed through high doses of radiation (Baskar et al., 2012). In certain instances, radiation therapy may be combined with hormone therapy, to treat moderate-risk and high-risk prostate cancers (Sekhoacha et al., 2022).

The patients diagnosed with stage IV or high-risk stage III are treated with androgen ablation, also termed ADT, either through surgical or pharmacological castration. The therapeutic mechanism of androgen ablation, also known as hormone therapy, is dependent on the use of first-generation anti-androgens such as bicalutamide, flutamide, and nilutamide. The mechanism of action of these therapeutics is based on suppressing testosterone production, which sustains AR signaling for tumor growth, thereby decreasing hormone levels (Crawford et al., 2015). These non-steroidal anti-androgens compete with androgen for binding AR, inhibiting its activation. There are also therapeutics that suppress testosterone synthesis such as goserelin, leuprorelin, and triptorelin. These are Gonadotropin-releasing hormone (GnRH), also known as Luteinizing-hormone-releasing hormone (LNRH), agonists, acting to inhibit testosterone synthesis directly by blocking the hypophysis receptors (Heidenreich et al., 2008). As previously mentioned, ADT benefits patients at first, decreasing testosterone and PSA levels, however, after 2-3 years, the disease relapses in a more aggressive characteristic, termed CRPC (Harris et al., 2009).

The first-line treatment option for CRPC patients is chemotherapeutic Docetaxel, which is an antimicrotubule agent. Docetaxel specifically attaches to the β-subunit of tubulin to suppress mitotic cell division and induce apoptosis (Zhu et al., 2013). Cabazitaxel is another chemotherapeutic that also suppresses mitotic cell division by targeting the N-terminal domain of the β-tubulin subunit. Cabazitaxel is a second-generation treatment

option and it is widely used for CRPC patients who develop resistance to Docetaxel (Crawford et al., 2015). In stage IV, castration resistance, second-generation anti-androgens are used to block re-activated AR signaling through altered testosterone synthesis mechanisms. Abiraterone is one of the effective second-generation anti-androgen that is FDA-approved for use in treating CRPC. It irreversibly and selectively inhibits CYP17A, which is a key enzyme in both classical and backdoor testosterone synthesis pathways (Molina & Belldegrun, 2011). Enzalutamide is another second-generation AR inhibitor that suppresses several steps of AR signaling. Enzalutamide is known to inhibit the nuclear translocation of AR, its binding to DNA, and its cofactors. Primarily, it competitively inhibits the binding of androgens such as DHT and testosterone to AR (Cookson et al., 2013).

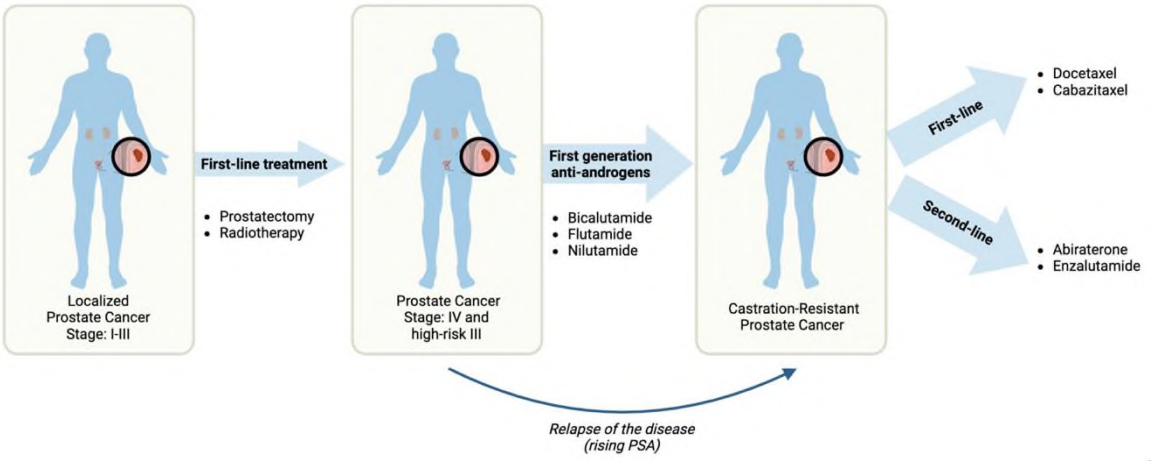


Figure 8. Treatment strategies for different stages of PCa. (Created with BioRender.com)

1.5. Pre-clinical Prostate Cancer Models

Pre-clinical cancer models, including *in vitro* and *in vivo* tumor representations, are widely used to investigate the molecular mechanisms of cancers, cell-to-cell interactions in the tumor microenvironment, and ultimately to search for novel and effective therapies. *In vitro* pre-clinical models are mainly based on the tumor cells that were originally isolated from cancer patients. Cell lines are powerful tools for molecular analyses since they are easy to manipulate both pharmacologically or genetically. However, they are not full representations of *in vivo* disease conditions. Therefore, genetically engineered

animal and human cell transplanted xenograft models are preferred in many experimental approaches.

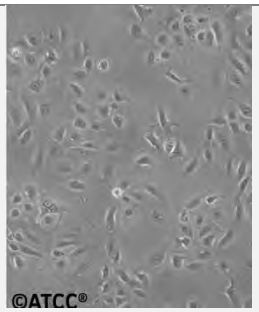
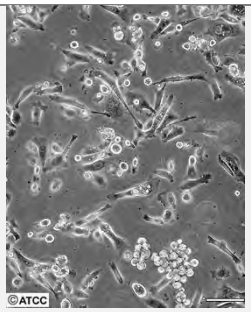
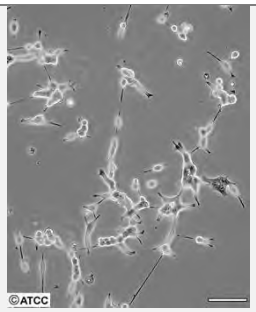
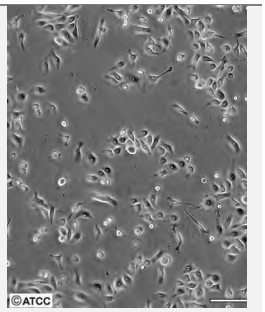
1.5.1. *In vitro* Prostate Cancer Models

There are three standard most frequently used PCa cell lines; DU145, PC3, and LNCaP (Table 2). DU145 cell line was established from the brain of a 69-year-old white male metastatic PCa patient in 1978 (Stone et al., 1978). The stage of the disease is suggested to be CRPC, since the patient had previously undergone orchiectomy, which is the removal of both testicles (Mickey et al., 1980). DU145 cell line shows an epithelial morphology and is not hormone-sensitive in that it does not express AR (Alimirah et al., 2006). The AR promoter is methylated in the DU145 cells (Sharma et al., 2016). The PC3 cell line is another cell line representing CRPC. It was established from a 62-year-old white male, who had previously undergone an orchiectomy and had bone metastasis. The PCa recurred after 4 months and was diagnosed to be grade IV prostatic adenocarcinoma (Kaighn et al., 1979). Likewise, PC3 cells also do not have an active AR in which AR mRNA can be detectable, but there are no detectable levels of AR protein (Edelstein et al., 1994). Consequently, both cell lines represent the most aggressive forms of PCa and are suitable to model CRPC *in vitro* since they are AR-negative and their growth is independent of androgens.

The LNCaP cell line, established from the left supraclavicular lymph node metastasis of a 50-year-old white male with metastatic PCa, is one of the most utilized PCa models (Horoszewicz et al., 1980). LNCaP cells are AR-positive and, therefore dependent on androgens for proliferation, however, similar to DU145 and PC3 cells, LNCaP cells were also isolated from a mCRPC patient. There are numerous subclones of the LNCaP cell line that are derived to mimic certain stages of the disease. For instance, LNCaP95 cells are derived from parental LNCaP cells by culturing them in the androgen-deprived medium for 6-42 months, inducing castration resistance (Pflug et al., 1999). These cells showed increased expression of AR-V7, which is the constitutively active form of AR regardless of ligand binding, than parental cells (Pflug et al., 1999; Zhu et al., 2020). The C4-2B cell line is another subclone of LNCaP. This cell line was developed from the bone metastasis of nude mice bearing tumors of LNCaP cells and the C4-2B cell line is

considered a valuable AR-positive mCRPC (Spans et al., 2014). Finally, the RWPE-1 benign cell line isolated from the peripheral prostate zone is also AR-positive and generally used as the healthy control cell line in PCa research.

Table 2. Most widely used cell lines in prostate cancer research.

Cell Line	*DU145*	*PC3*	*LNCaP*	*RWPE-1*
Morphology				
Origin	Brain metastasis	Bone Metastasis	Lymph node metastasis	Benign prostate
AR Status	Negative	Negative	Positive	Positive

1.5.2. *In vivo* Prostate Cancer Models

Tumor xenograft models, which involve the direct implantation of cancer cells into the model animal, are the most widely used *in vivo* models. Generally, there are three types of cell transplantation methods into immunodeficient mice; (1) cells can be transplanted subcutaneously, which provides easier observation of tumor growth; (2) cells can be transplanted into the actual pathophysiological site of the cancer, in the case of PCa, it is prostate; (3) cells can be transplanted under the kidney capsule, where there is enrichment in vascularity, providing the required nutrients (Figure 9) (Sailer et al., 2022). In the subcutaneous xenograft models, tumors become visible from the outside at the approximately second week of the cell transplantation, and this time depends on the tumorigenicity of the injected cell line (Sailer et al., 2022). Even though subcutaneous xenograft models provide easier monitoring of the tumor progression, the tumor

microenvironment, and the tissue architecture cannot reflect the exact human conditions, since subcutaneous site is not the original site for PCa tumor growth. In contrast to subcutaneous xenograft models, orthotopic models provide tumor generation at the actual pathophysiological site. These models are particularly useful in demonstrating tumor-stromal interactions as they mimic the prostate-specific microenvironment. This offers better insights into drug screening research. However, costly tumor growth monitoring approaches, such as ultrasonography and bioluminescent imaging techniques are necessary to track tumor progression (Anker et al., 2018; Lardizabal et al., 2018; Patel et al., 2000). Tumor xenograft models offer several advantages. For instance, cells can be genetically modified in the cell culture before transplantation, as cells from the culture are transplanted directly into the animals for tumor development (Aigner et al., 2002). Moreover, in addition to the drug-screening benefits of xenograft models, they are also useful tools to mimic the stages of the disease, thereby shedding light on the molecular mechanisms involved in the progression of the disease. Given that prostate cancer, castration resistance, or resistance to widely used chemotherapeutics can be studied in xenograft models by generating human tumors with desired genetic and morphological characteristics, researchers have a valuable tool for investigating these conditions.

Besides tumor xenograft PCa models, which involve the use of immunodeficient mice who are transplanted with human cancer cells, there are several genetically engineered mouse models (GEMM) of PCa (Figure 9). The transgenic adenocarcinoma of the mouse prostate (TRAMP) mice model, which was developed in 1996, is one of the important *in vivo* tools in PCa research (Gingrich et al., 1997). This model is introduced by the expression of both viral large and small SV40 large-T antigens, which causes the specific suppression of tumor suppressors in the prostate epithelium and these are pRb and p53 (Gingrich et al., 1997; Greenberg et al., 1994). PIN develops by approximately 18 weeks of age, and the invasive adenocarcinoma develops by 28 weeks of age. TRAMP mice model is particularly preferable for investigating the prevention and treatment of PCa as it provides a human-like pathology of PCa progression, starting from PIN lesions to malignant carcinoma (Gingrich et al., 1997; Greenberg et al., 1995; Maroulakou et al., 1994). The LADY mouse model is another widely used GEMM PCa model that was introduced in 1998 (Kasper et al., 1998). In the LADY mouse model, like TRAMP, SV40 T-antigen is utilized; however, upstream of it, there exists a larger fragment of the

probasin promoter containing additional androgen sequences (Kasper et al., 1998; Yan et al., 1997). In this model, the primary growth of the PCa tumor is androgen-dependent, and the molecular changes in the subsequent progression stages closely resemble those observed in human PCa (Kasper et al., 1998). In addition, metastases to distant organs, for instance, the lymph nodes, bones, and, liver are observed in the LADY model (Klezovitch et al., 2004). However, these metastases are primarily composed of neuroendocrine cells, a feature not typically observed in human PCa (Berman-Booty & Knudsen, 2015; Masumori et al., 2004). Finally, the third most widely-used GEMM PCa model is PTEN-deficient mice. As mentioned in section 1.3.2., loss of tumor suppressor PTEN is crucial in PCa initiation and progression. Hence, due to lethality, PTEN-null mice were developed through the deletion of PTEN in the prostatic epithelium rather than in the mice embryonic stem cells (Di Cristofano et al., 1998; Suzuki et al., 1998). PIN development is observed by 6 weeks of age in the PTEN null mice and by 10 months of age in the heterozygous PTEN deletion mice. Furthermore, in the homozygous PTEN knockout mice, invasive adenocarcinoma progresses by 9 weeks, and within the subsequent 3 weeks lymph node and lung metastases are observed (Saranyutanon et al., 2020). The PTEN knockout model is mostly used to investigate the PCa tumor microenvironment, especially the roles of the pro-inflammatory cytokine interleukin-17 (IL-17), which has been suggested to influence a favorable microenvironment for the growth of PCa, has been demonstrated (Q. Li et al., 2014; Onishi & Gaffen, 2010; Q. Zhang et al., 2014).

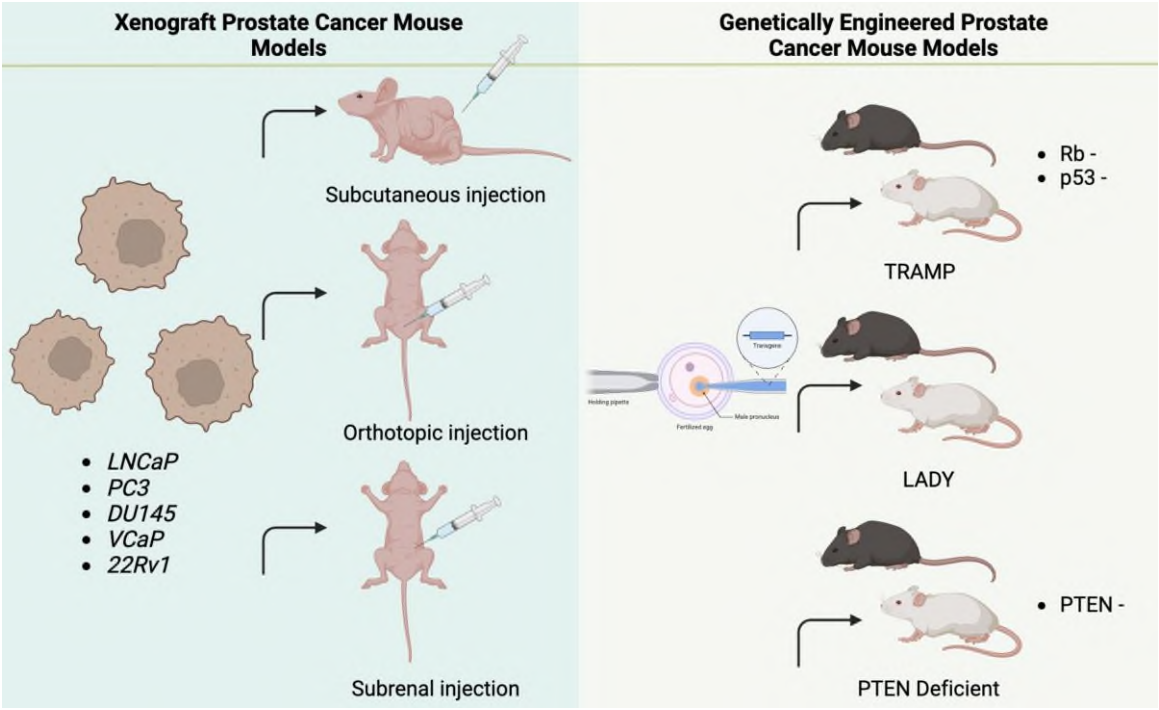


Figure 9. Most widely used *in vivo* prostate cancer models. (Created with BioRender.com)

1.6. CCL5/CCR5/STAT3/BCL-2 Signaling Axis

Chemokines, which are composed of four subfamilies, including the CC, CXC, CX3C, and XC, are chemotactic cytokines that mediate the migration of immune cells through interacting with their unique seven-transmembrane G protein-coupled receptors (GPCRs) (Chow & Luster, 2014). The classification of chemokines is dependent on the amino acid number within the first two cysteine residues (Nomiyama et al., 2013). Chemokines are small proteins that are 8-10 kDa and they are crucial regulators of cell positioning, angiogenesis, homeostasis, inflammation, immune response, chemotaxis, and migration (Hughes & Nibbs, 2018; Kranjc et al., 2019; López-Cotarelo et al., 2017). C-C chemokine ligand 5 (CCL5), also stated as Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTES), is a member of the C-C motif chemokine family and binds its receptor C-C chemokine receptor 5 (CCR5) with high-affinity (Aldinucci & Colombatti, 2014; Soria & Ben-Baruch, 2008). CCL5 might also bind to other chemokine receptors including CCR1, CCR3, and CCR4 (Appay & Rowland-Jones, 2001; Udi et al., 2013). Likewise, CCR5 is not only activated upon CCL5 binding, CCL3, CCL4, and

CCL8 might also bind with high-affinity (Zeng et al., 2022). Various cell types express CCR5 for instance, macrophages, T-cells, and dendritic cells. This chemokine receptor plays a crucial role in the inflammatory response (Oppermann, 2004; Vangelista & Vento, 2018). CCR5 is especially important in the entry of Human Immunodeficiency Virus (HIV) into the cell (Gavegnano et al., 2019).

CCR5 activation by CCL5 induces several downstream pathways such as MAPK, PI3K/Akt, JAK/STAT3, and NF-**κ**B (Figure 10) (Aldinucci et al., 2020; Hermida et al., 2017; C. Y. Huang et al., 2009; Jiao et al., 2018). In addition, CCL5, the ligand of CCR5, is the target gene of both STAT3 and NF-**κ**B, creating a feedback loop (Aldinucci et al., 2012; Appay & Rowland-Jones, 2001; Villarino et al., 2017). When CCL5 binds to the CCR5 receptor, PI3K becomes phosphorylated and subsequently phosphorylates Akt at the serine 473 position (Hermida et al., 2017). This activation of PI3K/Akt signaling regulates downstream pathways. For instance, BCL-2 becomes activated by suppression of its suppressor BAD, leading to inhibition of cell apoptosis (Deng et al., 2019). Moreover, activated Akt phosphorylates GSK-3, suppressing its function, and subsequently expression level of Cyclin D, a cell cycle regulator, is regulated (Hermida et al., 2017; J. Lin et al., 2020). CCL5/CCR5-PI3K/Akt signaling also positively regulates the mTOR pathway through the inhibition of Rheb which downregulates mTOR signaling. On the other hand, Akt can be directly activated by mTOR itself, promoting the activation of the downstream pathways (Mossmann et al., 2018). The MAPK pathway can be regulated through the CCL5/CCR5 signaling through the initiation of MEK phosphorylation (Mendoza et al., 2011; Samatar & Poulikakos, 2014). NF-**κ**B is also activated by CCL5/CCR5 by the direct phosphorylation of the p65 subunit at serine 536 (Dolcet et al., 2005). Additionally, upon CCL5 binding to CCR5, JAKs at adjacent receptors become trans-phosphorylated by moving toward each other, and STAT3 gets activated to induce gene expression downstream (Owen et al., 2019; Villarino et al., 2017).

In light of the molecular mechanism of the CCL5/CCR5 axis with its downstream partners, it is concluded that this signaling plays critical roles in the various cellular processes such as proliferation, migration, angiogenesis, metastasis, and survival (Abid et al., 2019; Jiao et al., 2019; Suarez-Carmona et al., 2019; S. W. Wang et al., 2012).

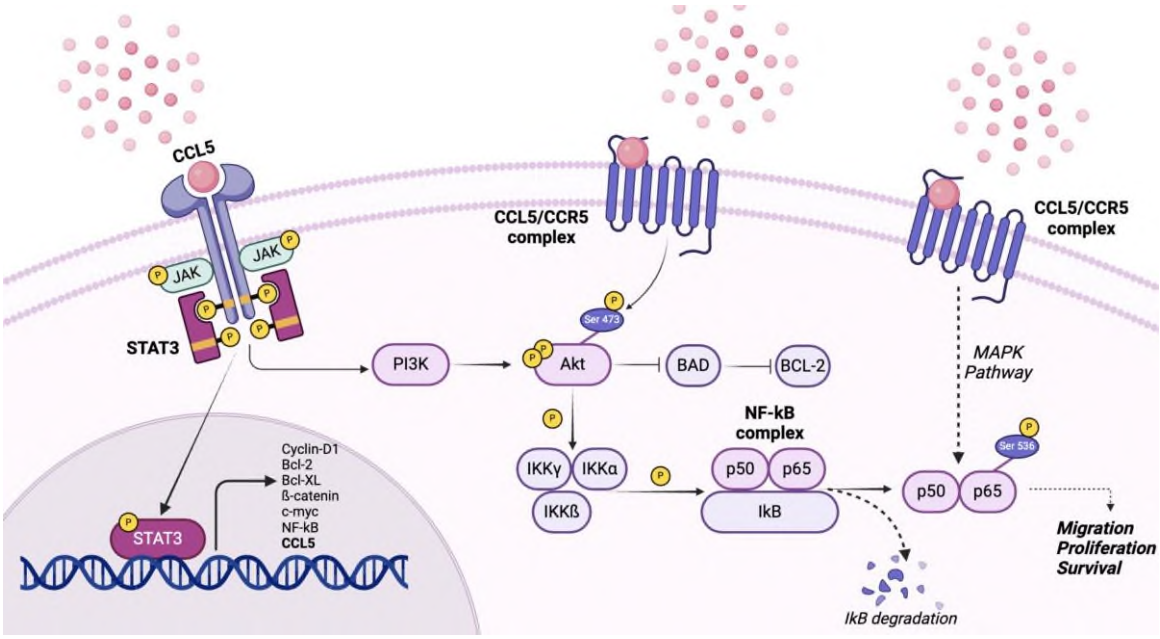


Figure 10. CCL5/CCR5/STAT3/BCL-2 signaling pathway. (Created with BioRender.com)

1.6.1. CCL5/CCR5/STAT3/BCL-2 Signaling Axis in Cancer

Chemokines are crucial regulators of cell trafficking and positioning, thus, they are of interest in cancer research. Neoplastic tissues are enriched in many types of chemokines. Besides the immune cell regulatory functions of chemokines, they can also directly promote the proliferation and survival of cancer cells and prevent apoptosis through inducing many downstream signaling pathways that are crucial for cell survival, proliferation, and metastasis. In the tumor microenvironment (TME), chemokines can be released from immune cells as well as cancer cells, contributing to tumor growth by the recruitment of different cell types to mediate anti-tumor and pro-tumor responses (Chow & Luster, 2014). Chemokine receptors are also overexpressed by cancer cells, thus creating a feedback loop, and activating downstream pathways in the cell for instance MAPK/ERK, PI3K/Akt, and JAK/STAT3. The cell division is promoted by the activation of these pathways while further preventing apoptosis through increasing the levels of anti-apoptotic molecules such as BCL-2 and BCL-XL while decreasing the level of pro-apoptotic BAD (Wani et al., 2014; Zhou et al., 2016).

CCL5 and CCR5, which are important inflammatory mediators, were demonstrated to be overexpressed in several cancerous tissue types, containing hematological malignancies, breast cancer, colorectal carcinoma, gastric adenocarcinoma, melanoma, pancreatic cancer, and prostate cancer. CCL5/CCR5 signaling has been clarified to promote tumor progression by promoting migration and growth of cancer cells, preventing apoptosis, supporting drug resistance, inducing angiogenesis, and recruiting immune and stromal cells (Aldinucci et al., 2020). CCR5 activation in response to CCL5 binding, induces proliferation of cancer cells in both autocrine and paracrine ways. CCR5 ligands secreted by T lymphocytes, fibroblasts, CAFs, and macrophages, lead to paracrine growth of cancer cells, whereas cancer cells that have functional CCR5 on them, secretes ligands for CCR5 and thereby induce proliferation in an autocrine manner (Casagrande et al., 2019; Vaday et al., 2006). CCR5 induces cell growth through stimulating the mTOR cascade, which further upregulates cyclin D1, which is a crucial regulator of the cell cycle (Murooka et al., 2009). Moreover, the JAK/STAT3 pathway is activated by the CCL5/CCR5 axis, resulting in cell division and anti-apoptotic signals (H. Ding et al., 2016). In addition to the tumor growth-inducing function of the axis, it also leads to the migration of cells through PI3K/Akt pathway followed by NF-**κ**B upregulation (S. W. Wang et al., 2012).

It was identified that CCL5/CCR5 promotes invasion through activating ERK and Rac transduction and elevating the release of MMP-2 and MMP-9 in PCa (Kato et al., 2013). Likewise, breast and colorectal cancer cells showed increased invasive phenotype with increased expression of CCR5 (Velasco-Velázquez et al., 2012; Cambien et al., 2011). It was also confirmed in lung cancer cells that, upon CCL5 binding, CCR5, PI3K/Akt/NF-**κ**B signaling was activated, increasing the migration ability of cells (C. Y. Huang et al., 2009). CCL5 and its association with angiogenesis was described in ovarian cancer research; angiogenesis was increased through upregulation of NF-**κ**B and STAT3, inducing ovarian carcinoma stem-like cells to differentiate into endothelial cells (S. Tang et al., 2016). In another study, it was determined that CCL5 participates in drug resistance of ovarian carcinoma cells through activating STAT3 and PI3K/Akt signaling pathways (B. Zhou et al., 2016).

1.6.2. CCL5/CCR5/STAT3/BCL-2 Signaling Axis in Prostate Cancer

Besides other cancer types mentioned above, the CCL5/CCR5 axis also plays a crucial role in PCa progression. CCL5 levels were determined to be overexpressed in the serum of PCa patients and it has been suggested that CCL5 expression is promoted by prostate-specific membrane antigen (PSMA) which has been defined as a diagnostic biomarker, through activating NF-κB that is known to increase CCL5 and IL-6 expression (R. Huang et al., 2020; Colombatti et al., 2009). Vaday et al. showed that expression of CCL5 mRNA and protein are present in three standard PCa cell lines (PC3, LNCaP, and DU145), while CCR5 was found to be on the cell surface, as well as in the intracellular pools of PCa cells. Furthermore, they demonstrated that when PCa cells are treated with CCL5, the cell proliferation was increased; and TAK-779, a CCR5 antagonist, was able to suppress CCL5-induced proliferation (Vaday et al., 2006). Consistent with these *in vitro* results of Vaday et al, another research underlined that mRNA expression of CCL5 and CCR5 was found in human PCa samples (König et al., 2004). Elevated levels of CCL5 in blood or tumor samples were found to be associated with poor prognosis of PCa (R. Huang et al., 2020). Additionally, PCa cell metastasis was found to be closely related to CCL5 secreted from cancer-associated fibroblasts (CAFs), and then macrophages are recruited that in turn promote metastasis (Yeh et al., 2016). Similarly, CCL5 was shown to contribute to PCa metastasis by inducing the β-catenin/STAT3 pathway. Further, enhanced expression of CCL5 was found to be significantly correlated with a high Gleason score, metastatic activity, and poor prognosis in PCa patients (R. Huang et al., 2020). Through the bioinformatics approach, it was described that CCR5 expression was 11.3 times greater in tumor tissues of the mouse PCa model compared to normal-matched tissue. The same study also confirmed that CCR5 was overexpressed (4 to 5 times) in PCa patients compared to normal controls (Sicoli et al., 2014). Lastly, the CCL5/CCR axis was shown to participate in drug resistance in PCa. Xiang et al. demonstrated that CCL5, through activating STAT3 signaling, promoted docetaxel resistance in PCa (Xiang et al., 2019).

CCR5 is a well-known co-receptor for the human immunodeficiency virus (HIV) type-1 and there are CCR5 antagonists used to treat AIDS like Maraviroc in the clinic (Oppermann, 2004; Gavegnano et al., 2019). Maraviroc was studied on various types of

cancers to suppress the CCL5/CCR5 signaling. In a study, when breast cancer mouse models were treated with maraviroc, survival was improved, and metastasis and angiogenesis were reduced (Sax et al., 2016). Maraviroc was able to reduce metastasis and improve the overall survival of mice with PCa (Zhao et al., 2018). In addition, PCa xenograft models were treated with Anibamine, which is a CCR5 inhibitor and resulted in reduced tumor growth (F. Zhang et al., 2012). Therefore, it is suggested that the CCL5/CCR5 axis might be considered an important target to develop an effective anti-tumor approach not only for PCa but for many other types of cancer. Therefore, CCL5/CCR5 signaling can activate many important cascades such as PI3K/Akt, MAPK, and JAK/STAT3, all of which were found to be upregulated in PCa, contributing to the progression of the disease (Figure 11).

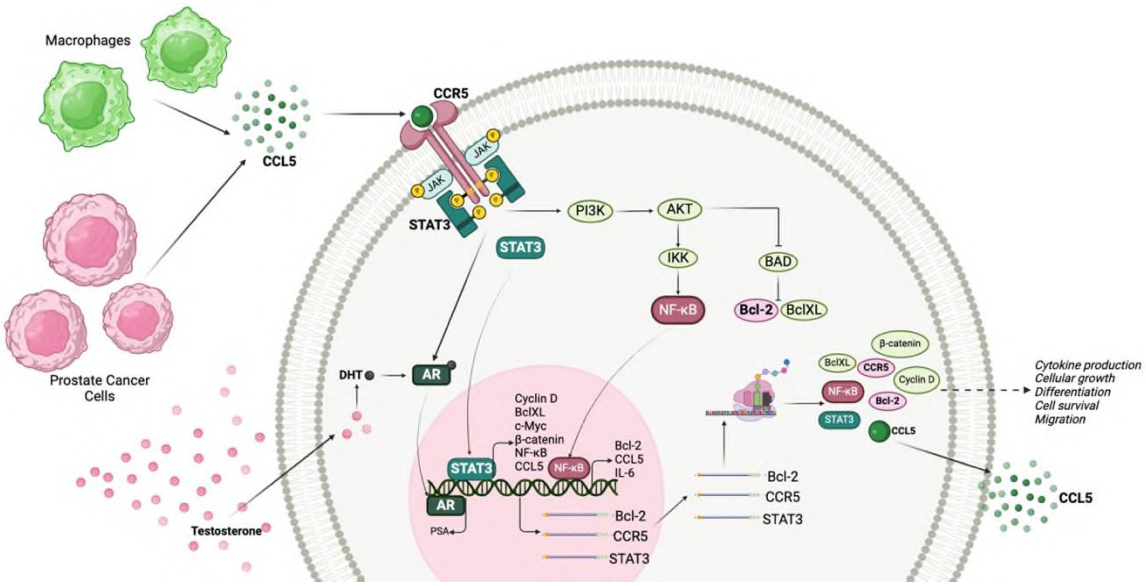


Figure 11. CCL5/CCR5/STAT3/BCL-2 signaling in prostate cancer. (Created with BioRender.com)

1.7. miRNAs and Their Biogenesis

A 22-nucleotide non-coding RNA, LIN-4, was identified in *Caenorhabditis elegans (C. elegans)* by Victor Ambros and Gary Ruvkun in 1993. LIN-4 was shown to be involved in the development of *C. elegans* by suppressing LIN14 protein at the post-transcriptional level (Ambros et al., 2003; Bartel, 2004; R. C. Lee et al., 1993; Wightman et al., 1993). In 2005, these small, 19-25 nucleotides long mRNA-regulators were identified as microRNAs (miRNAs), and their involvement in cancer progression was suggested (S. Lin & Gregory, 2015; J. Lu et al., 2005). miRNAs are evolutionary conserved, small non-coding RNA molecules, consisting of 18-22 nucleotides. They regulate gene expression by directly targeting and silencing the genes post-transcriptionally, binding specifically to the mostly 3' untranslated region (UTR) and rarely to the 5' UTR and coding sequences of their target mRNAs (Pillai et al., 2005). It is assessed that approximately 30% of human mRNAs are regulated by miRNAs. Recently, it has been found that miRNAs do not only bind to 3' UTR regions of mRNAs but might also bind to their 5' UTR regions and coding sequences. However, this is a rare event that works less effectively than binding to the 3'UTR regions (Lytle et al., 2007). The sequence of the miRNA that is complementary to the binding region of the mRNA is called the 'seed region' and mostly starts from the second nucleotide in the 5' end of the miRNA. The seed sequence, composed of approximately 7 nucleotides, is conserved, and the base pairing between the seed region of a miRNA and its target mRNA is crucial for perfect complementation, although the rest of the base pairing does not have to be perfect for translational repression and mRNA decay (Bartel, 2009).

The nomenclature for miRNAs is inconsistent, and classification rules have not yet been clearly identified. Nevertheless, generally, it is considered that mature miRNAs with identical sequences at nucleotides 2-8 are members of the same miRNA family (*e.g.* miR-27 family). Each miRNA, encoded by miRNA sisters belonging to the same family is distinguished with lettered suffixes (*e.g.* miR-27a and miR-27b). When two separate loci generate mature miRNAs with identical sequences, the name of the miRNA is supplemented with numeric suffixes at the end (*e.g.* miR-27b-1 and miR-27b-2). Two mature miRNAs are generated by the pre-miRNA; one from the 3' strand (called miR-

27a-3p) and one from the 5' strand (called miR-27a-5p). Generally, one of those arms is more functional and called the guide strand; whereas the other arm is degraded and is then called the passenger strand, which is also expressed with an asterisk at the end of the miRNA name (*e.g.* miR-27a*) (Ha & Kim, 2014). Usually, the -3p strand is more stable and since then called the guide strand (N. Fernandez et al., 2017; Treiber et al., 2017).

The miRNAs might be located in the introns of protein-coding or non-coding transcripts or the exonic regions of the genes. The miRNAs can be found in clusters that are either co-transcribed or individually transcribed (Ha & Kim, 2014). miRNA biogenesis and regulation of biogenesis are shown in Figure 12. The majority of human miRNAs are transcribed by RNA Polymerase II in the nucleus, generating a long RNA called primary-miRNA (pri-miRNA). A pri-miRNA is composed of single-stranded RNA regions at both 3' and 5' ends, a terminal loop, and a stem including nearly 35 base pairs. Then, pri-miRNA is processed in the nucleus with two processors; the nuclear RNase III Drosha and its cofactor DGCR8 form a complex called microprocessor to crop the stem loop (Ha & Kim, 2014). The microprocessor complex is positively regulated by GSK3β, TDP43, and ERK. GSK3β phosphorylates Drosha for its nuclear localization (X. Tang et al., 2010, 2011). TDP43 has been demonstrated to increase the stability of Drosha, while ERK has been shown to increase the stability of DGCR8, the cofactor of Drosha, (Di Carlo et al., 2013; Herbert et al., 2013). This nuclear processing creates a stem-loop structure of approximately 60 nucleotides in length. The hairpin-shaped RNA is termed pre-miRNA and it is transported to the cytoplasm by the protein exportin 5 (XPO5, also termed EXP5) to be processed further. A structure at the bottom of XPO5 strongly interacts with the 2 nucleotides located at the 3' overhang of the pre-miRNA, exporting it to the cytoplasm (Okada et al., 2009). In the cytoplasm, pre-mRNA is cleaved by the processor enzyme, Dicer, an RNase III type of endonuclease. Dicer, along with its cofactor TRBP, recognizes the 3' overhang of pre-mRNA as well as the cleavage site. The PAZ and RNase III domains, which are known to function as a molecular ruler, recognize this cleavage site that is typically positioned 21-25 nucleotides away from the 3' end. The pre-miRNA is cleaved near the terminal loop, the pre-miRNA hairpin, generating a small double-stranded RNA (dsRNA). The activity of Dicer can be directly regulated by its cofactor TRBP which can be phosphorylated by the MAPK ERK (Paroo et al., 2009). Subsequently, dsRNA generated after pre-miRNA processing, is loaded onto the RNA-

induced silencing complex (RISC), which involves AGO proteins, for subsequent unwinding and mRNA degradation (Hammond et al., 2001; Kawamata & Tomari, 2010; Tabara et al., 1999). There are primarily two rules for guide strand selection; (1) the strand that is thermodynamically more unstable at the 5' side, or (2) the strand that has a uracil nucleotide at the first position is selected as the guide strand, while the other strand, termed as the passenger strand, is degraded (Czech et al., 2009; Ghildiyal et al., 2010; H. Y. Hu et al., 2009; Lau et al., 2001; Okamura et al., 2009). In some cases, both the guide strand and the less abundant passenger strand of the mature miRNA are processed and actively take place in mRNA silencing (Chiang et al., 2010). For instance, the 5' strand of the miR-142 (termed miR-142-5p) was found to be the most abundant strand in the brain, testes, and ovaries, while the 3' strand is the dominant isoform in embryonic tissues (H. Wu et al., 2009).

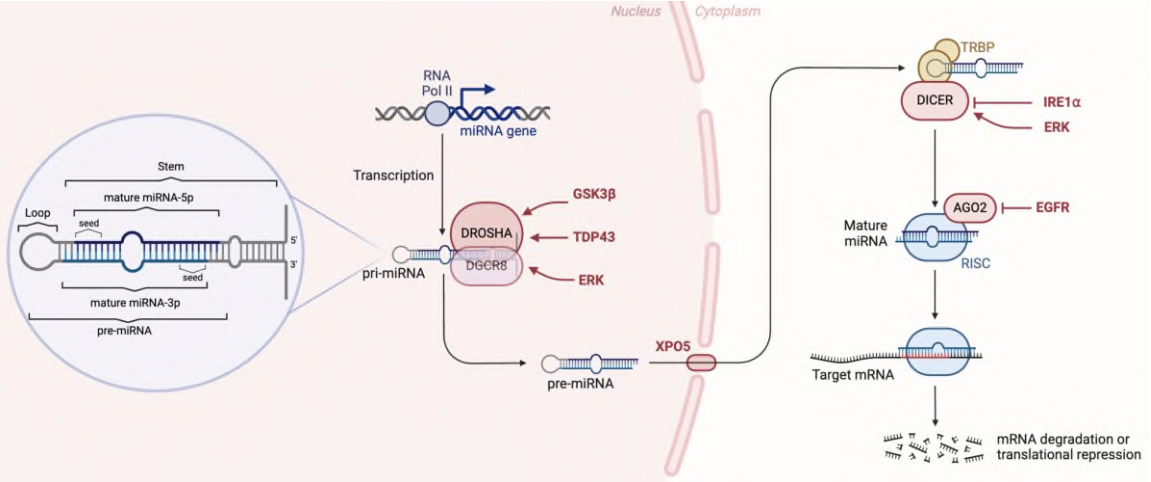


Figure 12. miRNA biogenesis & its regulation. (Created with BioRender.com)

1.7.1. miRNAs in Cancer

miRNAs are widely studied in the context of several diseases due to their regulatory roles in cellular processes. Recently, many miRNAs have been shown to participate in cell division, proliferation, migration, angiogenesis, and apoptosis. This makes them excellent diagnostic and prognostic markers, as well as therapeutic molecules in various cancer types. However, their functions in tumor cells remain to be partially explained. For an extended period, the expression of miRNAs was thought to be reduced in cancer cells due to the determination that genes encoding miRNAs are often placed in cancer-associated

genomic regions, potentially leading to their deletion (Rishabh et al., 2021a). However, it has been found that some miRNAs show significant overexpression in the tumor tissues, compared to normal tissues (C.-Z. Chen, 2005). Therefore, miRNAs in cancer are mainly classified based on their roles in tumorigenesis. There are tumor-suppressor miRNAs that target and downregulate the expression of oncogenes and oncogenic miRNAs also termed oncomiRs, that target and suppress the expression of genes involved in tumor suppression.

The lack of expression of certain miRNAs gives an increase to the expression of tumor-promoting genes. For instance, miR-16 and miR-15 were found to be acting as tumor suppressors and apoptosis regulators in chronic lymphocytic leukemia cells by targeting anti-apoptotic BCL-2 mRNA (Y. Chen et al., 2014). miRNAs can also regulate the DNA methylation process. For example, miR-143 has been shown to be reduced in colorectal cancer cells. This reduction in the miR-143 level enhances the activity of methyltransferase 3A DNA (DNMT3A), which correlates with an increased proliferative behavior of the cancer cells (Ng et al., 2009). In addition, in another study, the oncogene Raf1 was determined as the target gene of miR-143 in colorectal cancer (Corté et al., 2012). NF-**κ**B, a well-studied transcription factor, acting as an oncogene, was shown to be targeted by a tumor suppressor miRNA, miR146a, which has been found to be decreased in colorectal cancer (Y. Chen et al., 2014). On the other hand, selected miRNAs are over-expressed in the cancerous tissues, leading to downregulation of tumor suppressor genes. These kinds of miRNAs are called oncomiRs, such as the miR-10 family. Both miR-10a and miR-10b were found to be overexpressed in breast cancer, taking place in the development of the disease (Singh & Mo, 2013). In particular, miR-10b was found to be correlated with the increased expression of the HER-2 receptor. A cluster of oncomiRs, composed of six miRNAs; miR-17, miR-18, miR-19a, miR-20, and miR-92, has been demonstrated to inhibit Rbl2, a tumor suppressor gene (Rishabh et al., 2021b).

Many miRNAs have been identified to be involved in the molecular pathogenesis of PCa in the literature. For example, miR-21, which is one of the most common upregulated miRNAs in several cancer types, was found to be a predictive molecule for PCa recurrence after radical prostatectomy (T. Li et al., 2012). In addition, its increased levels

have been shown to be involved in castration resistance and worse disease outcomes with metastasis and high Gleason score (Cannistraci et al., 2014). miR-141 is another oncogenic miRNA in PCa and it was considered as a promising diagnostic and prognostic biomarker (Mitchell et al., 2008). miR-141, together with miR-375, is under investigation for the correlation between their expression patterns and radiation resistance in PCa (NCT02391051). It has been determined that oncogenic PI3K/Akt signaling can be positively regulated by the oncogenic miR-32, which was found to be overexpressed in CRPC tissues (Jalava et al., 2012). There are also tumor suppressor miRNAs identified in PCa, such as miR-224 and miR-452. The introduction of these two miRNAs to the PCa cell lines was shown to suppress the proliferation, migration, and invasion of DU145 and PC3 cells (Karatas et al., 2014). miR-30a, another tumor suppressor miRNA, whose role in tumor suppression was determined in CRPC, has been shown to target cell cycle protein cyclin E2 (L. Zhang et al., 2016).

Every single differentially expressed miRNA has the potential of being either a diagnostic, prognostic, or therapeutic molecule. In colorectal cancer, increased expression of miR-20a was found to be contributing to the resistance to chemotherapeutics like fluorouracil, oxaliplatin, and teniposide, by targeting BNIP2 (Chai et al., 2011). This study underlines that miRNAs are also involved in the drug-resistance mechanisms in cancer cells. Moreover, miRNA profiling studies introduced a novel era for cancer diagnosis. For example, the ThyraMIR test provides the discrimination of the types of thyroid cancer by evaluating the expression levels of 10 different miRNAs (Zedan et al., 2018). More importantly, miRNAs have a great therapy potential for cancer. It has been demonstrated that increased miR-29b levels in breast cancer, suppressed tumor growth through negatively regulating oncogenic signaling pathways involving KDM2A and Akt3 both in vitro and in vivo (Y. Li et al., 2017; Stahlhut & Slack, 2015). Since these small RNA molecules have demonstrated impressive success as therapeutics *in vitro*, several miRNA-based drugs have been evaluated in clinical trials. The first miRNA-based drug introduced to humans is MRX34, which is a liposomal nanoparticle containing synthetic double-stranded miR-34a (Ling et al., 2013a). miR-34a belongs to the well-studied miR-34 family, which has been determined as tumor suppressor miRNAs by regulating p53 (He et al., 2007; Raver-Shapira et al., 2007). In the phase-I clinical trial of MRX34 (NCT01829971), patients with various types of cancers, such as melanoma, multiple

myeloma, small-cell lung cancer, non-small-cell lung cancer, lymphoma, primary liver cancer, and renal cell carcinoma, were treated with the MRX34 (Ling et al., 2013b). However, upon death of 4 patients due to serious adverse effects, the clinical trial was terminated, even though the MRX34 trial had promising results and guiding consequences. It was determined that MRX34 was able to suppress the expression of miR-34 target genes and subsequently oncogenesis (Hong et al., 2020; Peltier et al., 2016). In addition, it was thought that the adverse effects were mainly because of the delivery system; therefore, by embracing a tumor-targeted delivery system, off-target toxicity of the drug might be eliminated. Another miRNA-based drug TargomiR, which is an encapsulated double-stranded miR-16, had better outcomes in the clinical trials (NCT02369198) (van Zandwijk et al., 2017). As mentioned before, miR-16 is a tumor suppressor miRNA, targeting anti-apoptotic BCL-2 (M., 2018). The TargomiR led to minor adverse effects, it was well tolerated overall and it showed promising tumor suppression (van Zandwijk et al., 2017).

1.7.2. miR-27 Family

The miR-27 family is one of the important members of miRNAs in cancer and it is composed of miR-27a and miR-27b. Their transcription is performed from the genes on different chromosomes. The miR-27a gene is located in an intergenic cluster on chromosome 19p13.13. miR-27a and miR-27b differ in their nucleotide sequences at the 3’ end, resulting in distinct target profiles (X. Li et al., 2019). The cluster of miR-27a contains miR-23a and miR-24-2; and these three different miRNAs in the cluster are synthesized from a single ~2159 nucleotide long pri-miRNA (Figure 13) (Chhabra et al., 2010). The expression of the miR-23a27a24-2 cluster was shown to be deregulated in various types of cancer (Liang et al., 2014). Each member of the cluster has diverse effects in cells, suggesting disease-dependent functions. Therefore, they should be investigated individually, as miR-23a27a24-2 cluster members have been shown to be differentially expressed. Through both transcriptional and post-transcriptional mechanisms, the expression of each member is independent of the expression of other cluster members (Buck et al., 2010). The mature miR-27a is 22 nucleotides long, and it has been shown

that miR-27a has crucial functions in oncogenesis, proliferation, apoptosis, chemotherapy resistance, and cell cycle regulation (J. Zhang et al., 2019). However, its molecular mechanism involving its target genes in various types of cancers has not yet been fully elucidated.

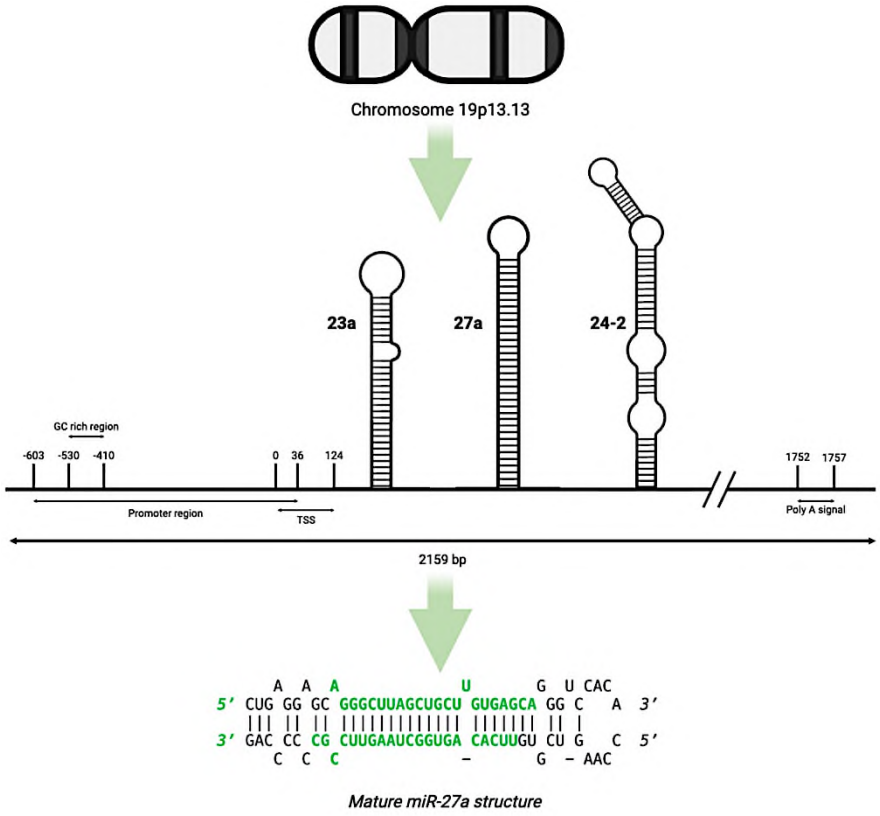


Figure 13. Transcription of miR-27a. (Created with BioRender.com)

1.7.3. miR-27a in Cancer

miR-27a has been found to have vital roles in many cellular processes of tumors, including proliferation, angiogenesis, apoptosis, invasion, and migration. In recent studies, miR-27a is considered as a novel biomarker and a therapeutic target in many types of cancer, including bladder cancer, gastric cancer, lung cancer, liver cancer, breast cancer, and prostate cancer. However, its functions are complex and disease and context-dependent. Even though miR-27a was first identified as an oncomiR, it has been shown that it can act as both an oncogene and a tumor suppressor. For instance, in breast cancer, it was found that miR-27a has a role as an oncogenic miRNA, involved in the cell cycle checkpoint regulation by increasing the percentage of cells in the G2/M stage (Mertens-Talcott et al., 2007). Several other studies supported its oncogenic function. miR-27a was determined as a potential therapeutic target as it was found to be upregulated in ovarian and prostate cancer cells (Z. Li et al., 2010; Fletcher et al., 2012). In addition, Li et al. described that miR-27a was overexpressed in hepatocellular carcinoma (HCC) tissues and cells. Moreover, they showed that downregulation of miR-27a promoted apoptosis, and thus decreased HCC cell proliferation (S. Li et al., 2015). In contrast to its oncogenic roles, Bao et al. (2014) showed that mir-27a was significantly downregulated in colorectal cancer and it was able to inhibit cell proliferation, promote apoptosis, and reduce cancer cell migration. Consistent with this tumor suppressive function, in esophageal squamous cell carcinoma (ESCC), miR-27a has been demonstrated to significantly inhibit the proliferation of ESCC cells by directly targeting a well-known oncogene KRAS (Jiang et al., 2015). Furthermore, Li et al. revealed the suppressive function of miR-27a in breast cancer cell proliferation by inhibiting the Wnt/B-catenin pathway (M. Li et al., 2018).

miR-27a has been identified to directly regulate some oncogenic signalings, such as PI3K/Akt, TGF-β, Wnt/β-catenin, and Ras/MEK/ERK. The pleckstrin homology (PH) domain and leucine-rich repeat protein phosphatase 2 (PHLPP2), a negative regulator of Akt, were reported as a target of miR-27a. The suppression of PHLPP2 through miR-27a causes the upregulation of oncogenic Akt with its downstream pathways (L. Ding et al., 2017). On the other hand, PI3K and anti-apoptotic BCL-2 at downstream were identified as novel targets of miR-27a, indicating a tumor-suppressive function (G. Liu et al., 2013). PI3K/Akt regulation by miR-27a underlines its conflicting regulatory function in cancer

cells. The negative regulator of Wnt/β-catenin signaling, secreted frizzled-related protein (SFRP), have shown to be targeted by miR-27a, pointing out an oncogenic function. It was reported that proliferation and migration were elevated by SFRP1 suppression and subsequent Wnt/β-catenin signaling activation when gastric cancer cells were transfected with miR-27a mimics (L.-Y. Kong et al., 2017; F. Wu et al., 2017). miR-27a has also been shown to be a positive regulator of RAS/MEK/ERK signaling pathways, promoting oncogenic processes. miR-27a was demonstrated to be targeting BTG2, increasing the activation of RAS/MEK/ERK signaling (L. Zhou et al., 2016). Furthermore, it was demonstrated that c-myc induces transcription of miR-23a27a24-2 cluster. This decrease in expression of SPRY2, in turn, activates MAPK signaling (X. Li et al., 2013). A well-known tumor suppressor TGF-β signaling has been indicated to be both directly and indirectly regulated by miR-27a. TGF-β was determined as a direct target of miR-27a, suppressing the TGF-β signaling pathway (Fang et al., 2018). SMAD2 and SMAD4, downstream TGF-β signaling members, were also shown to be suppressed by miR-27a, inhibiting cell cycle arrest mediated by TGF-β (Chae et al., 2017; Xu et al., 2017).

1.7.4. miR-27a-5p in Cancer

Even though the literature mainly focused on the -3p strand of the miR-27a, miR-27a-5p has also been evaluated as a crucial molecule in cancer cell proliferation, invasion, and apoptosis. Since two strands of a mature miRNA differ in their nucleotide sequences, they can exert distinct functions with different gene targets in various cancer cells.

Recently, miR-27a-5p was reported to function as a tumor suppressor in Wilms' tumors and non-small cell lung cancer (NSCLC) through targeting PBOV1 (W. Chen et al., 2022; Z.-T. Guo et al., 2022). The expression level of miR-27a-5p was shown to be decreased in Wilms' tumor tissues. Proliferation and migration of cells were decreased, while apoptosis was promoted by decreasing expression of PBOV1 when WiT49 and STA-WT3ab cells were transfected with miR-27a-5p mimics. Furthermore, Guo et al. (2022) indicated the tumor suppressive function of miR-27a-5p also *in vivo*. They found that miR-27a-5p overexpression inhibited tumor development; tumors generated with Wilms' tumor cells transfected with miR-27a-5p mimics showed smaller tumor sizes. Chen et al.

(2022) demonstrated that MALAT1 downregulated miR-27a-5p, causing to upregulation in the expression level of PBOV1 and promoting resistance of NSCLC cells to gemcitabine.

In head and neck squamous cell carcinoma (HNSCC), miR-27a-5p was identified as a tumor suppressor by targeting and downregulating EGFR, Akt1, and mTOR, all of which are well-known tumor drivers (X. Wu et al., 2013). Moreover, they did not only evaluate the tumor suppressor role of miR-27a-5p in the HNSCC, but also determined its function on the viability of PC3 PCa cells, HEC1A human endometrial adenocarcinoma cells, MIA PaCa pancreas cancer cells, and MDA-468 breast cancer cells. They found that when these cell lines were transfected with miR-27a-5p mimics, their viability decreased. Furthermore, they showed that overexpression of miR-27a-5p was able to increase apoptosis and decrease migration of HNSCC cells through suppressing the EGFR signaling pathway (X. Wu et al., 2013). In a more recent HNSCC study, a feedback loop between TP53 and miR-27a-5p was hypothesized. Chari et al. indicated *in vitro* and *in vivo* that mutated TP53 suppressed the miR-27a-5p promoter to downregulate its expression, promoting tumor growth (Chari et al., 2020).

PTEN, a negative regulator of PI3K/Akt signaling and tumor suppressor, was reported to be a target gene of miR-27a-5p in acute pancreatitis research, and they demonstrated that apoptosis was promoted and inflammatory response was suppressed when miR-27a-5p was upregulated (L. Kong et al., 2019). He et al. (2021) identified apyrimidinic endodeoxyribonuclease 1 (APEX1), which promotes gastric cancer development, as a target of miR-27a-5p. They observed significant downregulation in miR-27a-5p level in gastric cancer tissues and overexpression of this miRNA reduced the viability and migration of SGC-7901 cells. Moreover, decreased expression of miR-27a-5p, correlated with enhanced levels of APEX1 was shown to contribute to doxorubicin resistance in gastric cancer. This resistance was attributed to the activation of RAS/MEK/FOS and Akt/SMAD2 signaling pathways. The chemokine receptor CX3CR1 was reported to be targeted by miR-27a-5p in natural killer cells. They also clarified that the miR-27a-5p level was increased by TGF-β1 which was found to induce expression of the miR-23a27a24-2 cluster (Regis et al., 2017).

1.7.5. miR-27a in Prostate Cancer

There is limited and conflicting knowledge on the function of miR-27a in PCa. As it is clear in the literature, miR-27a may act differently in various types of cancers, and even for a single type of cancer, there are conflicting suggestions on its role. In the case of PCa, a part of the literature has resulted in miR-27a as an oncogene while the other part has been suggesting its tumor suppressive roles.

In 2012, Fletcher et al. first time showed that miR-27a acts as an oncomiR in PCa by directly targeting Prohibitin (PHB), which is known to negatively regulate AR activity, acting as a tumor suppressor (Fletcher et al., 2012). They also showed that miR-27a is both transcriptionally and post-transcriptionally regulated by androgen. AR itself was reported to bind to the miR-23a-27a-24-2 cluster promoter for transcriptional regulation of miR-27a. In addition, post-transcriptional regulation of the cluster was shown to be directed by androgens; with the active AR signaling, the Drosha-mediated conversion of pri-miR-23a-27a-24-2 to pre-miR-27a was determined to be increased. Furthermore, it was clarified that the oncogenic role of miR-27a is androgen-dependent since there was no PCa cell growth when miR-27a was induced in an androgen-depleted medium. In another research, transcriptional regulation of miR-27a by AR was confirmed in LNCaP cells treated with DHT, and miR-27a was found to be directly up-regulated by AR (Mo et al., 2013). It was also revealed that miR-27a supported PCa cell proliferation by promoting malignant phenotype by directly targeting ABCA1 and PDS5B, suggesting an oncogenic role for miR-27a. After radical prostatectomy, in the tumors of PCa patients who progressed into metastatic PCa, the miR-27a expression level was found to be overexpressed (Nam et al., 2018). Gao et al. demonstrated the diagnostic and prognostic potential of miR-27a in prostate cancer in which, they found that in the tumor tissues and sera of PCa patients, miR-27a was significantly overexpressed and this upregulation was found to be correlated with poor prognosis of the PCa patients (Gao et al., 2018). They further revealed that the proliferation of PCa cells was increased with miR-27a mimics, targeting SPRY2, while miR-27a inhibitors were able to suppress their proliferation. Additionally, miR-27a was also found to be related to chemotherapy resistance in PCa. Cao et al. showed that miR-27a was significantly overexpressed in tissues of PCa patients who were treated with chemotherapy agents compared to tissues of non-treated prostate

cancer patients (Cao et al., 2019). Moreover, they concluded that chemotherapy resistance is due to exosomal miR-27a produced by prostate fibroblasts, negatively regulating p53 expression. More recently, miR-27a expression was determined to be upregulated in a Brazilian cohort of PCa tumor tissues (Bernardes et al., 2022). Prigol et al. demonstrated that when HUVEC endothelial cells were exposed to PC3 exosomes, an increase in their angiogenic behavior was observed, which was attributed to the overexpression of miR-27a (Prigol et al., 2021).

On the other hand, Lin et al. demonstrated that primary prostate cancer tissues had reduced levels of miR-27a compared to normal-matched tissues where metastatic prostate cancer exhibited further decreased miR-27a levels, suggesting a tumor suppressive function (S. C. Lin et al., 2016). The *in vitro* data from the study further support the tumor-suppressive function of miR-27a. The enhanced expression of miR-27a significantly suppressed migration and invasion of PCa cell lines through negatively regulating COUP-TFII, FOXM1, and CENPF. These factors are known to be upregulated in PCa and further elevated in metastatic PCa, thus playing a role in regulating metastasis in PCa (Aytes et al., 2014; Laoukili et al., 2005; Qin et al., 2013; J. R. Testa et al., 1994). Furthermore, they showed that enzalutamide-resistant LNCaP cell clones had significantly decreased expression of miR-27a. Correlated with this, there was increased expression of COUP-TFII, CENPF, and FOXM1, compared to parental cell lines. When miR-27a is overexpressed and COUP-TFII, CENPF, or FOXM1 is suppressed, the efficacy of enzalutamide treatment was shown to be increased in these clones. This study highlights the role of miR-27a in drug resistance in PCa. Similarly, it was revealed that miR-27a expression level is decreased in PCa and metastatic PCa tumors (Ku et al., 2021). Consistent with this tumor-suppressive function, Wan et al. (2016) also showed miR-27a to act as a tumor suppressor. When miR-27a was overexpressed, the growth rate of PCa cells decreased, apoptosis induced, and migration repressed, whereas lower levels of miR-27a were observed in primary and metastatic PCa samples, especially tumors with high Gleason scores showed lower expression (Wan et al., 2016). Additionally, they reported that miR-27a is negatively regulated by oncogenic signaling PI3K/Akt in CRPC cells. LY294002, a PI3K/Akt pathway inhibitor, was shown to increase the expression of miR-27a and this in turn decreased the level of MP2K4 which was determined to be a direct target of miR-27a. Consistent with Fletcher et al. (2012) and Mo et al. (2013), they also

found that miR-27a expression is increased in response to androgen treatment as an early event. In another study, overexpression of miR-27a was reported to decrease the proliferation and invasion of PCa cells through suppressing KDM3A, YAP1, and TEAD1, all of which are c-myc regulators (Cui et al., 2020). They found that miR-27a is downregulated in response to ZNFX1 anti-sense RNA1 (ZFAS1), a novel lncRNA shown to be overexpressed in PCa tumors, contributing to poor clinical outcomes. The *in vivo* data from the study underlines the tumor-suppressive role of miR-27a through ZFAS1; when miR-27a agomir is intratumorally injected twice per week, tumor growth due to ZFAS1 overexpression was observed to be partially repressed.

The literature has mainly focused on the -3p strand of miR-27a in the context of PCa and the studies mentioned so far have investigated the function of miR-27a-3p. The knowledge on the function of miR-27a-5p is even more limited, with few studies. In 2013, Wu et al. (2013), mentioned in *section 1.7.4*, was first shown the tumor-suppressive potential of the -5p strand in PCa. They demonstrated that the viability of PC3 cells decreases with miR-27a-5p transfection. Similarly, Barros-Silva et al. (2018) demonstrated a tumor-suppressive function for miR-27a-5p. The expression level of miR-27a-5p was found to be downregulated in PCa and PIN tissues, compared to normal tissue. However, the miR-27a-5p expression was shown to be increased in CRPC tumors. This was attributed to the positive correlation between miR-27a-5p and MYC, which was found to be increased in CRPC. They demonstrated that the expression of miR-27a-5p is transcriptionally regulated by MYC; a binding site for c-myc within the promoter region of miR-27a-5p was identified. This transcriptional regulation by c-myc was found to be dependent on the methylation status of the miR-27a-5p promoter. It was indicated that in CRPC, the promoter of miR-27a-5p is hypomethylated and that c-myc can give rise to miR-27a-5p expression. However, despite the tumor-suppressive function of miR-27a-5p, the study hypothesized that the increase in the miR-27a-5p may not be sufficient to suppress tumor growth due to numerous other molecular alterations. Moreover, the level of miR-27a-5p was found to be lower in LNCaP cells, while PC3 cells, representing CRPC, have higher miR-27a-5p levels. miR-27a-5p overexpression was found to result in reduced viability of PCa cells and increased apoptosis rates. (Barros-Silva et al., 2018). Consistent with Wu et al. (2013) EGFR, Akt1, and mTOR were indicated as targets of miR-27a-5p.

1.8.Purpose of The Study

miRNAs are promising therapeutic candidates in cancer research; however, their functions and the signaling pathways that they regulate still remain to be understood. In the literature, the role of miR-27a in PCa has mostly been studied through the -3p strand, whereas the -5p strand was investigated in two studies; miR-27a-5p has been shown to reduce the viability of PCa cells, increased apoptosis, and its expression levels have been significantly decreased in PCa tumors (Barros-Silva et al., 2018; Wu et al., 2013). The function of miR-27a-5p on the development, progression, or drug resistance in PCa has yet to be fully elucidated, requiring further clarification. The regulatory relationship between miR-27a-5p and the EGFR/Akt/mTOR signaling pathway has been revealed, but all the genes targeted by this miRNA and other pathways it regulates have not been elucidated. The limited knowledge of the role of miR-27a-5p in PCa is not sufficient to enlighten the molecular mechanism, with its regulatory signaling pathways.

In this context, miR-27a-5p is suggested to act as a tumor suppressor by directly targeting CCR5, STAT3, and BCL-2, all of which are components of chemokine signaling, and downstream, the PI3K/Akt/NF-**κ**B pathway (Figure 14). It is well-known that both chemokine signaling and the PI3K/Akt/NF-**κ**B pathway promote not only PCa progression, but also various types of cancer progression by inducing anti-apoptotic molecules such as BCL-2 or by inducing gene expression through STAT3 to promote proliferation, migration, and cell survival. The purpose of the current study is to determine the target genes and molecular mechanism of miR-27a-5p, as well as to demonstrate the correlation between miR-27a-5p and CCR5/STAT3/BCL-2 signaling in prostate cancer.

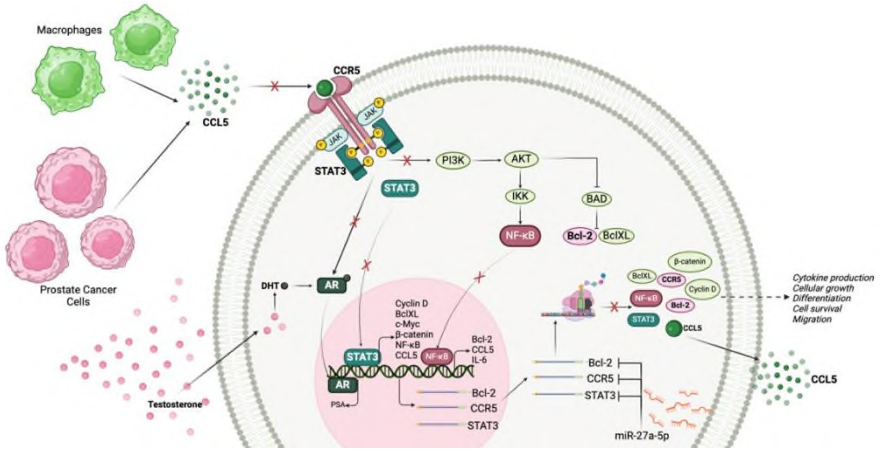


Figure 14. Hypothesis of the study. (Created with BioRender.com)

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Chemicals and Kits

Table 3. *List of the materials used in the study.*

Material/ Kit	Company	Catalog No	Application
Dulbecco's Modified Eagle's Medium (DMEM)	Gibco	41966-029	Cell culture
Roswell Park Memorial Institute 1640 (RPMI 1640)	Gibco	21875-034	
Keratinocyte serum-free medium (K-SFM)	Gibco	10724-011	
Fetal Bovine Serum (FBS)	PAN Biotech	P30-3306	
Bovine Pituitary Extract (BPE)	Gibco	13028-014	
Epidermal Growth Factor (EGF)	Gibco	10450-013	
Penicillin Streptomycin	Biological Industrie (BI)	BI-03-031-1B	
Non-essential Amino Acids (NEAA)	PAN Biotech	P08-32100	
Dulbecco's Phosphate Buffered Saline (DPBS)	PAN Biotech	P04-36500	
Trypsin-EDTA	Gibco	25200-056	
Cell culture dishes (25 cm^2, 75 cm^2)	TPP	90026, 90076	
Serological pipettes (5 mL, 10 mL)	CAPP	SP-5-C, SP-10-C	
96-well plates	TPP	92096	
24-well plates	TPP	92024	
6-well plates	TPP	92006	
Cryotubes (2 mL)	Isolab	091.11.102	
Thoma cell counting chamber			
Human Prostate Specific Antigen (PSA) ELISA Kit	Elabscience	E-EL-H0091	PSA determination
Formalin			

Cell viability detection kit-8 (CVDK-8)	EcoTech	CVDK-8	Viability assay
Cell Invasion Assay Kit	Merck	ECM550	Invasion assay
Annexin V-FITC/PI Apoptosis Kit	Elabscience	E-CK-A211	Apoptosis assay
hsa-miR-27a-5p mimic oligonucleotides	MedChemExpress	HY-R00499	Transfection
hsa-miR-27a-5p inhibitor oligonucleotides	MedChemExpress	HY-RI00499	
Negative control oligonucleotides	MedChemExpress	HY-R04602	
Opti-MEM	Gibco	31985062	
PolyFast Transfection Reagent	MedChemExpress	HY-K1014	
Lipofectamine RNAiMAX Reagent	Invitrogen	13778-030	
EcoPure Total RNA Kit	EcoTech	E2075	qRT-PCR
Absolute Ethanol	Merck	1009832500	
2-mercaptoethanol	Fisher Scientific	BP176	
cDNA Synthesis Kit (5X)	Abm Good		
miRNA cDNA Synthesis Kit	Abm Good		
qPCR SYBR Green Master Mix (2X)	Abm Good		
U6-2 primers	Abm Good	MPH00001	
PCR tubes (0.2 mL)	Axygen		
Filtered tips (10 µL, 200 µL, 1000 µL)	CAPP		General use
Tips (10 µL, 200 µL, 1000 µL)	CAPP		
Falcon tubes (15 mL, 50 mL)	CAPP		
Parafilm			
Eppendorf tubes (0.5 mL, 1.5 mL, 2.0 mL)	CAPP		

2.2. List of The Instruments

The instruments used in this study are shown in Table 4.

Table 4. *List of the instruments used in the study.*

Instrument	Company	Application
HEPA-filter laminar flow cabinet	Labconco	Cell culture
CO2 Incubator	Binder	
Inverted microscope	Zeiss	
Microcentrifuge	Thermo Fisher Scientific	General
Mini-spin	Ohaus	
Vortex	Ohaus	
Nano-drop	DeNovix	qRT-PCR
Rotor GeneQ	Qiagen	
Flow cytometry	CytExpert	Apoptosis assay

2.3. Methods

2.3.1. *In silico* Identification of The Target Genes of miR-27a-5p

Potential target genes of miR-27a-5p were predicted by using publicly available web servers, TargetScan, miRDIP, miRWalk, and miRDB. Then, in order to determine the genes predicted by all servers, a Venn diagram was generated with the gene lists. Binding energies and conformations upon binding between the identified common PCa-related target genes and miRNAs were determined with the StarMir tool. Pathway analysis was performed with the predicted target genes through the KEGG tool. The relevant web links for the utilized tools are shown in Table 5.

Table 5. *List of the utilized in silico tools.*

***in silico* tool**	**Web link**
TargetScan	https://www.targetscan.org/vert_72/
miRDIP	https://ophid.utoronto.ca/mirDIP/
miRWalk	http://mirwalk.umm.uni-heidelberg.de/search_mirnas/
miRDB	https://mirdb.org/
Venn diagram	https://bioinformatics.psb.ugent.be/webtools/Venn/
StarMir	https://sfold.wadsworth.org/cgi-bin/starmirWeb.pl
KEGG	https://www.kegg.jp/kegg/pathway.html

2.3.2. Prostate Cancer *in vivo* Model

Approximately 4-weeks-old BALB/c nude mice were used in the *in vivo* experiments. The ethical approval for the animal experimentation was obtained from Bezmialem Vakıf University. The animals were housed at Bezmialem Vakıf University Experimental Animals Lab in a pathogen-free facility. A group of nude mice was injected with $1.8X10^6$ PC3 cells (resuspended in saline) subcutaneously (right flank, n=7). Another group of mice was injected with $2X10^6$ LNCaP cells (resuspended in saline) subcutaneously (right flank, n=6). The last group of mice (control group) was injected with saline solution

subcutaneously (right flank, n=3). Body weight and tumor volume (lengthXwidth/2) were monitored three times a week. Blood samples were collected from the facial vein weekly to track plasma PSA levels, and plasma was isolated by centrifugation at 3000 rpm for 10 minutes. Approximately 4 weeks after injection, mice were anesthetized and underwent terminal cardiac bleeds. Tumors, key organs, and prostate tissues were rapidly dissected and tissues were snap-frozen with liquid N_2 or fixed in formalin. Snap-frozen tissues were stored at -80°C for RNA isolation. Tumors were examined via histopathological analyses and Gleason scores were determined at Bezmialem Vakıf University Histology Lab.

2.3.2.1.Determination of PSA Levels

A human PSA ELISA kit (Elabscience) was used to determine plasma PSA levels in the PCa mouse model. The manufacturer's protocol was followed with minor modifications. Briefly, 100 µL of previously serial diluted Standard Working Solution (Concentrations: 10 ng/mL -> 5 ng/mL -> 2.5 ng/mL) -> 1.25 ng/mL -> 0.63 ng/mL -> 0.31 ng/mL) were added in duplicates. 100 µL of plasma samples were added to corresponding wells and the plate was incubated overnight at 4°C. After incubation, the liquids were removed from the wells and 100 µL of previously diluted Biotinylated Detection Ab working solution was added to all wells. The plate was covered with the sealer and gently mixed. The plate was incubated on the shaker at 37°C for 75 minutes. Then, the liquids were removed from the cells and 350 µL of wash buffer was added to each well. The wash buffer was soaked for 1-2 minutes, liquids were aspirated and the plate was patted against a clean absorbent paper. The wash step was repeated 3 times. 100 µL previously diluted HRP conjugate working solution was added to each well and the plate was incubated on the shaker at 37°C for 30 minutes. The solution in each well was aspirated and the wash step was performed 3 times. 90 µL of substrate reagent was added to each well in the dark. It was incubated on the shaker at 37°C for approximately 25 minutes in the dark. Finally, 50 µL of Stop Solution was added to each well, and optical density (OD) was immediately determined with the microplate reader at 450 nm. By using the data from OD values of the Standard Working Solution, a standard curve was generated and PSA concentrations of samples were calculated using the equation obtained from the standard curve.

2.3.3. Cell culture

The cell lines used in this study were all maintained at 37°C, under 5% CO_2 in a humidified culture incubator. PC3 (CRL-1435, ATCC) and LNCaP (CRL-1740, ATCC) cell lines were maintained in RPMI 1640, the DU145 cell line (HTB-81, ATCC) was maintained in DMEM, RPMI and DMEM media were supplemented with 10% FBS and 1% penicillin-streptomycin and the Cells were washed twice with 2 mL PBS. Cells were detached from the surface of the flask by incubating with 1 mL of Trypsin-EDTA at 37°C for 3 minutes and then, trypsin was inactivated with 3 mL of complete medium. Cells were collected in a falcon tube and centrifuged at 1200 rpm for 5 minutes. The supernatant was discarded and the pellet was resuspended with fresh medium. Cells were seeded in new flasks, according to the desired dilution ratio and the media were renewed once in three days.

2.3.4. Transfection of PCa cells with miRNA Oligonucleotides

The oligonucleotide tubes were briefly centrifuged to ensure that the dried miRNA was at the bottom of the tube. The oligonucleotides were resuspended using nuclease-free water to generate a 20 μM stock solution. For 5 nmol oligonucleotide: 250 μL nuclease-free water was added. The resuspended oligonucleotides were stored at or below -20°C or -80°C.

According to the manufacturer's protocol, $0.3X10^6$ cells were seeded in a 6-well plate and incubated for 24 hours for the cells to reach 60-80% confluence before transfection. For duplicated wells, 9.37 μL Lipofectamine reagent will be diluted in 146.87 μL Opti-MEM medium. For each duplicate, 6.25 μL (200 nM per well) negative control oligonucleotides and 25 μL miRNA inhibitor and miRNA mimic (200 nM per well) were diluted in 150 μL Opti-MEM medium. Diluted miRNA was added to the diluted Lipofectamine reagent in a 1:1 ratio. The mixture was incubated for 5 minutes at room temperature. 125 μL of

the miRNA-lipid complexes were added per well. Cells were incubated for 24 hours at 37°C. qRT-PCR was performed to assess transfection efficiency.

2.3.5. RNA Isolation from Cell Culture

Total RNA was isolated from cells using the Total RNA Isolation Kit (EcoPure) according to the manufacturer's protocol. All procedure was performed on ice. Briefly, cells were trypsinized and centrifuged at 1200 rpm for 5 minutes. The pellet was lysed with 400 µL Lysis Binding Buffer supplemented with 4 µL β-mercaptoethanol. 400 µL of absolute ethanol was added to the lysate and mixed well by the vortex. The mixture was transferred to the columns and centrifuged at maximum speed for 30 seconds at room temperature. 400 µL Wash Buffer 1 was added to the column and centrifuged for 30 seconds at maximum speed. 500 µL Wash Buffer 2 was added to the column and centrifuged at maximum speed for 30 seconds. The flow was discarded. 200 µL Wash Buffer 2 was added to the column and centrifuged at maximum speed for 2 minutes. The columns were transferred to two 1.5 mL Eppendorf tubes. 75 µL of elution buffer was added to the center of the column and incubated for 1 minute at room temperature. Finally, it was centrifuged for 30 seconds at maximum speed to obtain RNA. Isolated RNA samples were quantified with the NanoDrop spectrophotometer. Samples that have an A260/A280 ratio greater than 2 were used for further protocols. RNA samples were stored at -80°C.

2.3.6. RNA Isolation from Frozen Tissue

Total RNA was isolated from cells using the Total RNA Isolation Kit (EcoPure) according to the manufacturer's protocol. All procedure was performed on ice. Briefly, up to 30 mg of frozen tissue samples were ground with the help of liquid nitrogen by using pre-cooled mortars and pestles. The ground tissues were lysed with 400 µL Lysis Binding Buffer supplemented with 4 µL β-mercaptoethanol. For complete homogenization, lysates were passed through a 25-gauge needle several times.

Homogenized lysates were centrifuged at maximum speed for 2 minutes at room temperature. Each supernatant was transferred to another Eppendorf tube and their volumes were recorded. The same volumes of absolute ethanol were added to the lysate and mixed well by vortex. The mixture was transferred to the columns and centrifuged at maximum speed for 30 seconds at room temperature. 400 µL Wash Buffer 1 was added to the column and centrifuged for 30 seconds at maximum speed. 500 µL Wash Buffer 2 was added to the column and centrifuged at maximum speed for 30 seconds. The flow was discarded. 200 µL Wash Buffer 2 was added to the column and centrifuged at maximum speed for 2 minutes. The columns were transferred to 1.5 mL Eppendorf tubes. 75 µL of elution buffer was added to the center of the columns and incubated for 1 minute at room temperature. Finally, it was centrifuged for 30 seconds at maximum speed to obtain RNA. Isolated RNA samples were quantified with NanoDrop spectrophotometer. Samples that have A260/A280 ratio of equal to or greater than 2 were used for further protocols. RNA samples were stored at -80°C.

2.3.7. Reverse Transcription for cDNA Synthesis

Complementary DNA (cDNA) was synthesized with the OneScript Plus cDNA Synthesis Kit. Synthesis was performed according to the manufacturer's protocol. Reaction tubes containing, 4 µL 5X RT Buffer, 1µL random primers, 1µL dNTP, 1µL RTase and 250 ng total RNA, were prepared, and nuclease-free H_2O (NFH_2O) were added to bring up the total volume to 20 µL. Tubes were briefly centrifuged. By using the Thermal Cycler (BioRad), the mixture was incubated at 52°C for 15 minutes for reverse transcription, and then, it was incubated at 85°C for 5 minutes for inactivation of reverse transcriptase. Synthesized cDNA was quantified with the NanoDrop spectrophotometer and cDNA samples that have an A260/A280 ratio of approximately 1.8 were used for qRT-PCR. cDNA samples were stored at -20°C.

Complementary DNA (cDNA) was synthesized with miRNA All In One cDNA Synthesis Kit. Synthesis was performed according to the manufacturer's protocol. Reaction tubes

containing, 10 µL 2X cDNA Super Mix, 2 µL Enzyme Mix, and 250 ng total RNA, were prepared and NFH_2O was added to bring up the total volume to 20 µL. Tubes were briefly centrifuged. By using the Thermal Cycler (BioRad), the mixture was incubated at 37°C for 30 minutes, at 50°C for 15 minutes, and then, at 85°C for 5 minutes. Synthesized cDNA was quantified with the NanoDrop spectrophotometer and cDNA samples that have an A260/A280 ratio of approximately 1.8 were used for qRT-PCR. cDNA samples were stored at -20°C.

2.3.8. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

qRT-PCR was performed by using qPCR SYBR Green Master Mix (2X) with Rotor GeneQ (Qiagen) machine, according to the defined protocol and condition in Table 6 and Table 7. Sequences of used forward and reverse primers are given in Table 8.

Table 6. *qRT-PCR conditions.*

Step	Temperature	Duration
Enzyme Activation	95°C	15 min
Denaturation	95°C	15 sec
Annealing/ Extension	60°C	60 sec

Table 7. *qRT-PCR protocol.*

Component	Amount
qPCR SYBR Green Master Mix (2X)	10 µL
Forward Primer (10 µM)	0.5 µL
Reverse Primer (10 µM)	0.5 µL
Template cDNA	1000 ng
Nuclease free H_2O	Up to 20 µL

Table 8. *Primer sequences. hsa; human, mmu; mouse*

Primer	Sequence
hsa-miR-27a-5p	F: 5' GGCTTAGCTGCTTGTGA 3'
mmu-miR-27a-5p	F: 5' GGCTTAGCTGCTTGTGAG 3'
hsa-CCR5	F: 5' TCTCTTCTGGGCTCCCTACAAC 3'
	R: 5' CCAAGAGTCTCTGTCACCTGCA 3'
mmu-CCR5	F 5' GTCTACTTTCTCTTCTGGACTCC 3'
	R 5' CCAAGAGTCTCTGTTGCCTGCA 3'
hsa-STAT3	F 5' CTTTGAGACCGAGGTGTATCACC 3'
	R 5' GGTCAGCATGTTGTACCACAGG 3'
mmu-STAT3	F 5' AGGAGTCTAACAACGGCAGCCT 3'
	R 5' GTGGTACACCTCAGTCTCGAAG 3'
hsa-BCL2	F 5' ATCGCCCTGTGGATGACTGAGT 3'
	R 5' GCCAGGAGAAATCAAACAGAGGC 3'
mmu-BCL2	F 5' CCTGTGGATGACTGAGTACCTG 3'
	R 5' AGCCAGGAGAAATCAAACAGAGG 3'
hsa-AR	F 5' ATGGTGAGCAGAGTGCCCTATC 3'
	R 5' ATGGTCCCTGGCAGTCTCCAAA 3'
mmu-AR	F 5' CCTTGGATGGAGAACTACTCCG 3'
	4 5' TCCGTAGTGACAGCCAGAAGCT 3'
β-actin Forward/Reverse	purchased from Applied Biological Materials
RNU6 Forward	purchased from Applied Biological Materials
Universal miRNA Reverse	purchased from Applied Biological Materials

2.3.9. Cell Viability Assay

The effect of miR-27a-5p on PCa cell viability will be determined by using CVDK-8 (EcoTech). According to the manufacturer's protocol, $3X10^4$ cells were seeded in a 96-well plate and incubated for 24 hours for the cells to reach 60-80% confluence before transfection. For each triplicate, 1.5 µL Lipofectamine reagent was diluted in 25 µL Opti-MEM medium. For each triplicate, 0.75 µL negative control oligonucleotides (200 nM per well) and 3 µL miRNA inhibitor and miRNA mimic (200 nM per well) were diluted in 25 µL Opti-MEM medium. Diluted oligonucleotides were added to the diluted Lipofectamine reagent in a 1:1 ratio. The mixtures were incubated for 5 minutes at room temperature. 10 µL of miRNA-lipid complexes were added per well. Cells were incubated for 24-48-72 hours at 37°C. 10 µL of CVDK-8 reagent was added to each well and the plates were incubated for 3 hours at 37°C. Absorbance was measured at 450 nm in the microplate reader.

2.3.10. Wound Healing Migration Assay

The effect of miR-27a-5p on PCa cell migration will be determined by a scratch wound-healing assay. $0.5X10^5$ cells were seeded in media containing 1% FBS into a 24-well plate and incubated for 24 hours for the cells to reach 60-80% confluence before transfection. For each duplicate, 3 µL Lipofectamine reagent will be diluted in 50 µL Opti-MEM medium. For each duplicate, 1 µL negative control oligonucleotides (200 nM per well) and 4 µL miRNA inhibitor and miRNA mimic (200 nM per well) were diluted in 50 µL Opti-MEM medium. Diluted oligonucleotides were added to the diluted Lipofectamine reagent in a 1:1 ratio. The mixtures were incubated for 5 minutes at room temperature. 50 µL of miRNA-lipid complexes were added per well. Cells were incubated for 24 hours for the cells to reach 95% confluence. A linear wound was generated with a 200 µL pipette tip. Wells were washed with PBS and fresh media were provided. Wound closure was determined by an inverted microscope at 6-hour time intervals (0-6-12-18-24 hr) with the help of the ImageJ program by using a plugin named Wound Healing Size Tool (Suarez-Arnedo et al., 2020).

2.3.11. Transwell Cell Invasion Assay

The effect of miR-27a-5p on PCa cell invasion was determined with the transwell cell invasion assay. Invasion chambers were placed in a 24-well plate and remained in the tissue culture hood for a while to adjust them to room temperature. 300 µL of serum-free medium was added to the interior of the inserts and invasion chambers were left for 1-2 hours at room temperature for the rehydration of the ECM layer. After that, the media in the inserts were carefully removed and 500 µL of media containing 10% FBS was added into the lower chamber. Cell suspensions containing $0.5X10^6$ DU145 or LNCaP cells/mL were prepared in the Opti-MEM medium and 300 µL of cell suspension was added into each insert. The chambers were incubated for 24 hours in the incubator. After 24 hours, cells were transfected with miR-27a-5p mimics and negative control oligonucleotides as stated in *section 2.3.10*. For each duplicate, 3 µL Lipofectamine reagent will be diluted in 50 µL Opti-MEM medium. For each duplicate, 1 µL negative control oligonucleotides (200 nM per well) and 4 µL miRNA inhibitor and miRNA mimic (200 nM per well) were diluted in 50 µL Opti-MEM medium. Diluted oligonucleotides were added to the diluted Lipofectamine reagent in a 1:1 ratio. The mixtures were incubated for 5 minutes at room temperature. 50 µL of miRNA-lipid complexes were added per well. Cells were further incubated for 24 hours and then, non-invading cells from the interior of the inserts were carefully removed by using a cotton-tipped swab. This step was repeated two times, without disturbing the polycarbonate membrane. 500 µL of staining solution was added to the unoccupied well of the plate and inserts were dipped into this solution for 20 minutes to stain invasive cells on the lower surface of the membrane. After 20 minutes, inserts were dipped in a beaker of water several times to rinse and inserts were air dried. Invaded cells were observed under the inverted microscope and counted by using the ImageJ program for quantitation (Schneider et al., 2012).. In addition, invaded cells were dissolved in 200 µL 10% acetic acid and 100 µL of the mixtures were transferred into a 96-well plate for colorimetric reading of OD at 560 nm.

2.3.12. Annexin V-FITC/PI Apoptosis Assay

The effect of miR-27a-5p on apoptosis of DU145 and LNCaP cells was assessed with an Annexin V-FITC/PI apoptosis assay. $1X10^5$ cells were seeded into a 24-well plate and incubated for 24 hours for the cells to reach 60-80% confluence before transfection. For each duplicate, 3 µL Lipofectamine reagent will be diluted in 50 µL Opti-MEM medium. For each duplicate, 1 µL negative control oligonucleotides (200 nM per well) and 4 µL miRNA inhibitor and miRNA mimic (200 nM per well) were diluted in 50 µL Opti-MEM medium. Diluted oligonucleotides were added to the diluted Lipofectamine reagent in a 1:1 ratio. The mixtures were incubated for 5 minutes at room temperature. 50 µL of miRNA-lipid complexes were added per well. Cells were incubated for 24 hours at 37°C and then, they were collected by trypsinization. The cell pellets were collected by centrifugation at 1300 rpm for 5 minutes. A group of cells were dissolved in PBS and they were not dyed to use them to determine the gate in the flow cytometry. The remaining group of cells were resuspended in 100 µL 1X Annexin V Binding working solution. 2.5 µL of Annexin V-FITC and 2.5 µL of PI were added to each tube. The tubes were gently vortexed and incubated at room temperature for 15-20 minutes in the dark. The, 400 µL of 1X Annexin V Binding working solution was added to each tube, and mixed. The cells were analyzed immediately in CytExpert flow cytometry. Annexin V-FITC was detected in the FITC channel and PI was detected in PerCP/Cy5.5 channel.

2.3.13. Statistical Analyses

Experiments were performed in duplicates unless otherwise stated. Statistical analyses were performed by using GraphPad Prism 9.0 software. Comparisons between the two groups were performed using Student's t-test. Correlations between miRNA expression and predicted target gene expressions were evaluated by the Pearson correlation coefficient (r) test. P-values <0.05 were considered as statistically significant . Significance is shown as follows: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ and ns – non-significant.

3. RESULTS

3.1. CCR5, STAT3, and BCL-2 were Identified as Potential Target Genes of miR-27a-5p *in silico*

2458 genes from TargetScan, 181 from miRDIP, 3610 from miRWalk, and 251 from miRDB were obtained as potential target genes of miR-27a-5p *in silico*. 28 genes that were predicted by each of the servers, were identified by the generation of the Venn diagram (Figure 15). 9 of them were found to be related to prostate cancer through the literature search, and the binding potential of miR-27a-5p to the 3'UTR sequence of these genes was determined by using StarMir and TargetScan tools. Binding regions at 5'UTR and coding sequence were ignored and the genes that have not 3'UTR binding were excluded, because of the fact that a miRNA should bind to the 3' UTR region of an mRNA for efficient mRNA suppression. According to the StarMir and TargetScan scores, NPM1, CCR5, STAT3, BCL-2, and BDNF were found to have a potential binding sequence in the 3'UTR for miR-27a-5p (Table 9). TRH mRNA did not show binding potential.

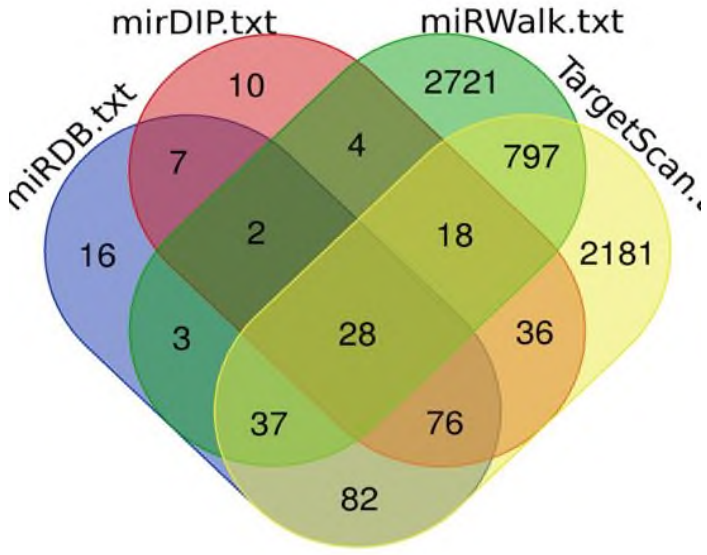


Figure 15. Venn diagram showing the number of target genes predicted by all servers.

Table 9. Binding scores of potential targets between miR-27a-5p.

	StarMir Scores		*TargetScan Scores*	
Gene	Logistic Probability Value	Free Energy (ΔG kcal/mol)	Context ++ Score Percentile	Context ++ Score
NPM1	0.849	-23	99	-0.62
CCR5	0.829	-25.2	98	-0.43
STAT3	0.803	-26.7	84	-0.2
BCL-2	0.741	-24.2	87	-0.22
BDNF	0.732	-25.4	93	-0.27
CYP19A1	0.666	-24.3	94	0.28
GIPC3	0.434	-29	68	-0.13
NF2	0.28	-29.7	90	-0.24
TRH	No 3' UTR binding			

The genes with Logistic Probability Values (StarMir) greater than 0.7 and Context ++ Score Percentile greater than 80 were subjected to pathway analysis. Consistent with pathway enrichment analysis (Figure 16), JAK-STAT and CCL5/CCR5 chemokine signaling pathways were found to be the most related pathways with target genes and also PCa. At the downstream of CCL5/CCR5 signaling STAT3 and BCL-2 were identified. Therefore, CCR5, STAT3, and BCL-2 genes were selected for further investigation (Table 10).

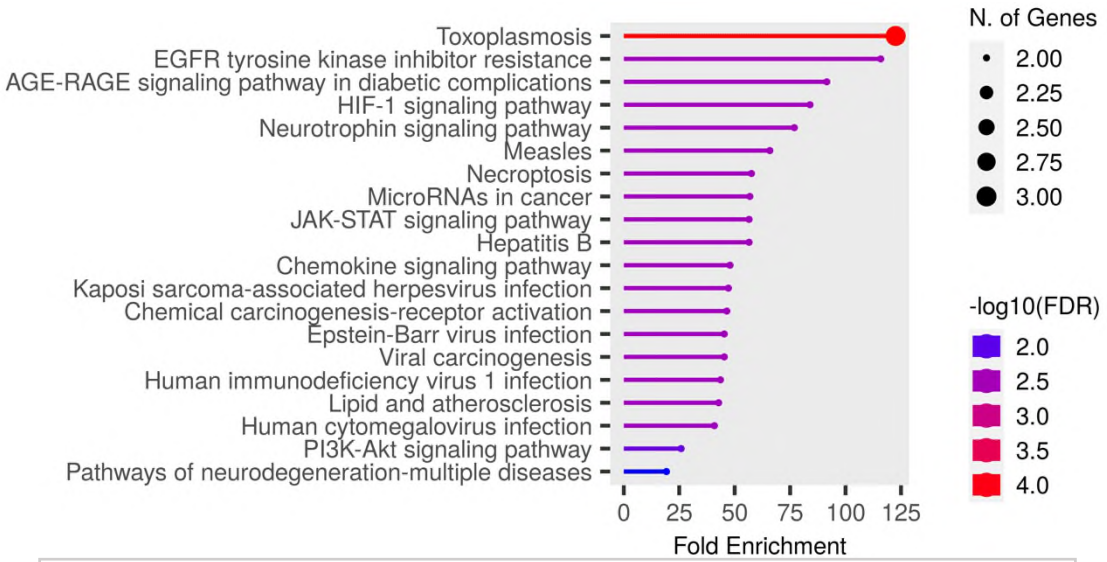
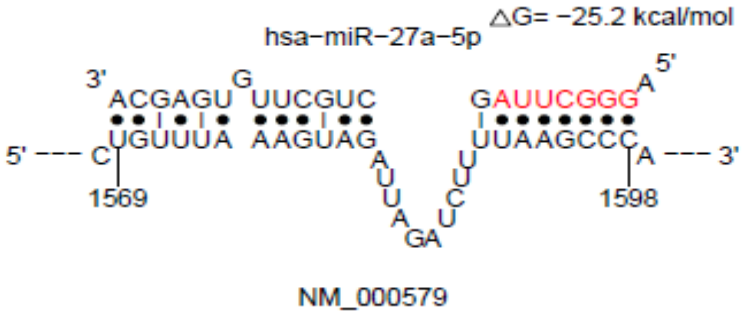
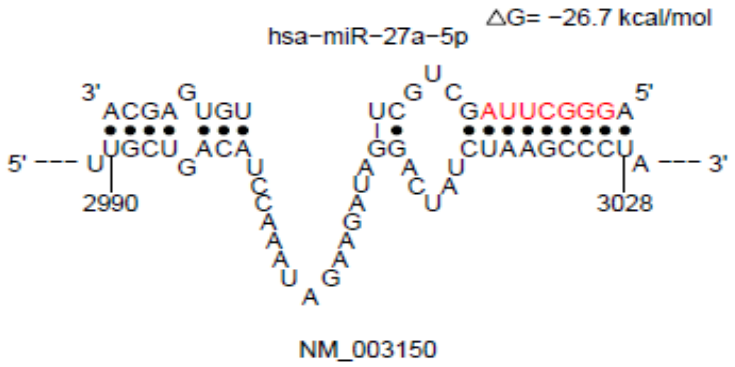
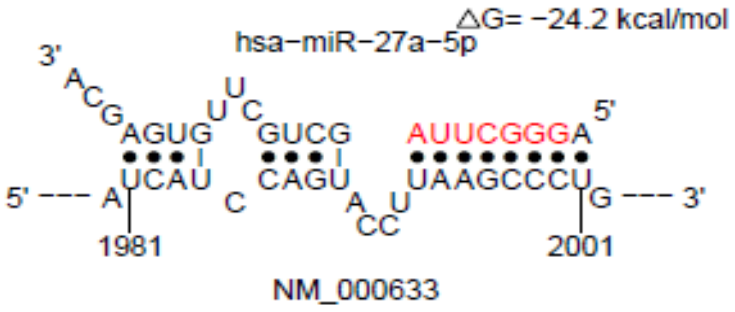


Figure 16. Pathway enrichment analysis.

Table 10. Predicted binding conformations between selected targets and miR-27a-5p. Red-colored nucleotides are seed sequences of miR-27a-5p.

Gene	*Binding Conformation*
CCR5	
STAT3	
BCL-2	

3.2.*In vivo* Prostate Cancer Model

There was not a significant change in the weights of both tumor and control groups throughout the experiment. Similarly, there were no significant changes in the weights of LNCaP-transplanted animals. On the other hand, animals transplanted with PC3 cells showed a significant decrease in body weight ($p<0.05$) (Figure 17).

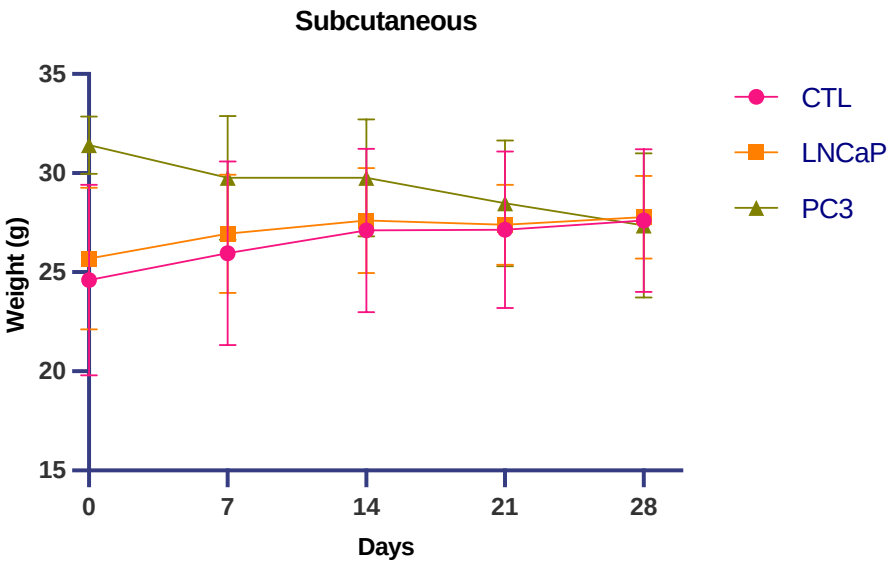


Figure 17. Body weight change of mice subcutaneously injected with either saline, PC3 cells, or LNCaP cells.

Subcutaneously generated PC3 tumors become visible on day 7, while LNCaP tumors appear on day 9 of post-cellular-transplantation. Tumor volumes of each group were significantly increased throughout the experiment. The tumor volume of PC3 animals was found to be significantly more increased than that of LNCaP animals on day 30 ($p<0.01$). This trend in tumor volume increase was also continued on day 35 (Figure 18, D). Therefore, after the animals were sacrificed, the PC3 animals had a significantly greater volume of tumors, compared to the tumor volumes of LNCaP animals.

AR expression level was determined in LNCaP tumors by qRT-PCR and it was found that AR was significantly upregulated in all LNCaP tumors with a fold change of 26.6 ($p<0.0001$), consistent with the tumor volume data (Figure 18, B).

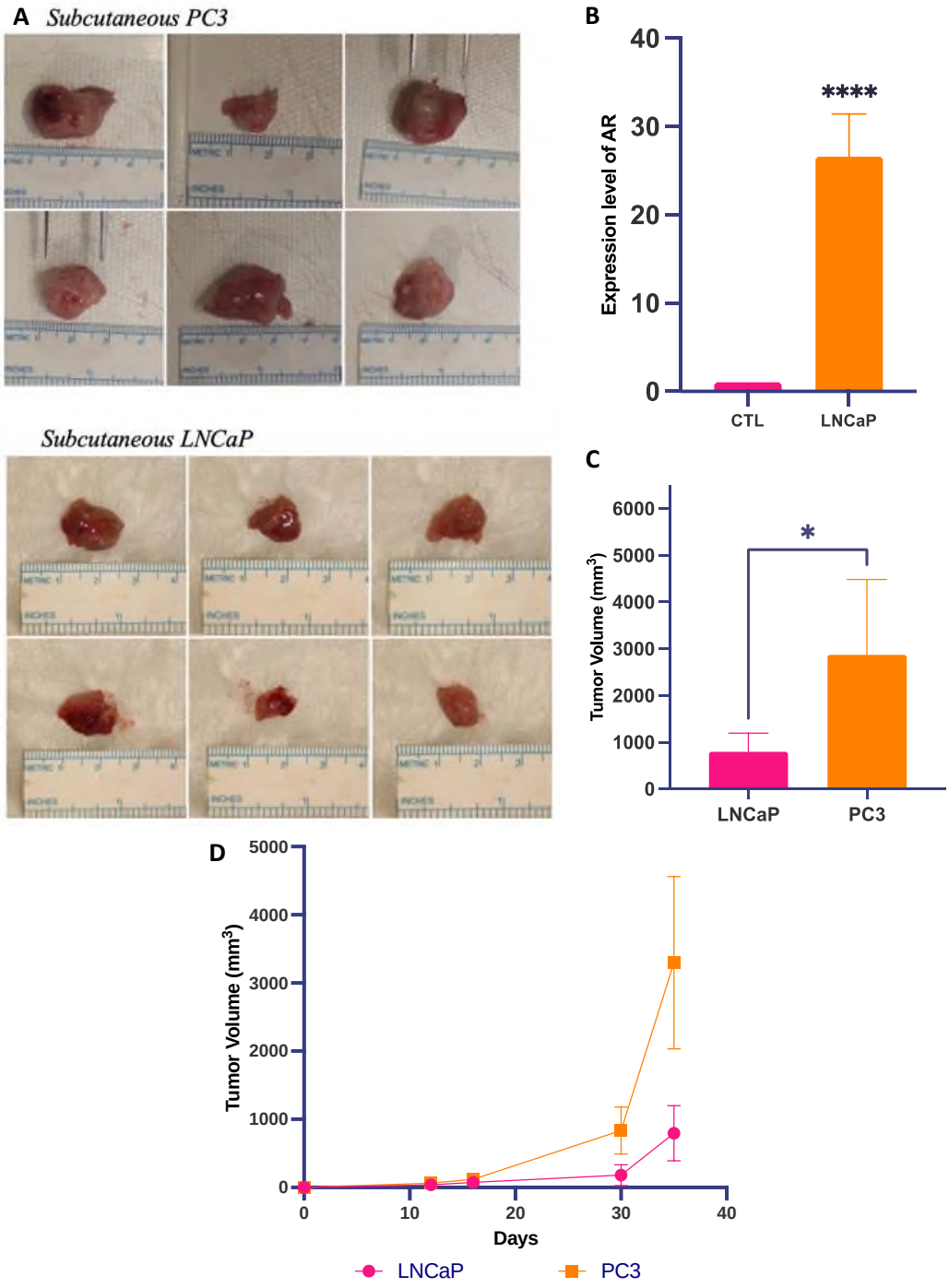


Figure 18. Tumors dissected from **A.** PC3 and LNCaP tumors. **B.** Expression level of AR in tumors generated with LNCaP cells. **C.** Sacrification tumor volume of animals. **D. G**rowth of PC3 and LNCaP tumors. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, and ns-non significant.

Plasma PSA levels were determined weekly in all animals throughout the experiment (Figure 19 & Table 11). LNCaP animals had greater PSA levels (674.25 pg/mL) in the third week, compared to PC3 animals (111.18 pg/mL) and then it was decreased in week 4.

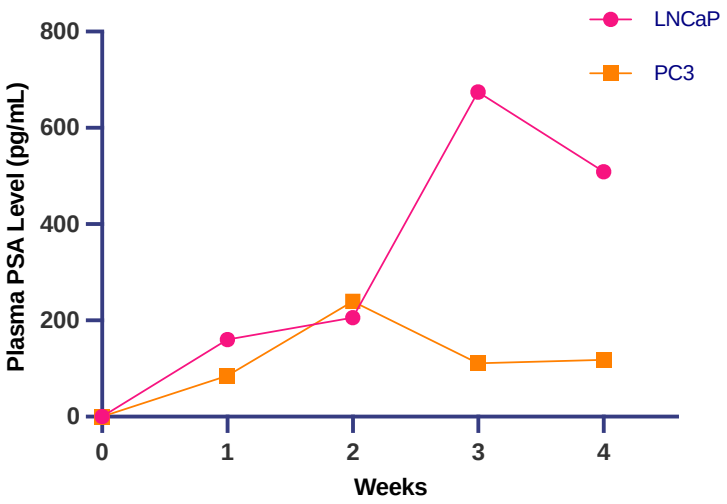


Figure 19. Plasma PSA levels (pg/mL) of animals throughout the experiment.

Table 11. Plasma PSA levels (pg/mL) of animals.

Group/ Week	*0*	*1*	*2*	*3*	*4*
PC3	0	85.2	239.9	111.2	118
LNCaP	0	160.4	205.5	674.2	509.2

Gleason scores of tumors were determined by histopathological analyses stated in Table 12. Muscular invasions with tumor cell infiltrations in the muscle tissues with high Gleason scores (5+5) were diagnosed in all subcutaneously generated tumors. A benign tissue was observed in one of the LNCaP animals.

Table 12. Histological evaluation of tumors with Gleason scores and diagnosis.

Route of Injection	*Cell Line*	*Individual Mice*	*Tumor Volume in 4th week (mm^3)*	*Gleason Score*	*Diagnosis*
Subcutaneous	LNCaP	#1	1370.5	5+5	Muscular invasion
		#2	686.9	Ø	
		#3	1065.6	5+5	Muscular invasion
		#4	909.1	5+5	Muscular invasion, necrosis in the muscle tissue, and reactive cells in the chondroid tissue
		#5	480.5	5+5	Muscular invasion
		#6	264	5+5	Lymph node metastasis
	PC3	#1	317.5	5+5	Tumor cell infiltration of the muscle tissue
		#2	3880.5	5+5	Lymph node metastasis
		#3	2068.8	5+5	Tumor cell infiltration of the muscle tissue, and lymph node metastasis
		#4	5477.9	5+5	Tumor cell infiltration of the muscle tissue, and lymph node metastasis
		#5	3331.5	5+5	Tumor cell infiltration of the muscle tissue, and lymph node metastasis
		#6	2805.3	Ø/ 5+5	Benign muscular tissue/ Lymph node metastasis
		#7	2225.1	5+5	Lymph node metastasis
	Saline (CTL)	#1		Ø	
		#2		Ø	
		#3		Ø	

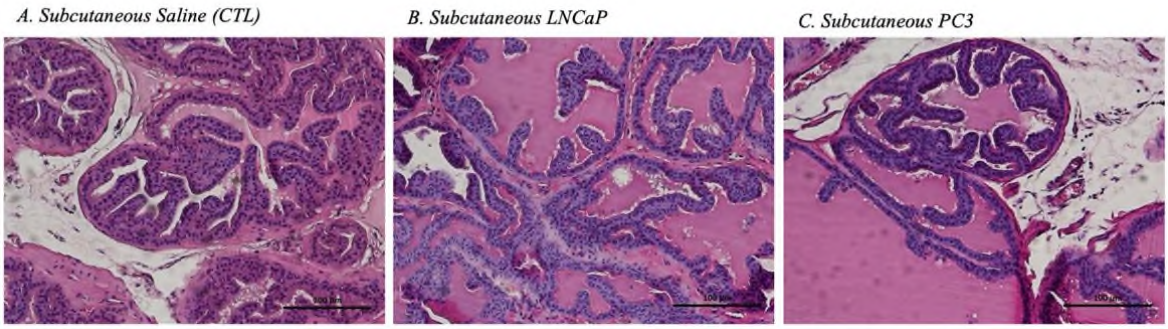


Figure 20. Representative histology images of tumors and healthy prostate tissues.

3.3.Expression Levels of miR-27a-5p and Predicted Target Genes *in vivo*

Expression levels of miR-27a-5p, CCR5, and BCL-2 were determined in LNCaP, and PC3 tumors by qRT-PCR (Table 13). Gene expression analyses showed that miR-27a-5p was significantly downregulated in LNCaP ($p<0.0001$), and PC3 ($p<0.0001$) tumors (Figure 21, A). There were no significant changes between LNCaP and PC3 tumors in the expression of miR-27a-5p.

Expression levels of CCR5, one of the predicted target genes of miR-27a-5p, were found to be significantly upregulated in both LNCaP and PC3 tumors (Figure 21, B). On the other hand, BCL-2 was found to be significantly overexpressed in only in LNCaP tumors (Figure 21, C). However, expression of BCL-2 was also higher in PC3 tumors in a non-significant way. In addition, STAT3 expression was not detectable in PC3 or LNCaP tumors.

Table 13. *In vivo* levels of miR-27a-5p, CCR5, and BCL-2 expressions as fold changes (FC).

Tumor Type	Subcutaneous		
	Saline	LNCaP	PC3
FC in exp. of CCR5	1.0	1.82	1.77
FC in exp. of BCL-2	1.0	4.13	4.53
FC in exp. of miR-27a-5p	1.0	0.57	0.42

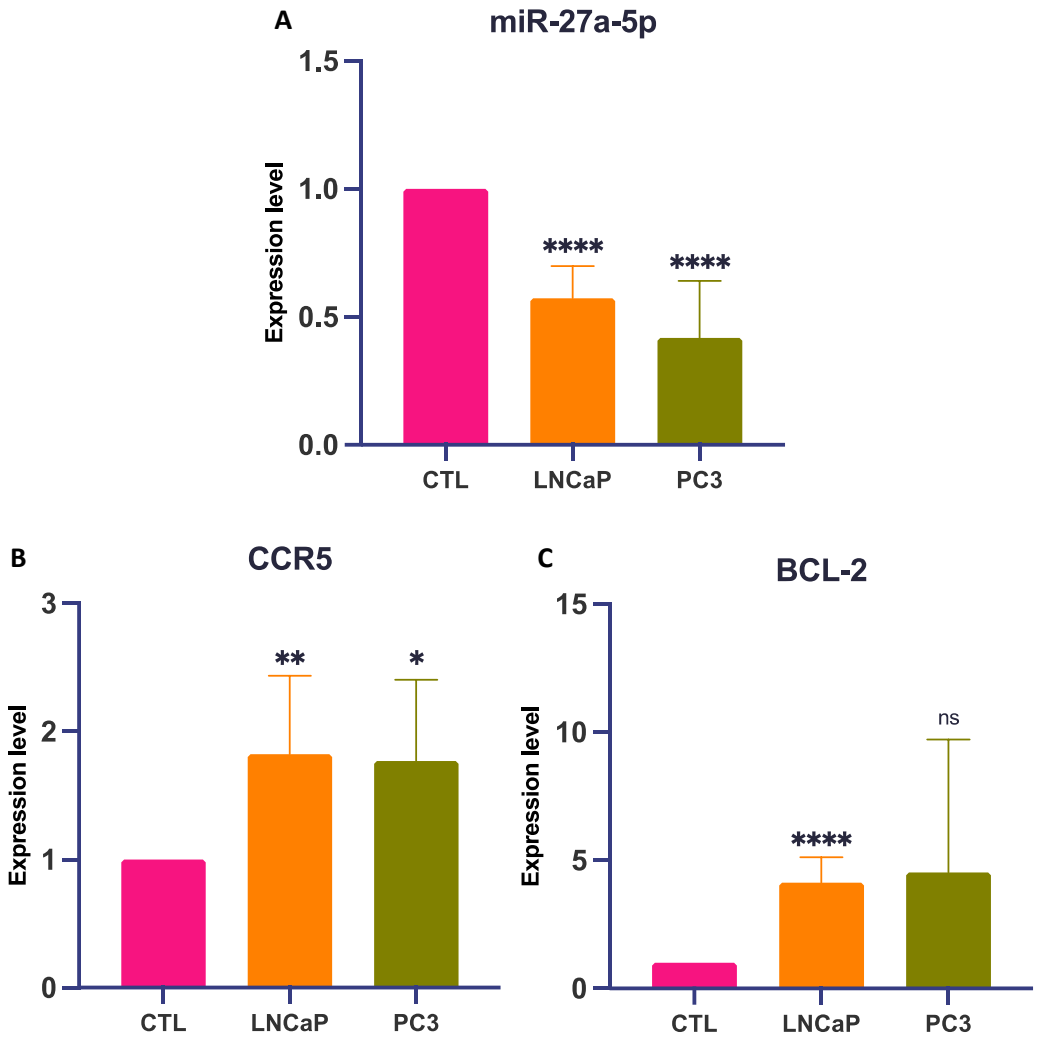


Figure 21. *In vivo* expression levels of target genes and miRNA. **A.** Expression level of miR-27a-5p; **B.** Expression level of CCR5; **C.** Expression level of BCL-2. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, and ns-non significant.

To evaluate the correlation between miR-27a-5p and predicted target genes (CCR5 & BCL-2), the *Pearson correlation coefficient (r)* was calculated for the expression data of matched individual animals (Figure 22). According to the statistical analyses and outlier removal, it was found that there's a significant incverse correlation between the expression of miR-27a-5p and that of CCR5 ($r = -0.8795$, $p<0.01$). There's also significant inverse correlation between the miR-27a-5p and BCL-2 gene expressions ($r = -0.8324$, $p<0.05$). Finally, the correlation between miR-27a-5p and AR was also significantly inverse ($r = -0.9263$, $p<0.01$).

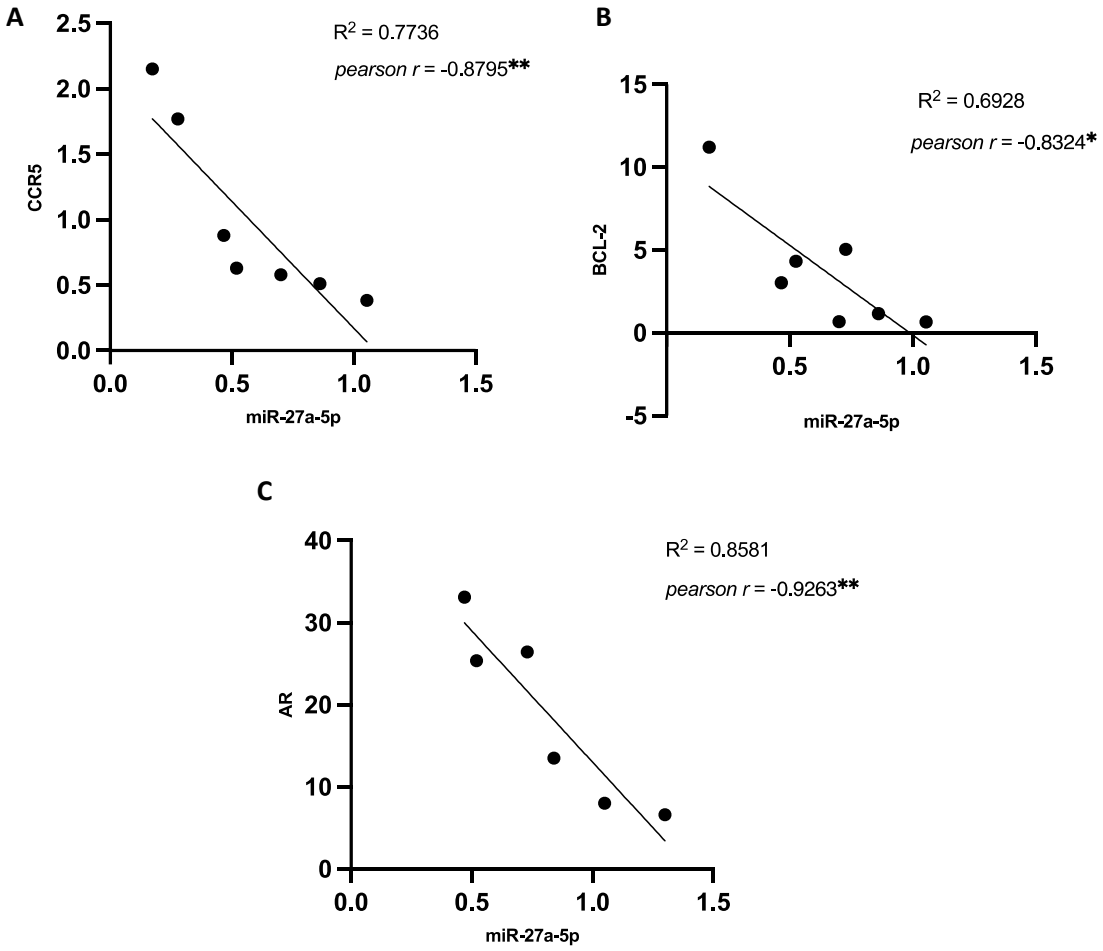


Figure 22. Correlation between the genes and miRNA. **A.** CCR5 vs miR-27a-5p; **B.** BCL-2 vs miR-27a-5p; **C.** AR vs miR-27-5p. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, and ns-non significant. r; Pearson correlation coefficient

3.4.Expression Levels of miR-27a-5p and Predicted Target Genes in Prostate Cancer Cell Lines

Expression levels of miR-27a-5p were determined with qRT-PCR in PC3, LNCaP, and DU145 prostate cancer cell lines, and compared to that of healthy prostate RWPE-1 cells (Figure 25). miR-27a-5p was found to be significantly downregulated in all PCa cell lines (Table 14). The lowest expression with a fold change of 0.07, was detected in the LNCaP cell line, even though it is not statistically significant when compared with other PCa cell lines. On the other hand, the highest expression (Fold Change: 0.27) was found in the DU145 cell line, though it is also not significant when compared with other PCa cell lines.

The expression levels of predicted target genes were determined with qRT-PCR (Figure 3, A). CCR5 was found to be significantly upregulated in LNCaP ($p<0.0001$), and PC3 ($p<0.05$) cell lines, compared to RWPE-1; fold changes are given in Table 14. On the other hand, the DU145 cell line was found to show the least expression of CCR5, and downregulation in the CCR5 level showed significance when compared to RWPE-1.

STAT3 was found to be significantly upregulated in DU145 ($p<0.01$), compared to RWPE-1. PC3 cells were found to be STAT3 negative, as it has been well-determined in the literature (Figure 23, B).

BCL-2 expression level was significantly decreased in DU145, compared to RWPE-1 cells (Figure 23, C). Nevertheless, its expression was determined to be significantly upregulated in PC3 and LNCaP cells, when compared to DU145 cell line, and the difference in expression of BCL-2 between PC3 and LNCaP cells is not significant.

Since the expression of all three predicted target genes present in DU145 and LNCaP cells, they were selected as model cell lines in the study to evaluate the role of miR-27a-5p.

Table 14. Fold changes in the gene expression of PCa cell lines.

Cell Lines	RWPE-1	PC3	DU145	LNCaP
FC in exp. of CCR5	1.0	1.2	0.8	2.6
FC in exp. of STAT3	1.0	0.0	3.9	0.5
FC in exp. of BCL-2	1.0	1.0	0.3	0.9
FC in exp. of miR-27a-5p	1.0	0.08	0.27	0.07

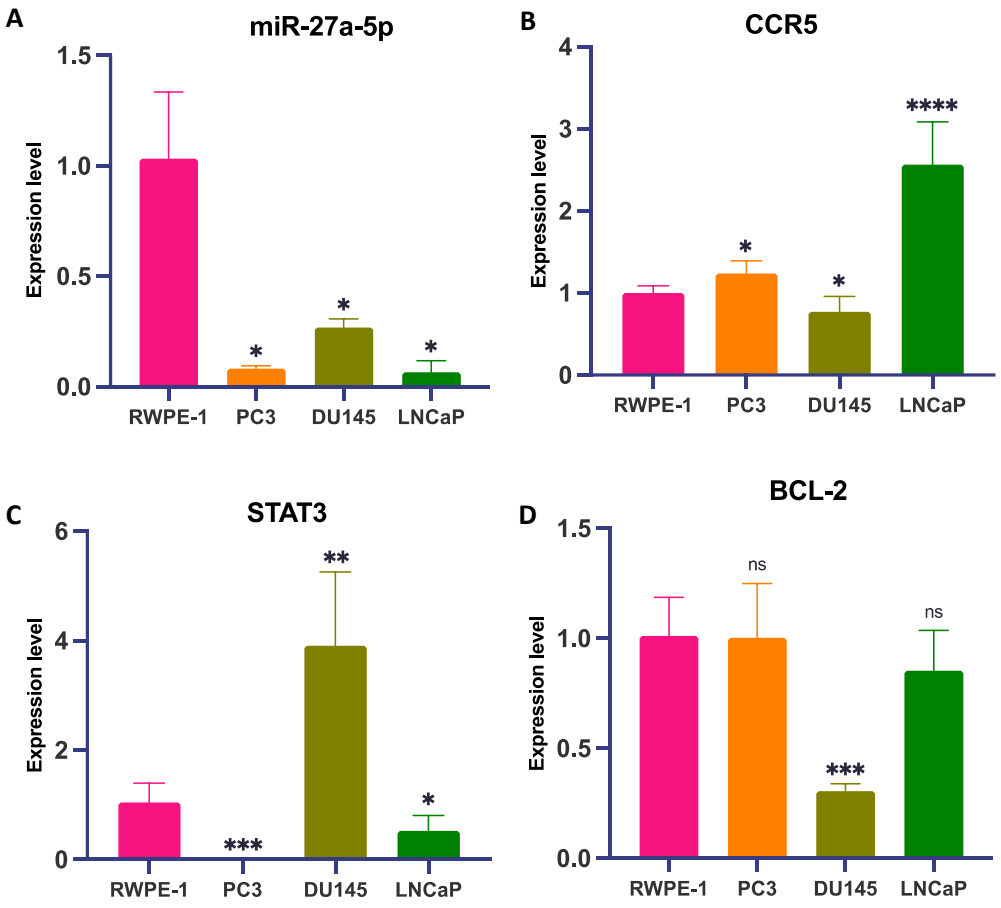


Figure 23. Expression levels of target genes in various PCa cell lines. **A.** Expression level of miR-27a-5p; **B.** Expression level of CCR5; **C.** Expression level of STAT3; **D.** Expression level of BCL-2. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, and ns-non significant.

3.5. Transfection

3.5.1. Transfection Efficiency

Oligonucleotide transfection efficiencies in DU145 and LNCaP cells were determined by qRT-PCR (Table 15). It was shown that 200 nM miR-27a-5p mimic oligonucleotides significantly increased the levels of miR-27a-5p in both DU145 ($p<0.0001$; Figure 24, A) and LNCaP ($p<0.05$; Figure 24, B) cell lines when compared to the cells transfected with sham oligonucleotides. In contrast, 200 nM miR-27a-5p inhibitors significantly decreased the levels of miR-27a-5p in both DU145 ($p<0.01$) and LNCaP ($p<0.05$) cell lines when compared to the cells transfected with sham oligonucleotides.

Table 15. Fold changes in the gene expression of transfected PCa cell lines.

Cell Lines	DU145	LNCaP
Sham	1.0	1.0
miR-27a-5p mimics	2.8	33.9
miR-27a-5p inhibitors	0.25	0.5

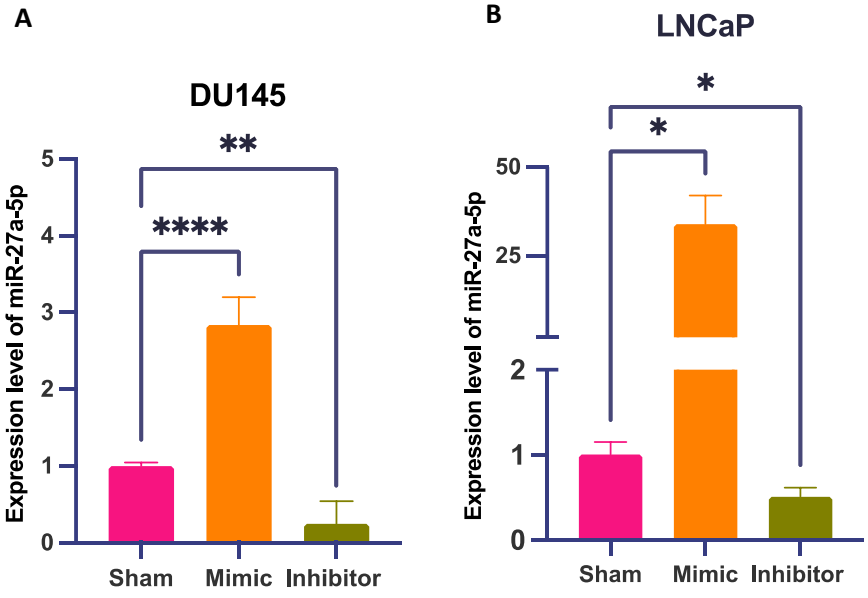


Figure 24. Expression levels of miR-27a-5p in transfected PCa cell lines. **A.** Expression level of miR-27a-5p in DU145 cells; **B.** Expression level of miR-27a-5p in LNCaP cells. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, and ns-non significant.

3.5.2. Target Gene Expression Analyses

The expression levels of miR-27a-5p's predicted target genes, STAT3, CCR5, and BCL-2 were determined in DU145 and LNCaP cells transfected with miR-27a-5p mimic, inhibitor, and sham oligonucleotides (Table 16).

DU145 cells transfected with miR-27a-5p mimic oligonucleotides showed a significant decrease in STAT3 and BCL-2 expression levels with fold changes of 0.18 ($p<0.01$) and 0.37 ($p<0.05$), respectively (Figure 25, A and C). On the other hand, DU145 cells transfected with miR-27a-5p inhibitor oligonucleotides showed a significant increase in STAT3 expression level with a fold change of 1.3 ($p<0.01$), whereas miR-27a-5p inhibitors did not cause any significant change in the expression of BCL-2. In contrast, the CCR5 expression level was found to be significantly increased in DU145 cells transfected with miR-27a-5p mimics and inhibitors with fold changes of 3.18 ($p<0.01$) and 2.48 ($p<0.001$), respectively (Figure 25, B).

LNCaP cells transfected with miR-27a-5p mimic oligonucleotides were determined to have significantly decreased STAT3, BCL-2, and AR levels with fold changes of 0.53 ($p<0.01$), 0.27 ($p<0.0001$), and 0.34 ($p<0.0001$), respectively (Figure 26, A, C, and D). Transfection of LNCaP cells with miR-27a-5p inhibitors did not cause any significant changes in the expressions of STAT3, BCL-2, and CCR5, while miR-27a-5p inhibitors significantly increased the expression of AR ($p<0.05$). In addition, overexpression of miR-27a-5p led to a significant increase in the expression of CCR5 ($p<0.0001$) (Figure 26, B).

Table 16. Fold changes in the expression of target genes.

Cell Lines	**DU145**			**LNCaP**			
Genes	STAT3	CCR5	BCL-2	STAT3	CCR5	BCL-2	AR
Sham	1.0	1.0	1.0	1.0	1.0	1.0	1.0
miR-27a-5p mimics	0.18	3.18	0.37	0.53	1.36	0.27	0.34
miR-27-5p inhibitors	1.3	2.48	0.94	1.17	0.87	1.14	1.77

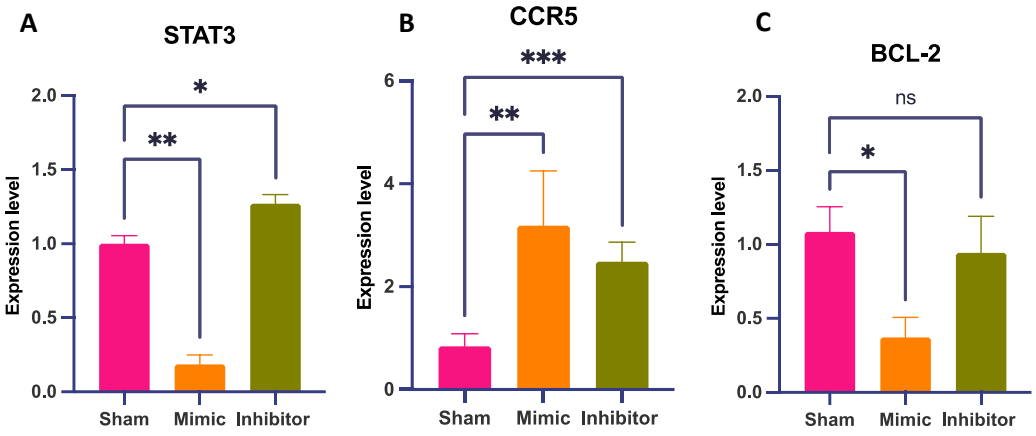


Figure 25. Expression levels of target genes in transfected DU145 cell line. **A.** Expression level of STAT3; **B.** Expression level of CCR5; **C.** Expression level of BCL-2. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, and ns-non significant.

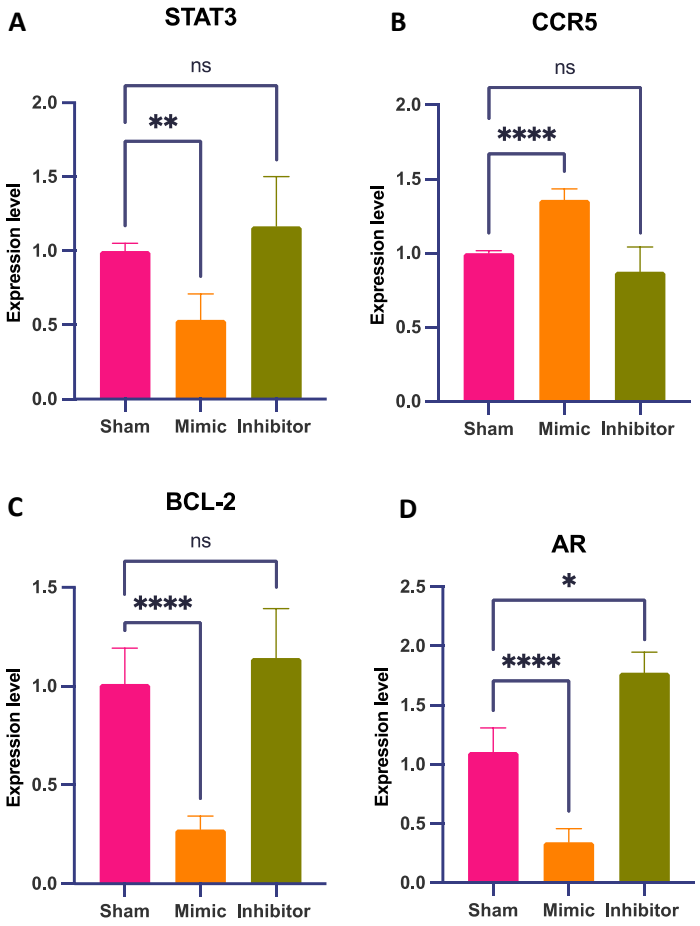


Figure 26. Expression levels of target genes in transfected LNCaP cell line. **A.** Expression level of STAT3; **B.** Expression level of CCR5; **C.** Expression level of BCL-2; **D.** Expression level of AR. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, and ns-non significant.

3.6. The Effect of miR-27a-5p on The Viability of PCa Cell Lines

The effect of miR-27a-5p on the viability of PCa cell lines was determined by the CVDK-8 cell viability assay. Overexpression of miR-27a-5p significantly decreased the cell viability of DU145 and LNCaP cells in 24 hours to 54.3% ($p<0.0001$) and 60.7% ($p<0.05$), respectively (figure 27, A). A significant change was not observed in the viability of DU145 cells between 24 hours and 48 hours of transfection with miR-27a-5p mimics (Figure 27, B). Even though it is non-significant, miR-27a-5p mimics showed a higher anti-proliferative effect on DU145 cells than LNCaP cells. In contrast, the viability of PC3 cells was not affected by miR-27a-5p overexpression (Cell viability = 93.8%, $p=0.5509$). DU145 ($p<0.01$) and LNCaP ($p<0.05$) cell lines were found to be significantly more sensitive to the miR-27a-5p overexpression, compared to PC3 cells. Downregulation of miR-27a-5p did not cause any significant change in the viability of DU145 and LNCaP cells. Moreover, inhibition of miR-27a-5p in PC3 cells resulted in significantly increased cell viability to 120.4% ($p<0.01$) in 24 hours.

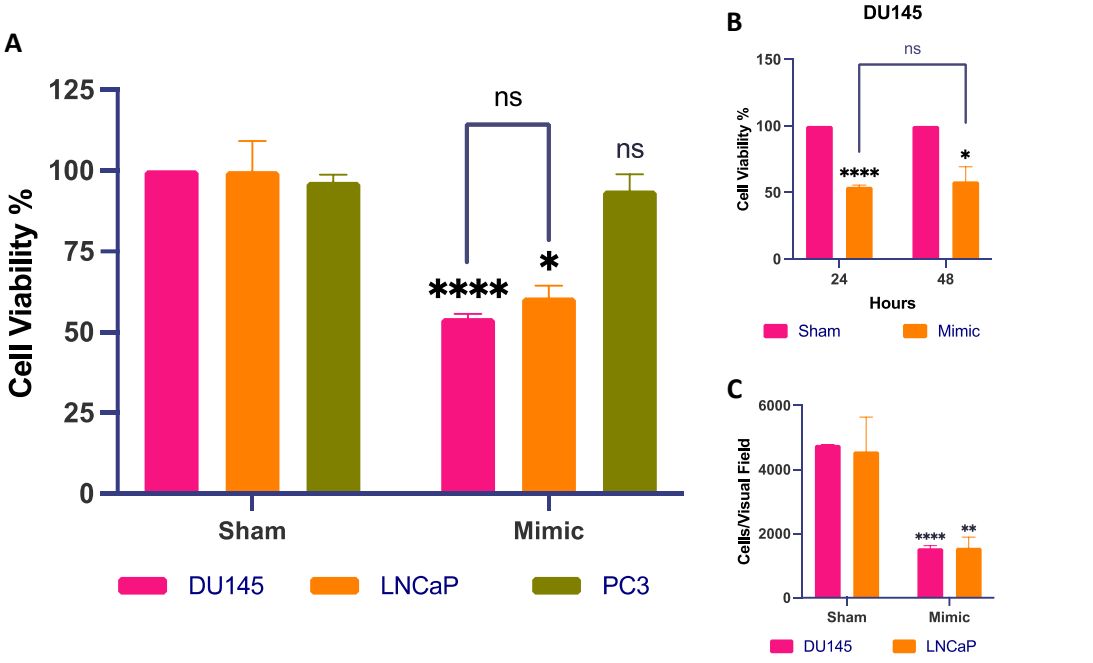


Figure 27. The effect of miR-27a-5p on the cell viability of PCa cell lines. **A.** Cell viability of DU145, LNCaP, and PC3 cell lines transfected with miR-27a-5p mimics; **B.** Cell viability of DU145 cells at 24-48 hours. **C.** Quantification of cell viability of DU145 and LNCaP cell lines through counting cells/visual field. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, and ns-non significant.

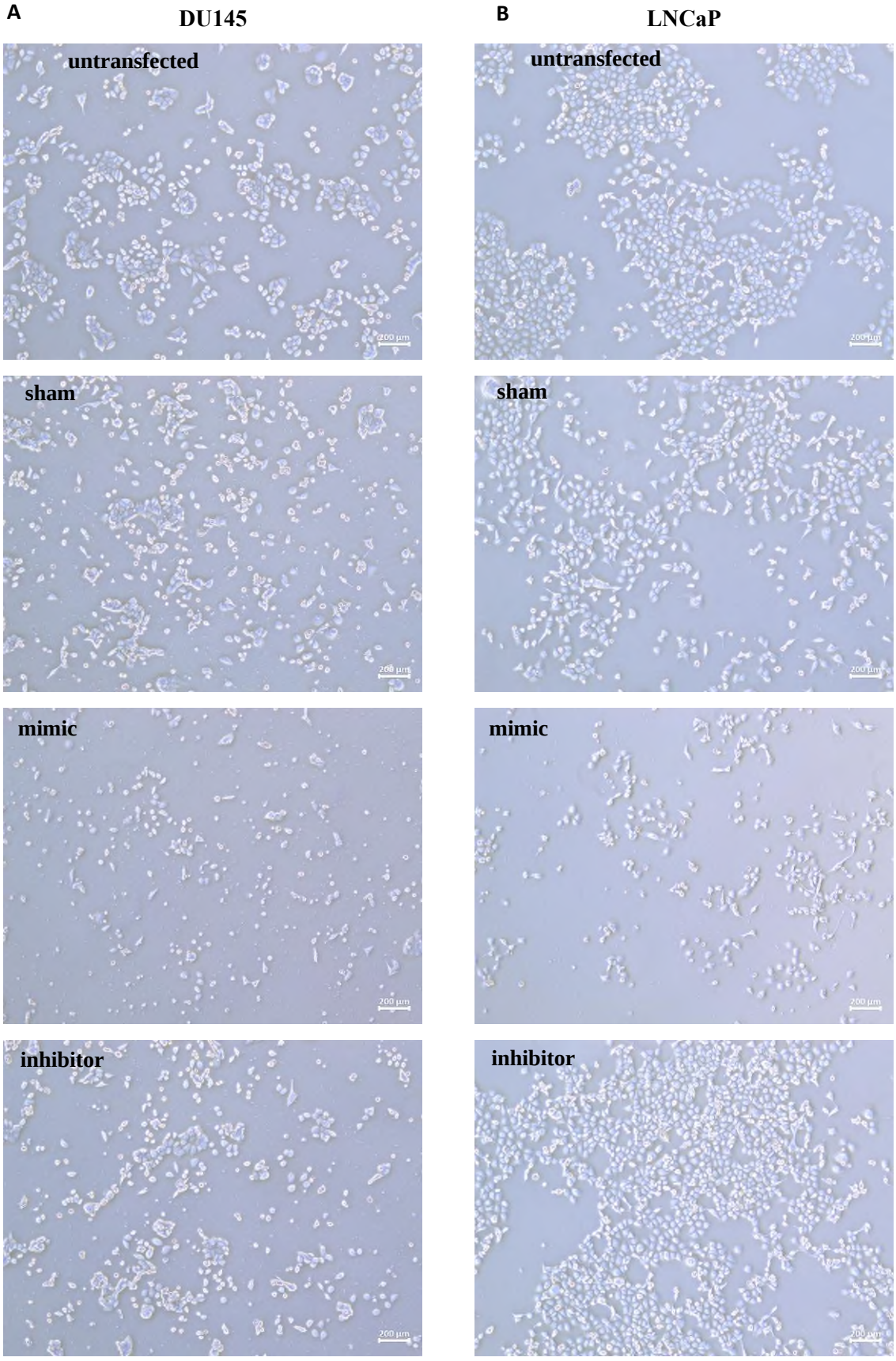


Figure 28. Representative images of PCa cells transfected with miR-27a-5p mimic, inhibitor, and sham oligonucleotides. **A.** Cell viability of DU145 cells; **B.** Cell viability of LNCaP cells.

3.7. The Effect of miR-27a-5p on The Migration of PCa Cell Lines

The wound-healing scratch assay was performed to quantify the role of miR-27a-5p in suppressing the migration of PCa cell lines. DU145 and LNCaP cells transfected with miRNA mimics and sham were incubated for 24 hours after a wound was induced, and migration of the cells was determined under the microscope with 6-hour time intervals for 24 hours.

Migration of both DU145 and LNCaP cells was impaired with miR-27a-5p overexpression. Wounds generated in the miRNA mimic groups did not heal for 24h, at which time sham and non-transfected groups were healed more. It was revealed that wound closure between 6 and 24 hours in DU145 and LNCaP cells transfected with miR-27a-5p mimics was significantly less than that of the cells transfected with negative control oligonucleotides. At 24 hours, the wound closure percentages of DU145 and LNCaP cells transfected with sham were 21.4% and 40.3%, respectively, whereas the wound closures of the cells transfected with mimics were 12.4% ($p<0.05$) and 20.14% ($p<0.05$), respectively (Figure 29, A and C).

Likewise, the migration rate of both cell lines was decreased with miR-27a-5p overexpression, compared to the sham group. The migration rate of DU145 cells transfected with miR-27a-5p mimics was significantly less than the migration rate of the sham group between 6 and 24 hours (Figure 29, B). On the other hand, the decrease in migration rate of LNCaP cells transfected with miR-27a-5p mimics was only significant in the 12 and 18 hours, compared to the sham group (Figure 29, D).

In addition, the migration ability of LNCaP cells was significantly greater than that of DU145 cells, in which the wounds generated in untransfected LNCaP cells and LNCaP cells in the culture medium with 10% FBS were near-completely healed after 24 hours; while the wound closure of untransfected DU145 cells and DU145 cells in the culture medium with 10% FBS were 35.7% and 25.3%, respectively (Figure 30 and 31).

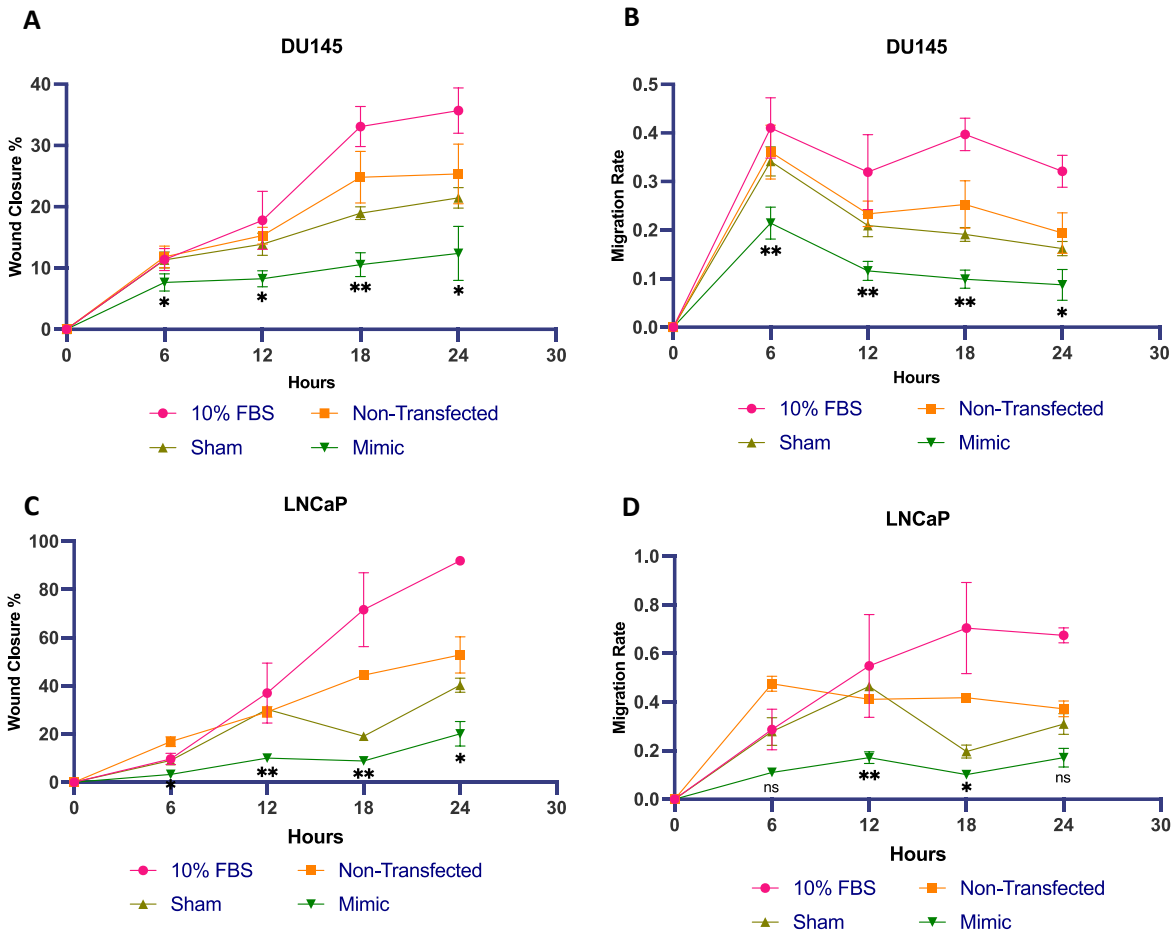


Figure 29. The effect of miR-27a-5p on the migration of PCa cell lines. **A.** Wound closure of DU145 cell line; **B.** Migration rate of DU145 cell line; **C.** Wound closure of LNCaP cell line; **D.** Migration rate of LNCaP cell line. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, and ns-non significant.

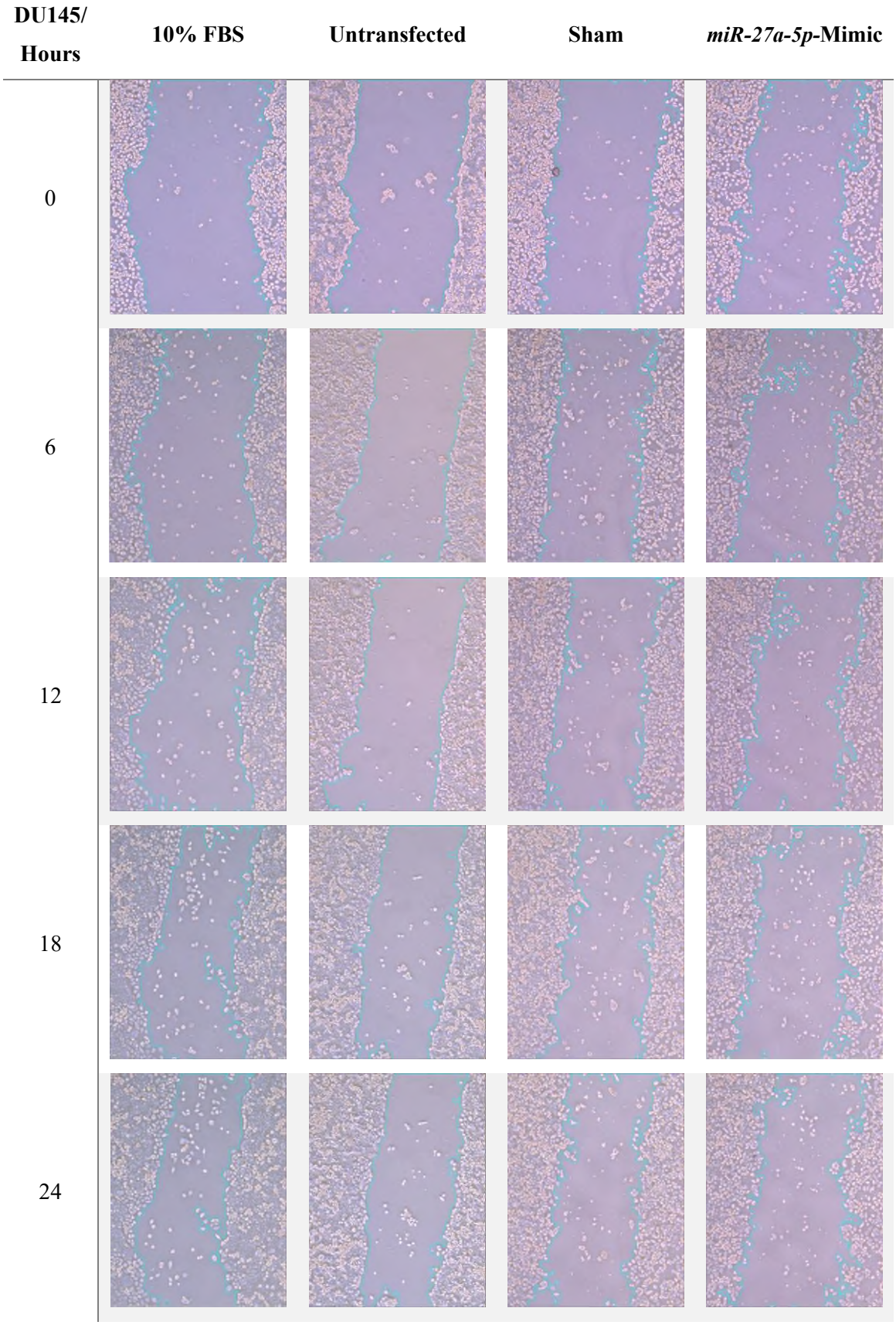


Figure 30. Representative images of DU145 cells' migration in response to miR-27a-5p overexpression at 0-6-12-18-24 hours.

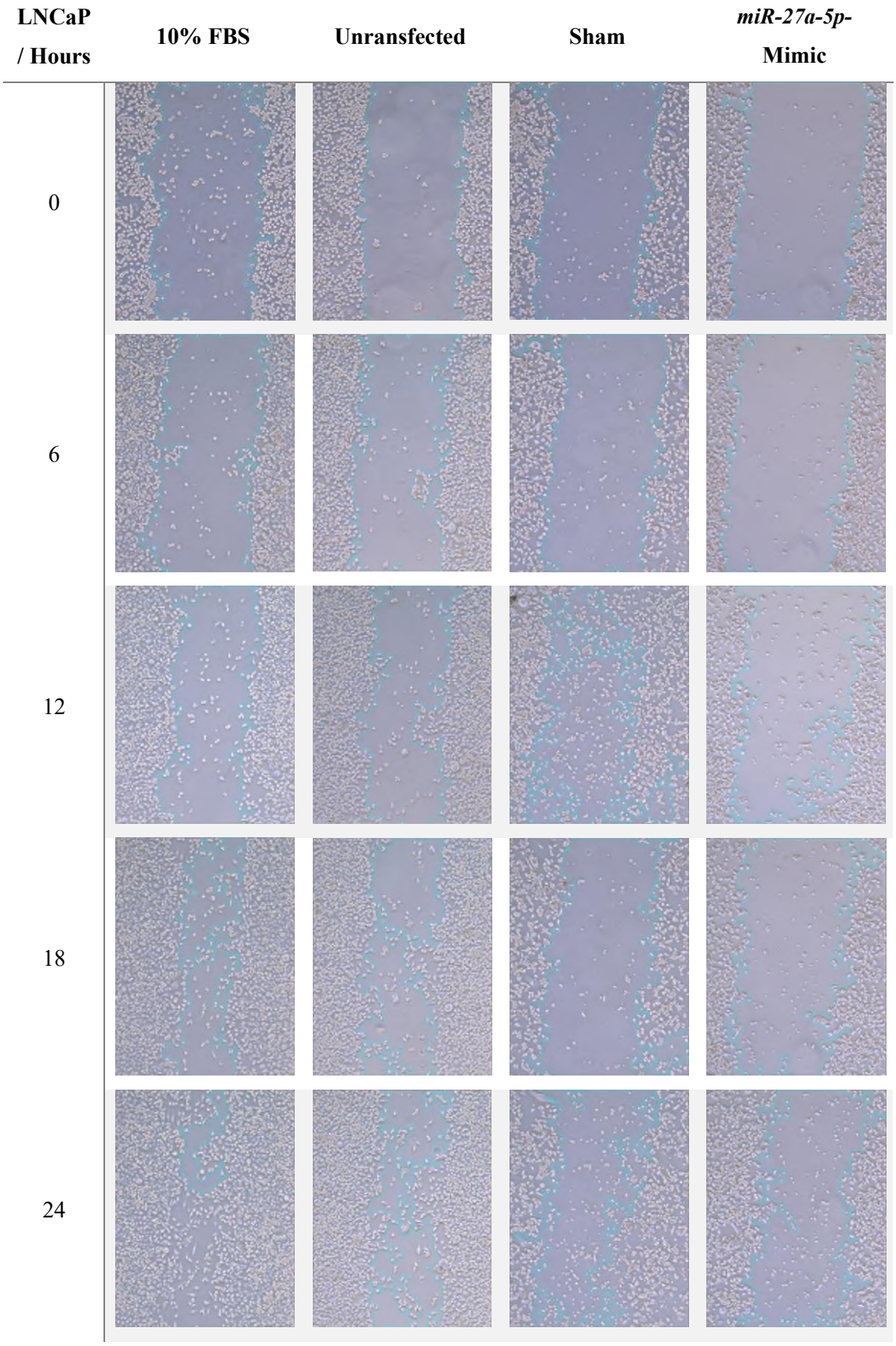


Figure 31. Representative images of LNCaP cells' migration in response to miR-27a-5p overexpression at 0-6-12-18-24 hours.

3.8. The Effect of miR-27a-5p on The Invasion of PCa Cell Lines

The invasion-suppressor potential of miR-27a-5p in PCa cell lines was evaluated with a transwell cell invasion assay. For this purpose, cells were seeded into the inserts in serum-starved media, containing an 8 μm pore size polycarbonate membrane with an ECMatrix and transfected with miR-27a-5p mimics and negative control oligonucleotides. The invaded cells were visualized, counted, and dissolved after 24 hours for quantification. It was found that miR-27a-5p overexpression was able to suppress invasion of both DU145 and LNCaP cells, compared to sham (Figure 33, A, and B).

After 24 hours, the invaded number of DU145 cells was significantly less in the mimic group (Cells/Visual Field = 1527.5, $p<0.05$) than in the sham group (Cells/Visual Field = 4868.5) (Figure 32, B). Also, the absorbance percentage of the dissolved cells was less in the mimic group than sham (Figure 32, A). Similarly, after 24 hours, there were significantly fewer invaded cells in the LNCaP mimic group (Cells/Visual Field = 7264.25, $p<0.01$) than in the sham group (Cells/Visual Field = 18254.4) (Figure 32, D). In accordance with this, the absorbance percentage of the dissolved cells was less in the mimic group (Figure 32, C). In addition, the invasion potential of LNCaP cells was observed to be greater than DU145 cells.

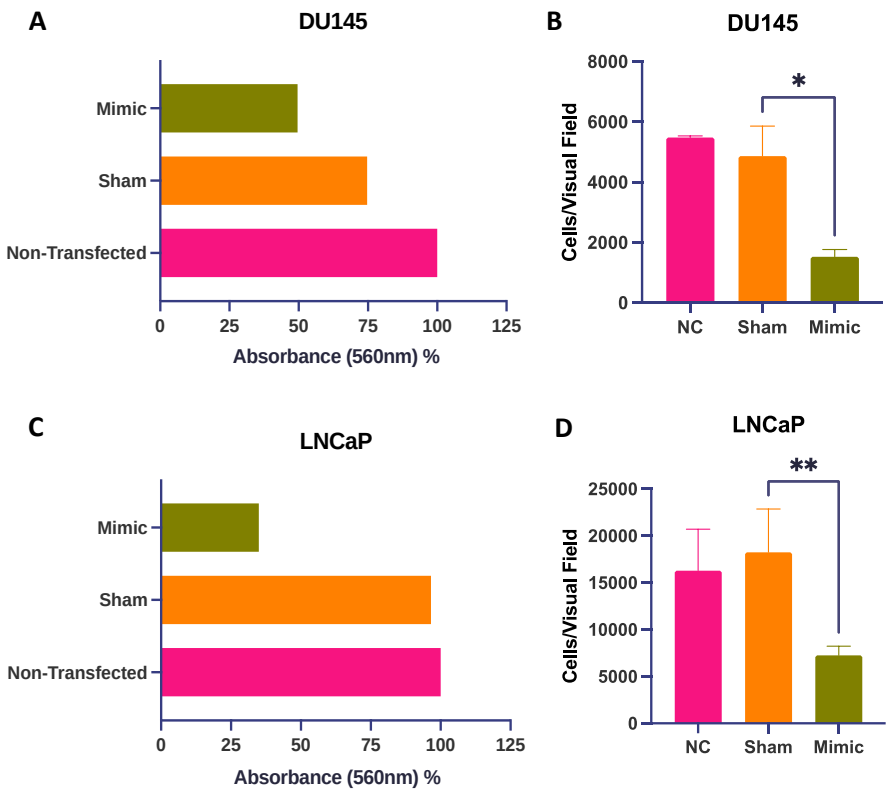


Figure 32. The effect of miR-27a-5p on the invasion of PCa cell lines. **A.** Absorbance of invaded DU145 cells; **B.** Invaded cell number of DU145 cells; **C.** Absorbance of invaded LNCaP cells; **D.** Invaded cell number of LNCaP cells. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, and ns-non significant.

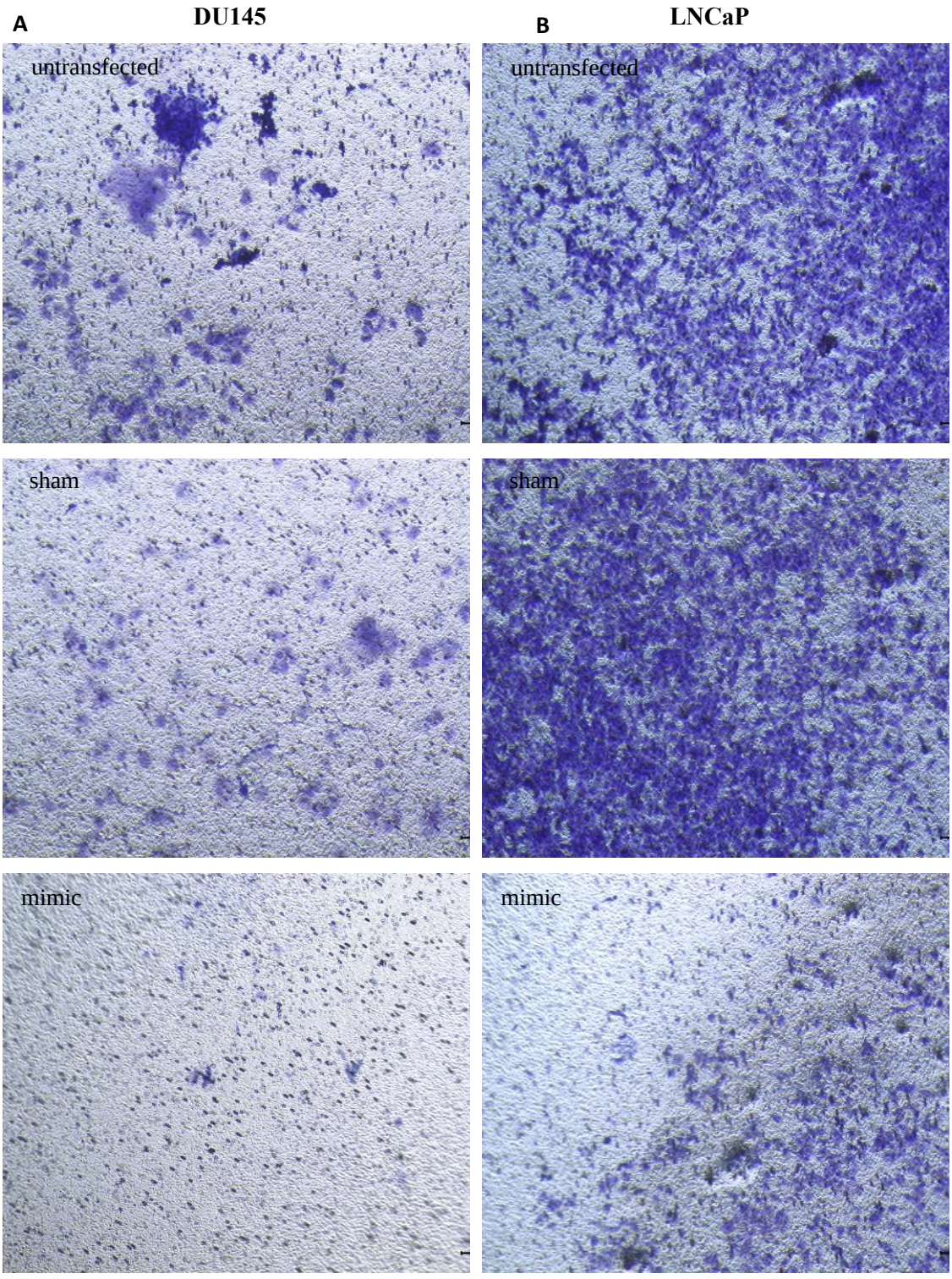


Figure 33. Representative images of PCa cells' invasion in response to transfection with miR-27a-5p mimic, and sham oligonucleotides. **A.** Invasion of DU145 cells; **B.** Invasion of LNCaP cells.

3.9. The Effect of miR-27a-5p on The Apoptosis of PCa Cell Lines

Annexin V-FITC/PI apoptosis assay was utilized under flow cytometry, to identify apoptotic and necrotic cells in response to miR-27a-5p overexpression. By Annexin V-FITC and PI staining analysis, induced miR-27a-5p overexpression resulted in an increased number of early and late apoptotic cells and also necrotic cells in both DU145 and LNCaP cell lines. The early and late apoptotic cell percentages in DU145 cells transfected with sham were 16.92% and 7.32%, respectively, whereas these apoptotic cell percentages increased to 18.17% and 11.88% in DU145 cells transfected with miR-27a-5p mimics (Figure 34, A, and B). Furthermore, apoptosis induction in response to miR-27a-5p overexpression was found to be greater in LNCaP cells; the early and late apoptotic cell percentages in LNCaP cells transfected with sham were 10.5% and 2.79%, respectively, while these apoptotic cell percentages increased to 17.93% and 9.13% with miR-27a-5p overexpression (Figure 34, C, and D).

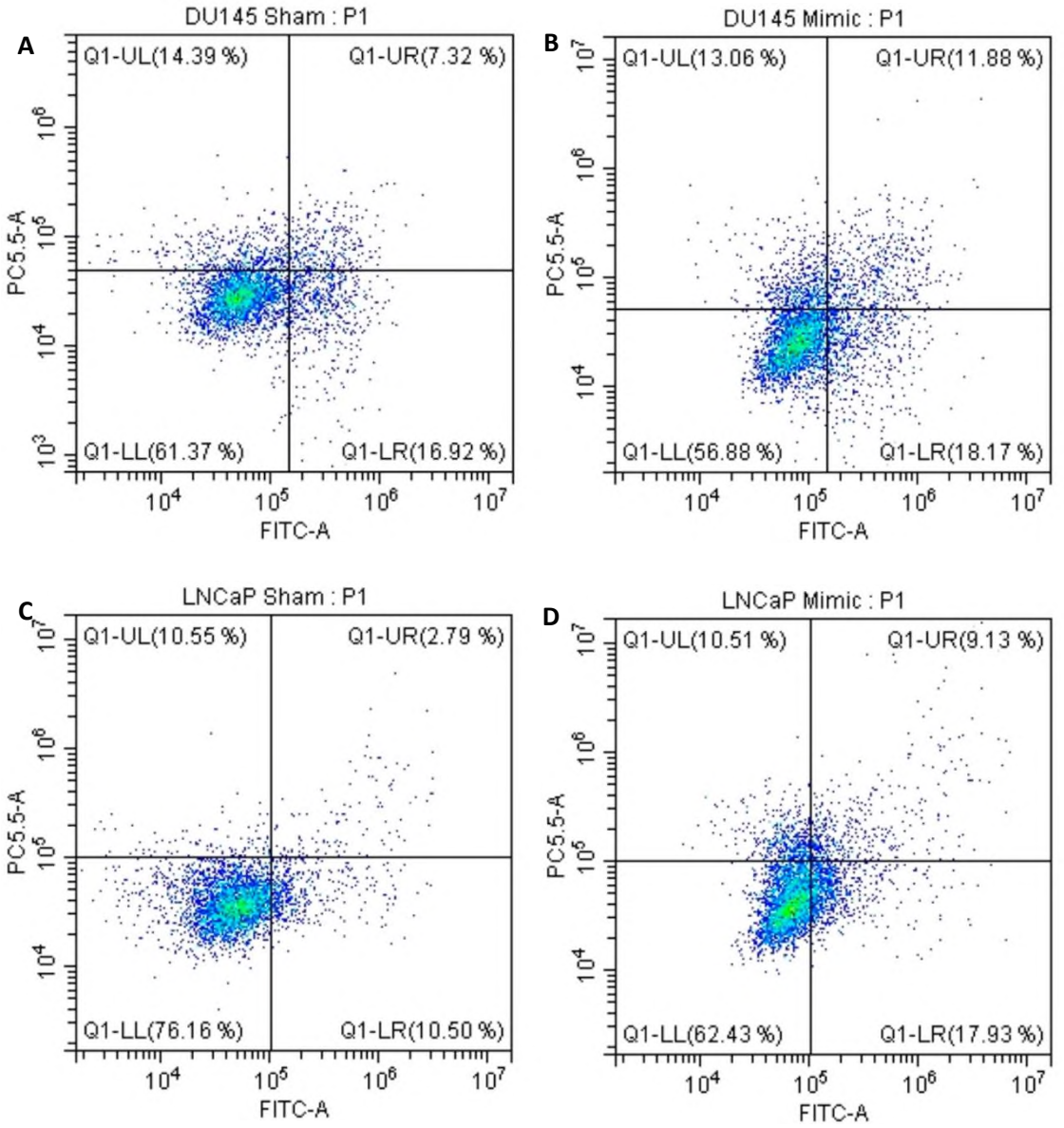


Figure 34. The effect of miR-27a-5p on the apoptosis of PCa cell lines; **A.** Apoptotic cells in DU145 cells transfected with sham oligonucleotides; **B.** Apoptotic cells in DU145 cells transfected with miR-27a-5p mimics; **C.** Apoptotic cells in LNCaP cells transfected with sham oligonucleotides; **D.** Apoptotic cells in LNCaP cells transfected with miR-27a-5p mimics.

4. DISCUSSION

In the present era, miRNAs have become promising therapeutic targets in cancer due to their participation in cellular processes that are crucial for tumor growth and metastasis to distant organs. The downregulation or upregulation of certain miRNAs favors the activation of oncogenes, leading to alterations in these pathways that result in proliferation, cell survival, migration, invasion, angiogenesis, and suppression of apoptosis. miR-27a family is one of the promising miRNA families with crucial roles in PCa progression. However, there is limited information on the functions of miR-27a-5p in PCa, in which most of these studies have been focused on the -3p strand. The expression of miR-27a, with its cluster miR-23a27a24-2, was shown to be regulated by AR itself both transcriptionally and post-transcriptionally, highlighting its significance in PCa (Fletcher et al., 2012). In this work, the -3p strand of miR-27a was identified as an oncomir. On the other hand, the tumor suppressive role of miR-27a-5p in PCa was stated in another study. Barros-Silva et al. (2018) showed that forced expression of miR-27a-5p suppressed malignant phenotype by inhibiting the proliferation of LNCaP and PC3 cells while promoting apoptosis (Barros-Silva et al., 2018). In addition, the potential target genes of the miRNA have also been given in the same study, in which they have identified binding sites for miR-27a-5p in EGFR, Akt1, and mTOR mRNAs *in silico*. Nevertheless, it is a fact that a single miRNA can directly target several mRNAs, thus, the genes and pathways that are directly regulated by miR-27a-5p still need to be fully elucidated. Therefore, in the framework of this thesis, target genes of miR-27a-5p were investigated and the pathways that might be potentially regulated by miR-27a-5p were revealed. For this purpose, potential target genes were predicted with *in silico* approaches, then the correlation between these predicted genes and miR-27a-5p was investigated *in vivo* and finally, functions of miR-27a-5p were determined *in vitro*.

The binding potential between miR-27a-5p and predicted target genes was evaluated by StarMir and TargetScan tools, with the consideration of literature knowledge that the seed sequence of miRNA should be perfectly complementary with the 3'UTR sequence of mRNAs. In this manner, the genes that showed seedless complementarity or that showed complementarity at the coding sequence or 5'UTR sequence were ignored. NPM1, CCR5,

STAT3, and BCL-2 mRNAs demonstrated the most probability of binding between the seed sequence of miR-27a-5p and 3'UTR sequences of them. According to the following pathway analyses, it was observed that STAT3 and BCL-2 are the downstream partners of CCL5/CCR5 chemokine signaling that is known to activate various pathways, such as PI3K/Akt/BCL-2, NF-kB, and JAK/STAT, playing roles in proliferation, apoptosis, and cell survival. In addition, CCR5, STAT3, and BCL-2 have been determined to play key roles in prostate cancer, especially in castration or drug resistance. Therefore, it was hypothesized that miR-27a-5p might act as a tumor suppressor in PCa by directly regulating components of the CCL5/CCR5 signaling pathway, STAT3, and BCL-2, suppressing proliferation, migration, and survival.

To support this hypothesized regulatory role of miR-27a-5p, which is established by *in silico* analyses, expression levels of miR-27a-5p and its predicted target genes were determined in tumor tissues from *in vivo* PCa models generated with transplantation of LNCaP or PC3 cells subcutaneously. According to histopathology examination of tumors, all malign PCa tumors showed high Gleason scores (5+5) which indicates a very abnormal cell look and that cancer cells tend to proliferate and metastasize. PC3 animals showed the most neoplastic characteristics as indicated by, the greatest volumes of tumors, and the significant decrease in body weight. AR expression level was found to be significantly upregulated in all LNCaP tumors. As a result, it can be concluded that subcutaneously generated tumors are prostatic neoplasms. Consistent with this, the expression level of miR-27a-5p was significantly decreased in all tumors. Since PC3 tumors showed a greater neoplastic characteristic, it was anticipated to have the lowest expression level of miR-27a-5p. However, no significant difference was determined in the expression of miR-27a-5p between LNCaP and PC3 tumors, even though its expression was found to be slightly decreased in PC3 tumors. It was interesting to demonstrate a significant inverse correlation between AR and miR-27a-5p. Herein, there might be two hypothetical alternative mechanisms; even though Fletcher et al. (2012) demonstrated the positive transcriptional regulation of miR-27a with its cluster by AR, AR might negatively regulate the -5p arm of miR-27a through post-transcriptional mechanisms; second of all, miR-27a-5p might be directly regulating AR, though, no

binding potential between seed sequence of miR-27a-5p and 3'UTR sequence of AR was observed *in silico* analyses (data not shown).

Expressions of predicted target genes were also determined in these tumors and both CCR5 and BCL-2 were found to be upregulated in subcutaneously generated tumors. It is evident from the gene expression data of tumors that miR-27a-5p was downregulated, highlighting its tumor suppressive function, while predicted target genes, CCR5 and BCL-2, were found to be overexpressed in these tumors. The Pearson correlation tests emphasized that there are significant inverse correlations between miR-27a-5p and predicted target genes CCR5, and BCL-2, as well as AR. These inverse correlations shown for CCR5, BCL-2, and AR indicate a direct regulatory function of miR-27a-5p in these oncogenic signaling pathways. However, gene expressions should be investigated in a larger sample size of *in vivo* experiments to confirm whether there is a significant negative regulation of CCR5, BCL-2, and AR by miR-27a-5p.

Expression levels of miR-27a-5p and its predicted target genes were determined in the PCa cell lines: PC3, DU145, and LNCaP. All PCa cell lines showed significantly decreased expression of miR-27a-5p, compared to RWPE-1 cells. As expected, STAT3 expression was found to be significantly upregulated in DU145 cells; PC3 cells were found to be negative for STAT3, as is clear in the literature. In terms of BCL-2, it is interesting to find downregulation in its expression level since it is an anti-apoptotic gene that has been demonstrated to play an oncogenic role in PCa. However, BCL-2 expression was found to be significantly upregulated in PC3 and LNCaP cells, compared to every other PCa cell line. Finally, CCR5 expression was detected in all PCa cell lines, though, it was only significantly upregulated in LNCaP cells. Overall, these expression profiling in various types of PCa cell lines underlines the inverse correlation between miR-27a-5p and predicted genes, CCR5, STAT3, and BCL-2 while also highlighting the potential tumor suppressive role of miR-27a-5p in PCa. Both *in vivo* and *in vitro* gene expression profiles underline the tumor suppressive potential of miR-27a-5p, as long as its potential to regulate the CCL5/CCR5 signaling pathway by directly targeting CCR5, STAT3, and BCL-2.

The molecular mechanism of miR-27a-5p in PCa and its association with predicted target genes were evaluated *in vitro*. Since DU145 cells were found to have overexpressed STAT3 levels and LNCaP cells showed expression of BCL-2, STAT3, and overexpression of CCR5, they were selected to investigate the proposed hypothesis further. For this purpose, PCa cells were transfected with either miR-27a-5p mimics or inhibitors, and transfection efficiency was determined with qRT-PCR, compared to the cells transfected with negative control oligonucleotides. We determined that in DU145 cells, when miR-27a-5p is overexpressed, STAT3 expression is decreased, whereas when miR-27a-5p is suppressed, STAT3 expression is increased. LNCaP cells also showed a similar response to the overexpression and suppression of miR-27a-5p, where miR-27a-5p mimics suppressed STAT3 mRNA levels. However, miR-27a-5p inhibitors resulted in non-significantly increased STAT3 levels, which might be due to the fact that the growth of LNCaP cells is not dependent on STAT3, while the growth of DU145 cells is STAT3-dependent. These results suggest a direct regulation of STAT3 mRNA by miR-27a-5p, however, it should be further confirmed with a luciferase assay. In a similar manner, BCL-2 mRNA level was also found to be reduced with miR-27a-5p overexpression in both DU145 and LNCaP cell lines, highlighting the regulatory correlation between miR-27a-5p and anti-apoptotic BCL-2, as it was also consistent with *in vivo*.

On the other hand, CCR5, which was determined to be the second most probable target gene of miR-27a-5p *in silico* and whose expression level was found to be inversely correlated with miR-27a-5p *in vivo*, did not show a negative regulation in response to miR-27a-5p. CCR5 mRNA level showed even a positive regulation in response to miR-27a-5p. In DU145 and LNCaP cells, when miR-27a-5p is overexpressed, the CCR5 level was determined to be increased, indicating a positive correlation between miR-27a-5p and CCR5. However, it was also interesting to determine that, in DU145 cells, when miR-27-5p was suppressed, the CCR5 level was also increased. This result emphasizes that the regulation of CCR5 might be independent of miR-27a-5p. The positive correlation between them might be due to the indirect regulatory mechanisms or a feedback loop that is caused by the tumor suppressive function of miR-27a-5p, in which CCR5 is enhanced to compensate for the inactivated growth-promoting and anti-apoptotic pathways, because CCR5 is known to activate various growth-promoting pathways, including

PI3K/Akt, MAPK, JAK/STAT3, and NF-κB (Aldinucci et al., 2020; Hermida et al., 2017; C. Y. Huang et al., 2009; Jiao et al., 2018).

In addition to above findings, the significant inverse correlation found between AR and miR-27a-5p in the tumors generated with the LNCaP cell line was confirmed *in vitro*. We demonstrated that LNCaP cells transfected with miR-27a-5p mimics had significantly reduced AR mRNA levels, while when the miRNA is suppressed, AR mRNA level was increased, emphasizing a negative regulation of AR by miR-27a-5p. A positive correlation between AR and miR-27a has been shown, in which AR induces the expression of miR-27a cluster in PCa and miR-27a-3p directly targets and suppresses PHB, a negative regulator of AR (Fletcher et al., 2012). On the other hand, for the first time, we showed that AR mRNA is suppressed in PCa by the -5p strand of the miR-27a, a miRNA with multifaceted functions involving both strands. Thus, it can be concluded that miR-27a-5p is not only a promising therapeutic candidate for primary PCa, but also CRPC and drug-resistant PCa, since the development of resistance in PCa involves the induction of AR-variants and current lack of effective inhbitors of these variants is the main issue today. The decrease found in the AR mRNA level by miR-27a-5p overexpression also suggests a potential for the suppression of AR-variants with this miRNA.

For the first time, we demonstrated that miR-27a-5p negatively regulates the genes that are identified to participate in the growth, migration, invasion, and apoptosis of PCa cell lines. Therefore, the function of miR-27a-5p in these cellular processes was also confirmed. Anti-proliferative effect of miR-27a-5p was determined with the cell viability assay and it was found that both DU145 and LNCaP cells transfected with miR-27a-5p mimics showed decreased cell viability. Consistent with the study of Barros-Silva et al. (2018), the viability of LNCaP cells was decreased when miR-27a-5p was overexpressed, while the viability of PC3 cells was increased by transfection with miR-27a-5p inhibitors. The viability of PC3 cells was not affected by miR-27a-5p overexpression, which is a conflicting result considering the study of X. Wu et al. (2013). They have found that miR-27a-5p overexpression resulted in decreased viability of PC3 cells, which was attributed to the downregulation in EGFR, Akt1, and mTOR signaling (X. Wu et al., 2013). However, in that study, EGFR, Akt1, and mTOR signaling downregulation by miR-27a-

5p was not shown in PC3 cells, but in HNSCC cells. Regarding our hypothesis that is inhibiting cell viability through negative regulation of STAT3, BCL-2, and AR, and considering that PC3 cells are negative for STAT3 and AR, it was anticipated that miR-27a-5p would not have any anti-proliferative effect on PC3 cell line. Furthermore, this result also suggests that the anti-proliferative effect of miR-27a-5p might be dependent on STAT3, which is expressed in both DU145 and LNCaP cells and was suppressed in response to miR-27a-5p overexpression.

STAT3 signaling is activated by growth factors and cytokines and upon phosphorylation, STAT3 is dimerized, translocating into the nucleus to induce the expression of genes that regulate cell death, apoptosis, migration, and invasion (Sadrkhanloo et al., 2023). STAT3 is not only crucial to promote the proliferation of cancer cells, but it is also crucial to sustain cell survival mechanisms and migration. Therefore, targeting STAT3 signaling has been attracting attention to suppress cancer progression. Due to the role of STAT3 in promoting migration and invasion of the cancer cells, we have investigated the function of miR-27a-5p, which targets and suppresses STAT3 mRNA, in these processes. For the first time, we demonstrated that miR-27a-5p inhibits migration and invasion of DU145 and LNCaP cells. However, we did not reveal that this effect of miR-27a-5p in repression migration and invasion of PCa cells is STAT3 dependent or independent. Yet, we demonstrated its anti-proliferative effects in two STAT3-positive cell lines, DU145 and LNCaP, whereas it did not show any antiproliferative effect in the STAT3-negative cell line, PC3. Therefore, this supports the idea that the tumor suppressor function of miR-27a-5p is STAT3 dependent. On the other hand, overexpression of miR-27a-5p in DU145 cells resulted in a 42% and 68% decrease in migration and invasion, respectively, while in LNCaP cells, there were 50% and 60% decreases in migration and invasion respectively. We have shown that even though STAT3 expression is present in LNCaP cells, it is not overexpressed. Thus, the growth or migration of these cells is not only dependent on STAT3. The finding that miR-27a-5p is nearly equally effective in suppressing migration and invasion of DU145 and LNCaP cells might be attributed to the evidence of AR suppression by miR-27a-5p. AR acts as a transcription factor and it has been shown to induce the expression of genes that play crucial roles in PCa metastasis (Imamura & Sadar, 2016; Ko et al., 2015). Regarding this knowledge and consistent with the

effect of miR-27a-5p in cell viability, it can be concluded that miR-27a-5p suppresses migration and invasion of PCa cell lines in an AR-dependent manner, in addition to STAT3. Therefore, it can be concluded that the function of miR-27a-5p is context-dependent, where it can function as a tumor suppressor in the presence of STAT3 or AR expression.

BCL-2 family proteins mediate mitochondrial apoptosis and anti-apoptotic BCL-2 has been shown to be upregulated in PCa patients, preventing apoptosis, and promoting cell survival. Enhanced levels of BCL-2 in PCa patients have been demonstrated to be correlated with aggressive disease formation, metastasis, resistance to therapies, and high Gleason score (Pilling et al., 2019; Soliman et al., 2021). Well-documented oncogenic signaling pathway PI3K/Akt suppresses the phosphorylation of BAD, which is a negative regulator of BCL-2, and activated BCL-2 represses BH3-only proteins, inhibiting activation of BAX and BAK to prevent cells from undergoing apoptosis. Our target gene expression findings signify a regulatory role for miR-27a-5p in apoptosis through suppressing anti-apoptotic BCL-2 mRNA. Thereby, we assessed the apoptotic response of the PCa cell lines to miR-27a-5p overexpression. We demonstrated that both early and late apoptotic cell percentages are increasing with miR-27a-5p upregulation in both DU145 and LNCaP cells. Nevertheless, the early apoptotic cell percentage of DU145 cells transfected with miR-27a-5p mimics slightly increased. Whereas, late apoptotic cell percentage increased by a considerable amount in the DU145 cell line. In contrast to DU145 cells, miR-27a-5p upregulation was found to be more successful at increasing early and late apoptotic cells in LNCaP cells. Similar to DU145 cells, the increase in the late apoptotic cell percentage was greater in also LNCaP cells. This different apoptotic response of two PCa cell lines might be due to AR status, in which the growth and survival of LNCaP cells are dependent on AR. AR has been identified to regulate intrinsic apoptosis pathways by the repression of apoptotic genes. AR has been determined to inhibit apoptosis in LNCaP cells through enhancing anti-apoptotic p21 mRNA and protein levels (S. Lu et al., 1999). In addition, AR signaling has also been shown to suppress pro-apoptotic BAD by activating MAPK and enhancing the phosphorylation of ERK-1/2, leading to apoptosis inhibition (Nguyen et al., 2005; Simões et al., 2013). Therefore, it was anticipated to determine a higher apoptotic response in AR-positive LNCaP cells,

compared to AR-negative DU145 cells. Furthermore, this higher apoptotic response in LNCaP cells might also be due to the BCL-2 expression level, where LNCaP cells were found to have significantly higher BCL-2 levels compared to DU145 cells.

In conclusion, according to the findings of this study, miR-27a-5p acts as a tumor-suppressor miRNA in PCa through negatively regulating STAT3, BCL-2, and AR. We highlighted that miR-27a-5p suppresses proliferation, migration, and invasion, while promoting apoptosis of PCa cells, which underlines its potential to be used as a novel miRNA therapeutic in primary PCa. Besides primary PCa, it also has the potential to inhibit the growth of CRPC and drug-resistant PCa due to its AR-suppressive function. The molecular mechanism of miR-27a-5p in PCa was revealed in this study, however, its function in resistant and more aggressive forms of PCa should be further clarified.

5. CONCLUSION

To conclude, we revealed the molecular mechanism of miR-27a-5p with its target genes and its context-dependent tumor-suppressor function in PCa. It was demonstrated that STAT3, BCL-2, and AR are negatively regulated by miR-27a-5p. We identified binding regions for miR-27a-5p in 3' UTR regions of STAT3 and BCL-2 mRNAs *in silico* and the direct targeting potential was further confirmed *in vitro* by qRT-PCR. However, it should also be clarified with luciferase assay that STAT3 and BCL-2 are the direct targets of miR-27a-5p. In addition, the regulatory correlation between miR-27a-5p and AR should be studied extensively. We could not identify a binding region for miR-27a-5p in the 3' UTR region of AR *in silico*, whereas, we showed that AR mRNA is negatively regulated by miR-27a-5p. It should be clarified whether this miRNA directly targets AR mRNA, as well as its binding regions. If AR is a direct target of miR-27a-5p, then this miRNA becomes an important therapeutic not only for primary PCa but also CRPC and drug-resistant PCa. In addition, the effect of miR-27a-5p should also be investigated on AR-variants in the future, because since it negatively regulates AR, it has a high potential in regulating variants.

Ultimately, a tumor-suppressive function was demonstrated for miR-27a-5p by suppressing STAT3, BCL-2, and AR in PCa. We determined that miR-27a-5p suppresses 3 out of the 6 hallmarks of PCa; proliferation, migration, and invasion, and resisting cell death. We underline the therapeutic importance of miR-27a-5p in PCa, while shedding light on the molecular mechanisms behind this tumor-suppressor function. This comprehensive *in silico*, *in vitro*, and *in vivo* work provides a promising therapeutic candidate in PCa with a precise molecular basis.

6. REFERENCES

Abate-Shen, C., Shen, M. M., & Gelmann, E. (2008). Integrating differentiation and cancer: The Nkx3.1 homeobox gene in prostate organogenesis and carcinogenesis. *Differentiation*, *76*(6), 717–727. https://doi.org/10.1111/J.1432-0436.2008.00292.X

Abeshouse, A., Ahn, J., Akbani, R., Ally, A., Amin, S., Andry, C. D., Annala, M., Aprikian, A., Armenia, J., Arora, A., Auman, J. T., Balasundaram, M., Balu, S., Barbieri, C. E., Bauer, T., Benz, C. C., Bergeron, A., Beroukhim, R., Berrios, M., … Zmuda, E. (2015a). The Molecular Taxonomy of Primary Prostate Cancer. *Cell*, *163*(4), 1011–1025. https://doi.org/10.1016/J.CELL.2015.10.025

Abeshouse, A., Ahn, J., Akbani, R., Ally, A., Amin, S., Andry, C. D., Annala, M., Aprikian, A., Armenia, J., Arora, A., Auman, J. T., Balasundaram, M., Balu, S., Barbieri, C. E., Bauer, T., Benz, C. C., Bergeron, A., Beroukhim, R., Berrios, M., … Zmuda, E. (2015b). The Molecular Taxonomy of Primary Prostate Cancer. *Cell*, *163*(4), 1011–1025. https://doi.org/10.1016/J.CELL.2015.10.025

Abeshouse, A., Ahn, J., Akbani, R., Ally, A., Amin, S., Andry, C. D., Annala, M., Aprikian, A., Armenia, J., Arora, A., Auman, J. T., Balasundaram, M., Balu, S., Barbieri, C. E., Bauer, T., Benz, C. C., Bergeron, A., Beroukhim, R., Berrios, M., … Zmuda, E. (2015c). The molecular taxonomy of primary prostate cancer. *Cell*, *163*(4), 1011. https://doi.org/10.1016/J.CELL.2015.10.025

Abid, S., Marcos, E., Parpaleix, A., Amsellem, V., Breau, M., Houssaini, A., Vienney, N., Lefevre, M., Derumeaux, G., Evans, S., Hubeau, C., Delcroix, M., Quarck, R., Adnot, S., & Lipskaia, L. (2019). CCR2/CCR5-mediated macrophage–smooth muscle cell crosstalk in pulmonary hypertension. *European Respiratory Journal*, *54*(4), 1802308. https://doi.org/10.1183/13993003.02308-2018

Agoulnik, I. U., & Weigel, N. L. (2006). Androgen receptor action in hormone-dependent and recurrent prostate cancer. *Journal of Cellular Biochemistry*, *99*(2), 362–372. https://doi.org/10.1002/JCB.20811

Aigner, A., Renneberg, H., Bojunga, J., Apel, J., Nelson, P. S., & Czubayko, F. (2002). Ribozyme-targeting of a secreted FGF-binding protein (FGF-BP) inhibits proliferation of prostate cancer cells in vitro and in vivo. *Oncogene*, *21*(37), 5733–5742. https://doi.org/10.1038/SJ.ONC.1205560

Aldinucci, D., Borghese, C., & Casagrande, N. (2020a). The CCL5/CCR5 Axis in Cancer Progression. *Cancers 2020, Vol. 12, Page 1765*, *12*(7), 1765. https://doi.org/10.3390/CANCERS12071765

Aldinucci, D., Borghese, C., & Casagrande, N. (2020b). The CCL5/CCR5 Axis in Cancer Progression. *Cancers*, *12*(7), 1–30. https://doi.org/10.3390/CANCERS12071765

Aldinucci, D., & Colombatti, A. (2014). The inflammatory chemokine CCL5 and cancer progression. *Mediators of Inflammation*, *2014*. https://doi.org/10.1155/2014/292376

Aldinucci, D., Gloghini, A., Pinto, A., Colombatti, A., & Carbone, A. (2012). The role of CD40/CD40L and interferon regulatory factor 4 in Hodgkin lymphoma microenvironment. *Leukemia & Lymphoma*, *53*(2), 195–201. https://doi.org/10.3109/10428194.2011.605190

Alimirah, F., Chen, J., Basrawala, Z., Xin, H., & Choubey, D. (2006). DU-145 and PC-3 human prostate cancer cell lines express androgen receptor: Implications for the androgen receptor functions and regulation. *FEBS Letters*, *580*(9), 2294–2300. https://doi.org/10.1016/J.FEBSLET.2006.03.041

Ambros, V., Lee, R. C., Lavanway, A., Williams, P. T., & Jewell, D. (2003). MicroRNAs and Other Tiny Endogenous RNAs in C. elegans. *Current Biology*, *13*(10), 807–818. https://doi.org/10.1016/S0960-9822(03)00287-2

Anker, J. F., Mok, H., Naseem, A. F., Thumbikat, P., & Abdulkadir, S. A. (2018). A Bioluminescent and Fluorescent Orthotopic Syngeneic Murine Model of Androgen-dependent and Castration-resistant Prostate Cancer. *JoVE (Journal of Visualized Experiments)*, *2018*(133), e57301. https://doi.org/10.3791/57301

Antonarakis, E. S., Keizman, D., Zhang, Z., Gurel, B., Lotan, T. L., Hicks, J. L., Fedor, H. L., Carducci, M. A., De Marzo, A. M., & Eisenberger, M. A. (2012). An immunohistochemical signature comprising PTEN, MYC, and Ki67 predicts progression in prostate cancer patients receiving adjuvant docetaxel after prostatectomy. *Cancer*, *118*(24), 6063–6071. https://doi.org/10.1002/CNCR.27689

Appay, V., & Rowland-Jones, S. L. (2001a). RANTES: a versatile and controversial chemokine. *Trends in Immunology*, *22*(2), 83–87. https://doi.org/10.1016/S1471-4906(00)01812-3

Appay, V., & Rowland-Jones, S. L. (2001b). RANTES: a versatile and controversial chemokine. *Trends in Immunology*, *22*(2), 83–87. https://doi.org/10.1016/S1471-4906(00)01812-3

Aurilio, G., Cimadamore, A., Mazzucchelli, R., Lopez-Beltran, A., Verri, E., Scarpelli, M., Massari, F., Cheng, L., Santoni, M., & Montironi, R. (2020). Androgen Receptor Signaling Pathway in Prostate Cancer: From Genetics to Clinical Applications. *Cells*, *9*(12). https://doi.org/10.3390/CELLS9122653

Aytes, A., Mitrofanova, A., Lefebvre, C., Alvarez, M. J., Castillo-Martin, M., Zheng, T., Eastham, J. A., Gopalan, A., Pienta, K. J., Shen, M. M., Califano, A., & Abate-Shen, C. (2014). Cross-species regulatory network analysis identifies a synergistic interaction between FOXM1 and CENPF that drives prostate cancer malignancy. *Cancer Cell*, *25*(5), 638–651. https://doi.org/10.1016/J.CCR.2014.03.017

Barfeld, S. J., Urbanucci, A., Itkonen, H. M., Fazli, L., Hicks, J. L., Thiede, B., Rennie, P. S., Yegnasubramanian, S., DeMarzo, A. M., & Mills, I. G. (2017). c-Myc Antagonises the Transcriptional Activity of the Androgen Receptor in Prostate Cancer Affecting Key Gene Networks. *EBioMedicine*, *18*, 83–93. https://doi.org/10.1016/J.EBIOM.2017.04.006

Barros-Silva, D., Costa-Pinheiro, P., Duarte, H., Sousa, E. J., Evangelista, A. F., Graça, I., Carneiro, I., Martins, A. T., Oliveira, J., Carvalho, A. L., Marques, M. M., Henrique, R., & Jerónimo, C. (2018). MicroRNA-27a-5p regulation by promoter methylation and MYC signaling in prostate carcinogenesis. *Cell Death & Disease 2018 9:2*, *9*(2), 1–15. https://doi.org/10.1038/s41419-017-0241-y

Bartel, D. P. (2004). MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. *Cell*, *116*(2), 281–297. https://doi.org/10.1016/S0092-8674(04)00045-5

Bartel, D. P. (2009). MicroRNAs: Target Recognition and Regulatory Functions. *Cell*, *136*(2), 215–233. https://doi.org/10.1016/J.CELL.2009.01.002

Baskar, R., Lee, K. A., Yeo, R., & Yeoh, K. W. (2012). Cancer and Radiation Therapy: Current Advances and Future Directions. *International Journal of Medical Sciences*, *9*(3), 193–199. https://doi.org/10.7150/IJMS.3635

Beaulieu, M. E., Castillo, F., & Soucek, L. (2020). Structural and Biophysical Insights into the Function of the Intrinsically Disordered Myc Oncoprotein. *Cells 2020, Vol. 9, Page 1038*, *9*(4), 1038. https://doi.org/10.3390/CELLS9041038

Berman-Booty, L. D., & Knudsen, K. E. (2015). Models of neuroendocrine prostate cancer. *Endocrine-Related Cancer*, *22*(1), R33–R49. https://doi.org/10.1530/ERC-14-0393

Bernardes, J. G. B., Fernandes, M. R., Rodrigues, J. C. G., Vinagre, L. W. M. S., Pastana, L. F., Dobbin, E. A. F., Medeiros, J. A. G., Junior, L. B. D., Bernardes, G. M., Bernardes, I. M. M., Dos Santos, N. P. C., Demachki, S., & Burbano, R. M. R. (2022). Association of Androgenic Regulation and MicroRNAs in Acinar Adenocarcinoma of Prostate. *Genes*, *13*(4), 622. https://doi.org/10.3390/GENES13040622/S1

Bhartiya, D., & Singh, J. (2015). FSH–FSHR3–stem cells in ovary surface epithelium: basis for adult ovarian biology, failure, aging, and cancer. *Reproduction*, *149*(1), R35–R48. https://doi.org/10.1530/REP-14-0220

Bohrer, L. R., Liu, P., Zhong, J., Pan, Y., Angstman, J., Brand, L. J., Dehm, S. M., & Huang, H. (2013). FOXO1 binds to the TAU5 motif and inhibits constitutively active androgen receptor splice variants. *The Prostate*, *73*(10), 1017–1027. https://doi.org/10.1002/PROS.22649

Buck, A. H., Perot, J., Chisholm, M. A., Kumar, D. S., Tuddenham, L., Cognat, V., Marcinowski, L., Dölken, L., & Pfeffer, S. (2010). Post-transcriptional regulation

of miR-27 in murine cytomegalovirus infection. *RNA*, *16*(2), 307. https://doi.org/10.1261/RNA.1819210

Cai, C., Chen, S., Ng, P., Bubley, G. J., Nelson, P. S., Mostaghel, E. A., Marck, B., Matsumoto, A. M., Simon, N. I., Wang, H., Chen, S., & Balk, S. P. (2011). Intratumoral De Novo steroid synthesis activates androgen receptor in castration-resistant prostate cancer and is upregulated by treatment with CYP17A1 inhibitors. *Cancer Research*, *71*(20), 6503–6513. https://doi.org/10.1158/0008-5472.CAN-11-0532

Cambien, B., Richard-Fiardo, P., Karimdjee, B. F., Martini, V., Ferrua, B., Pitard, B., Schmid-Antomarchi, H., & Schmid-Alliana, A. (2011). CCL5 neutralization restricts cancer growth and potentiates the targeting of PDGFRβ in colorectal carcinoma. *PloS One*, *6*(12). https://doi.org/10.1371/JOURNAL.PONE.0028842

Cannistraci, A., Di Pace, A. L., De Maria, R., & Bonci, D. (2014). MicroRNA as New Tools for Prostate Cancer Risk Assessment and Therapeutic Intervention: Results from Clinical Data Set and Patients' Samples. *BioMed Research International*, *2014*. https://doi.org/10.1155/2014/146170

Cao, Z., Xu, L., & Zhao, S. (2019). Exosome-derived miR-27a produced by PSC-27 cells contributes to prostate cancer chemoresistance through p53. *Biochemical and Biophysical Research Communications*, *515*(2), 345–351. https://doi.org/10.1016/J.BBRC.2019.05.120

Carey, A. M., Pramanik, R., Nicholson, L. J., Dew, T. K., Martin, F. L., Muir, G. H., & Morris, J. D. H. (2007). Ras-MEK-ERK signaling cascade regulates androgen receptor element-inducible gene transcription and DNA synthesis in prostate cancer cells. *International Journal of Cancer*, *121*(3), 520–527. https://doi.org/10.1002/IJC.22715

Carroll, P. A., Freie, B. W., Mathsyaraja, H., & Eisenman, R. N. (2018a). The MYC transcription factor network: balancing metabolism, proliferation and oncogenesis. *Frontiers of Medicine 2018 12:4*, *12*(4), 412–425. https://doi.org/10.1007/S11684-018-0650-Z

Carroll, P. A., Freie, B. W., Mathsyaraja, H., & Eisenman, R. N. (2018b). The MYC transcription factor network: balancing metabolism, proliferation and oncogenesis. *Frontiers of Medicine 2018 12:4*, *12*(4), 412–425. https://doi.org/10.1007/S11684-018-0650-Z

Carver, B. S., Chapinski, C., Wongvipat, J., Hieronymus, H., Chen, Y., Chandarlapaty, S., Arora, V. K., Le, C., Koutcher, J., Scher, H., Scardino, P. T., Rosen, N., & Sawyers, C. L. (2011). Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. *Cancer Cell*, *19*(5), 575–586. https://doi.org/10.1016/J.CCR.2011.04.008

Casagrande, N., Borghese, C., Visser, L., Mongiat, M., Colombatti, A., & Aldinucci, D. (2019). CCR5 antagonism by maraviroc inhibits Hodgkin lymphoma

microenvironment interactions and xenograft growth. *Haematologica*, *104*(3), 564–575. https://doi.org/10.3324/HAEMATOL.2018.196725

Castro, E., Jugurnauth-Little, S., Karlsson, Q., Al-Shahrour, F., Piñeiro-Yañez, E., Van de Poll, F., Leongamornlert, D., Dadaev, T., Govindasami, K., Guy, M., Eeles, R., & Kote-Jarai, Z. (2015). High burden of copy number alterations and c-MYC amplification in prostate cancer from BRCA2 germline mutation carriers. *Annals of Oncology*, *26*(11), 2293–2300. https://doi.org/10.1093/ANNONC/MDV356

Chae, D. K., Ban, E., Yoo, Y. S., Kim, E. E. K., Baik, J. H., & Song, E. J. (2017). MIR-27a regulates the TGF-β signaling pathway by targeting SMAD2 and SMAD4 in lung cancer. *Molecular Carcinogenesis*, *56*(8), 1992–1998. https://doi.org/10.1002/MC.22655

Chai, H., Liu, M., Tian, R., Li, X., & Tang, H. (2011). miR-20a targets BNIP2 and contributes chemotherapeutic resistance in colorectal adenocarcinoma SW480 and SW620 cell lines. *Acta Biochimica et Biophysica Sinica*, *43*(3), 217–225. https://doi.org/10.1093/ABBS/GMQ125

Chan, S. C., Li, Y., & Dehm, S. M. (2012). Androgen Receptor Splice Variants Activate Androgen Receptor Target Genes and Support Aberrant Prostate Cancer Cell Growth Independent of Canonical Androgen Receptor Nuclear Localization Signal. *Journal of Biological Chemistry*, *287*(23), 19736–19749. https://doi.org/10.1074/JBC.M112.352930

Chao, O. S. P., & Clément, M. V. (2006). Epidermal growth factor and serum activate distinct pathways to inhibit the BH3 only protein BAD in prostate carcinoma LNCaP cells. *Oncogene 2006 25:32*, *25*(32), 4458–4469. https://doi.org/10.1038/sj.onc.1209421

Chari, N. S., Ivan, C., Le, X., Li, J., Mijiti, A., Patel, A. A., Osman, A. A., Peterson, C. B., Williams, M. D., Pickering, C. R., Caulin, C., Myers, J. N., Calin, G. A., & Lai, S. Y. (2020). Disruption of TP63-miR-27a* Feedback Loop by Mutant TP53 in Head and Neck Cancer. *JNCI: Journal of the National Cancer Institute*, *112*(3), 266–277. https://doi.org/10.1093/JNCI/DJZ097

Chen, C.-Z. (2005). MicroRNAs as oncogenes and tumor suppressors. *The New England Journal of Medicine*, *353*(17), 1768–1771. https://doi.org/10.1056/NEJMP058190

Chen, W., Tan, X., Yang, Q., Fang, Z., & Xu, Y. (2022). MALAT1 enhances gemcitabine resistance in non-small cell lung cancer cells by directly affecting miR-27a-5p/PBOV1 axis. *Cellular Signalling*, *94*, 110326. https://doi.org/10.1016/J.CELLSIG.2022.110326

Chen, Y., Fu, L. L., Wen, X., Liu, B., Huang, J., Wang, J. H., & Wei, Y. Q. (2014). Oncogenic and tumor suppressive roles of microRNAs in apoptosis and autophagy. *Apoptosis 2014 19:8*, *19*(8), 1177–1189. https://doi.org/10.1007/S10495-014-0999-7

Chhabra, R., Dubey, R., & Saini, N. (2010). Cooperative and individualistic functions of the microRNAs in the miR-23a~27a~24-2 cluster and its implication in human diseases. *Molecular Cancer*, *9*, 232. https://doi.org/10.1186/1476-4598-9-232

Chiang, H. R., Schoenfeld, L. W., Ruby, J. G., Auyeung, V. C., Spies, N., Baek, D., Johnston, W. K., Russ, C., Luo, S., Babiarz, J. E., Blelloch, R., Schroth, G. P., Nusbaum, C., & Bartel, D. P. (2010). Mammalian microRNAs: experimental evaluation of novel and previously annotated genes. *Genes & Development*, *24*(10), 992–1009. https://doi.org/10.1101/GAD.1884710

Choo, R., Klotz, L., Danjoux, C., Morton, G. C., Deboer, G., Szumacher, E., Fleshner, N., Bunting, P., & Hruby, G. (2002). Feasibility Study: Watchful Waiting For Localized Low To Intermediate Grade Prostate Carcinoma With Selective Delayed Intervention Based On Prostate Specific Antigen, Histological And/Or Clinical Progression. *The Journal of Urology*, *167*(4 I), 1664–1669. https://doi.org/10.1016/S0022-5347(05)65174-9

Chow, M. T., & Luster, A. D. (2014). Chemokines in Cancer. *Cancer Immunology Research*, *2*(12), 1125. https://doi.org/10.1158/2326-6066.CIR-14-0160

Coleman, D. J., Gao, L., Schwartzman, J., Korkola, J. E., Sampson, D., Derrick, D. S., Urrutia, J., Balter, A., Burchard, J., King, C. J., Chiotti, K. E., Heiser, L. M., & Alumkal, J. J. (2019). Maintenance of MYC expression promotes de novo resistance to BET bromodomain inhibition in castration-resistant prostate cancer. *Scientific Reports 2019 9:1*, *9*(1), 1–9. https://doi.org/10.1038/s41598-019-40518-5

Collin, S. M. (2013). Folate and B12 in Prostate Cancer. *Advances in Clinical Chemistry*, *60*, 1–63. https://doi.org/10.1016/B978-0-12-407681-5.00001-5

Colombatti, M., Grasso, S., Porzia, A., Fracasso, G., Scupoli, M. T., Cingarlini, S., Poffe, O., Naim, H. Y., Heine, M., Tridente, G., Mainiero, F., Ramarli, D., & Hotchin, N. (2009). *The Prostate Specific Membrane Antigen Regulates the Expression of IL-6 and CCL5 in Prostate Tumour Cells by Activating the MAPK Pathways 1*. https://doi.org/10.1371/journal.pone.0004608

Cookson, M. S., Roth, B. J., Dahm, P., Engstrom, C., Freedland, S. J., Hussain, M., Lin, D. W., Lowrance, W. T., Murad, M. H., Oh, W. K., Penson, D. F., & Kibel, A. S. (2013). Castration-Resistant Prostate Cancer: AUA Guideline. *The Journal of Urology*, *190*(2), 429–438. https://doi.org/10.1016/J.JURO.2013.05.005

Corté, H., Manceau, G., Blons, H., & Laurent-Puig, P. (2012). MicroRNA and colorectal cancer. *Digestive and Liver Disease*, *44*(3), 195–200. https://doi.org/10.1016/J.DLD.2011.10.010

Crawford, E. D., Higano, C. S., Shore, N. D., Hussain, M., & Petrylak, D. P. (2015a). Treating Patients with Metastatic Castration Resistant Prostate Cancer: A Comprehensive Review of Available Therapies. *The Journal of Urology*, *194*(6), 1537–1547. https://doi.org/10.1016/J.JURO.2015.06.106

Crawford, E. D., Higano, C. S., Shore, N. D., Hussain, M., & Petrylak, D. P. (2015b). Treating Patients with Metastatic Castration Resistant Prostate Cancer: A Comprehensive Review of Available Therapies. *The Journal of Urology*, *194*(6), 1537–1547. https://doi.org/10.1016/J.JURO.2015.06.106

Cui, X., Piao, C., Lv, C., Lin, X., Zhang, Z., & Liu, X. (2020). ZNFX1 anti-sense RNA 1 promotes the tumorigenesis of prostate cancer by regulating c-Myc expression via a regulatory network of competing endogenous RNAs. *Cellular and Molecular Life Sciences*, *77*(6), 1135–1152. https://doi.org/10.1007/S00018-019-03226-X/FIGURES/8

Culig, Z., Hobisch, A., Cronauer, M. V., Radmayr, R., Bartsch, G., Klocker, H., Hittmair, A., & Trapman, J. (1994). Androgen Receptor Activation in Prostatic Tumor Cell Lines by Insulin-like Growth Factor-I, Keratinocyte Growth Factor, and Epidermal Growth Factor. *Cancer Research*, *54*(20), 5474–5478.

Czech, B., Zhou, R., Erlich, Y., Brennecke, J., Binari, R., Villalta, C., Gordon, A., Perrimon, N., & Hannon, G. J. (2009). Hierarchical Rules for Argonaute Loading in Drosophila. *Molecular Cell*, *36*(3), 445–456. https://doi.org/10.1016/j.molcel.2009.09.028

Dai, C., Heemers, H., & Sharifi, N. (2017). Androgen Signaling in Prostate Cancer. *Cold Spring Harbor Perspectives in Medicine*, *7*(9), a030452. https://doi.org/10.1101/CSHPERSPECT.A030452

Dang, C. V. (2012). MYC on the Path to Cancer. *Cell*, *149*(1), 22–35. https://doi.org/10.1016/J.CELL.2012.03.003

Dang, C. V. (2016a). A Time for MYC: Metabolism and Therapy. *Cold Spring Harbor Symposia on Quantitative Biology*, *81*(1), 79–83. https://doi.org/10.1101/SQB.2016.81.031153

Dang, C. V. (2016b). A Time for MYC: Metabolism and Therapy. *Cold Spring Harbor Symposia on Quantitative Biology*, *81*(1), 79–83. https://doi.org/10.1101/SQB.2016.81.031153

Dang, C. V., O'Donnell, K. A., Zeller, K. I., Nguyen, T., Osthus, R. C., & Li, F. (2006). The c-Myc target gene network. *Seminars in Cancer Biology*, *16*(4), 253–264. https://doi.org/10.1016/J.SEMCANCER.2006.07.014

Deng, S., Dai, G., Chen, S., Nie, Z., Zhou, J., Fang, H., & Peng, H. (2019). Dexamethasone induces osteoblast apoptosis through ROS-PI3K/AKT/GSK3β signaling pathway. *Biomedicine & Pharmacotherapy*, *110*, 602–608. https://doi.org/10.1016/J.BIOPHA.2018.11.103

Di Carlo, V., Grossi, E., Laneve, P., Morlando, M., Dini Modigliani, S., Ballarino, M., Bozzoni, I., & Caffarelli, E. (2013). TDP-43 regulates the microprocessor complex activity during in vitro neuronal differentiation. *Molecular Neurobiology*, *48*(3), 952–963. https://doi.org/10.1007/S12035-013-8564-X/FIGURES/7

Di Cristofano, A., Pesce, B., Cordon-Cardo, C., & Pandolfi, P. P. (1998). Pten is essential for embryonic development and tumour suppression. *Nature Genetics*, *19*(4), 348–355. https://doi.org/10.1038/1235

Ding, H., Zhao, L., Dai, S., Li, L., Wang, F., & Shan, B. (2016). CCL5 secreted by tumor associated macrophages may be a new target in treatment of gastric cancer. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *77*, 142–149. https://doi.org/10.1016/J.BIOPHA.2015.12.004

Ding, L., Zhang, S., Xu, M., Zhang, R., Sui, P., & Yang, Q. (2017). MicroRNA-27a contributes to the malignant behavior of gastric cancer cells by directly targeting PH domain and leucine-rich repeat protein phosphatase 2. *Journal of Experimental and Clinical Cancer Research*, *36*(1), 1–13. https://doi.org/10.1186/S13046-017-0516-2/FIGURES/6

Dolcet, X., Llobet, D., Pallares, J., & Matias-Guiu, X. (2005). NF-kB in development and progression of human cancer. *Virchows Archiv*, *446*(5), 475–482. https://doi.org/10.1007/S00428-005-1264-9/FIGURES/5

Dong, X. Y., Chen, C., Sun, X., Guo, P., Vessella, R. L., Wang, R. X., Chung, L. W. K., Zhou, W., & Dong, J. T. (2006). FOXO1A is a candidate for the 13q14 tumor suppressor gene inhibiting androgen receptor signaling in prostate cancer. *Cancer Research*, *66*(14), 6998–7006. https://doi.org/10.1158/0008-5472.CAN-06-0411

Duffy, M. J., O'Grady, S., Tang, M., & Crown, J. (2021a). MYC as a target for cancer treatment. *Cancer Treatment Reviews*, *94*, 102154. https://doi.org/10.1016/J.CTRV.2021.102154

Duffy, M. J., O'Grady, S., Tang, M., & Crown, J. (2021b). MYC as a target for cancer treatment. *Cancer Treatment Reviews*, *94*, 102154. https://doi.org/10.1016/J.CTRV.2021.102154

Edelstein, R. A., Carr, M. C., Caesar, R., Young, M., Atala, A., & Freeman, M. R. (1994). Detection of human androgen receptor mRNA expression abnormalities by competitive PCR. *DNA and Cell Biology*, *13*(3), 265–273. https://doi.org/10.1089/DNA.1994.13.265

Epstein, J. I. (2018). Prostate cancer grading: a decade after the 2005 modified system. *Modern Pathology*, *31*, 47–63. https://doi.org/10.1038/MODPATHOL.2017.133

Fang, F., Huang, B., Sun, S., Xiao, M., Guo, J., Yi, X., Cai, J., & Wang, Z. (2018). miR-27a inhibits cervical adenocarcinoma progression by downregulating the TGF-βRI signaling pathway. *Cell Death & Disease 2018 9:3*, *9*(3), 1–14. https://doi.org/10.1038/s41419-018-0431-2

Ferlay, J., Parkin, D. M., & Steliarova-Foucher, E. (2010). Estimates of cancer incidence and mortality in Europe in 2008. *European Journal of Cancer*, *46*(4), 765–781. https://doi.org/10.1016/J.EJCA.2009.12.014

Fernandez, N., Cordiner, R. A., Young, R. S., Hug, N., MacIas, S., & Cáceres, J. F. (2017). Genetic variation and RNA structure regulate microRNA biogenesis. *Nature Communications 2017 8:1*, *8*(1), 1–12. https://doi.org/10.1038/ncomms15114

Fernandez, P. C., Frank, S. R., Wang, L., Schroeder, M., Liu, S., Greene, J., Cocito, A., & Amati, B. (2003). Genomic targets of the human c-Myc protein. *Genes & Development*, *17*(9), 1115–1129. https://doi.org/10.1101/GAD.1067003

Fletcher, C. E., Dart, D. A., Sita-lumsden, A., Cheng, H., Rennie, P. S., & Bevan, C. L. (2012). Androgen-regulated processing of the oncomir MiR-27a, which targets Prohibitin in prostate cancer. *Human Molecular Genetics*, *21*(14), 3112–3127. https://doi.org/10.1093/HMG/DDS139

Gamble, S. C., Odontiadis, M., Waxman, J., Westbrook, J. A., Dunn, M. J., Wait, R., Lam, E. W. F., & Bevan, C. L. (2004). Androgens target prohibitin to regulate proliferation of prostate cancer cells. *Oncogene*, *23*(17), 2996–3004. https://doi.org/10.1038/SJ.ONC.1207444

Gao, W., Hong, Z., Huang, H., Zhu, A., Lin, S., Cheng, C., Zhang, X., Zou, G., & Shi, Z. (2018). miR-27a in serum acts as biomarker for prostate cancer detection and promotes cell proliferation by targeting Sprouty2. *Oncology Letters*, *16*(4), 5291. https://doi.org/10.3892/OL.2018.9274

Gavegnano, C., Savarino, A., Owanikoko, T., & Marconi, V. C. (2019a). Crossroads of Cancer and HIV-1: Pathways to a Cure for HIV. *Frontiers in Immunology*, *10*, 479342. https://doi.org/10.3389/FIMMU.2019.02267/BIBTEX

Gavegnano, C., Savarino, A., Owanikoko, T., & Marconi, V. C. (2019b). Crossroads of Cancer and HIV-1: Pathways to a Cure for HIV. *Frontiers in Immunology*, *10*, 2267. https://doi.org/10.3389/FIMMU.2019.02267/BIBTEX

Gelmann, E. P. (2016). Molecular Biology of the Androgen Receptor. *Https://Doi.Org/10.1200/JCO.2002.10.018*, *20*(13), 3001–3015. https://doi.org/10.1200/JCO.2002.10.018

Ghildiyal, M., Xu, J., Seitz, H., Weng, Z., & Zamore, P. D. (2010). Sorting of Drosophila small silencing RNAs partitions microRNA* strands into the RNA interference pathway. *RNA*, *16*(1), 43–56. https://doi.org/10.1261/RNA.1972910

Gingrich, J. R., Barrios, R. J., Kattan, M. W., Nahm, H. S., Finegold, M. J., & Greenberg, N. M. (1997). Androgen-independent prostate cancer progression in the TRAMP model. *Cancer Research*, *57*(21), 4687–4691.

Gleason, D. F. (1992). Histologic grading of prostate cancer: A perspective. *Human Pathology*, *23*(3), 273–279. https://doi.org/10.1016/0046-8177(92)90108-F

Global Cancer Observatory. (n.d.). Retrieved March 4, 2024, from https://gco.iarc.fr/en

González-Prieto, R., Cuijpers, S. A., Kumar, R., Hendriks, I. A., Co Vertegaal, A., & An Gonz Alez-Prieto, R. (2020). Structural and Biophysical Insights into the Function of the Intrinsically Disordered Myc Oncoprotein. *Cells 2020, Vol. 9, Page 1038*, *9*(4), 1038. https://doi.org/10.3390/CELLS9041038

Grad, J. M., Zeng, X. R., & Boise, L. H. (2000). Regulation of Bcl-xL: a little bit of this and a little bit of STAT. *Current Opinion in Oncology*, *12*(6), 543–549. https://doi.org/10.1097/00001622-200011000-00006

Greenberg, N. M., Demayo, F., Finegold, M. J., Medina, D., Tilley, W. D., Aspinall, J. O., Cunha, G. R., Donjacour, A. A., Matusik, R. J., & Rosen, J. M. (1995). Prostate cancer in a transgenic mouse. *Proceedings of the National Academy of Sciences*, *92*(8), 3439–3443. https://doi.org/10.1073/PNAS.92.8.3439

Greenberg, N. M., DeMayo, F. J., Sheppard, P. C., Barrios, R., Lebovitz, R., Finegold, M., Angelopoulou, R., Dodd, J. G., Duckworth, M. L., Rosen, J. M., & Matusik, R. J. (1994). The rat probasin gene promoter directs hormonally and developmentally regulated expression of a heterologous gene specifically to the prostate in transgenic mice. *Molecular Endocrinology (Baltimore, Md.)*, *8*(2), 230–239. https://doi.org/10.1210/MEND.8.2.8170479

Grossmann, M. E., Huang, H., & Tindall, D. J. (2001). Androgen Receptor Signaling in Androgen-Refractory Prostate Cancer. *JNCI: Journal of the National Cancer Institute*, *93*(22), 1687–1697. https://doi.org/10.1093/JNCI/93.22.1687

Guo, Y., Pan, W., Liu, S., Shen, Z., Xu, Y., & Hu, L. (2020). ERK/MAPK signalling pathway and tumorigenesis. *Experimental and Therapeutic Medicine*, *19*(3). https://doi.org/10.3892/ETM.2020.8454

Guo, Z.-T., Yu, Q., Miao, C., Ge, W., & Li, P. (2022). MicroRNA-27a-5p Inhibits Proliferation, Migration, and Invasion and Promotes Apoptosis of Wilms' Tumor Cell by Targeting PBOV1. *Molecular and Cellular Biology*, *42*(8). https://doi.org/10.1128/MCB.00397-21

Ha, M., & Kim, V. N. (2014). Regulation of microRNA biogenesis. *Nature Reviews Molecular Cell Biology 2014 15:8*, *15*(8), 509–524. https://doi.org/10.1038/nrm3838

Hammond, S. M., Boettcher, S., Caudy, A. A., Kobayashi, R., & Hannon, G. J. (2001). Argonaute2, a Link Between Genetic and Biochemical Analyses of RNAi. *Science*, *293*(5532), 1146–1150. https://doi.org/10.1126/SCIENCE.1064023

Harris, W. P., Mostaghel, E. A., Nelson, P. S., & Montgomery, B. (2009a). Androgen deprivation therapy: progress in understanding mechanisms of resistance and optimizing androgen depletion. *Nature Clinical Practice Urology 2009 6:2*, *6*(2), 76–85. https://doi.org/10.1038/ncpuro1296

Harris, W. P., Mostaghel, E. A., Nelson, P. S., & Montgomery, B. (2009b). Androgen deprivation therapy: progress in understanding mechanisms of resistance and

optimizing androgen depletion. *Nature Clinical Practice Urology 2009 6:2*, *6*(2), 76–85. https://doi.org/10.1038/ncpuro1296

Harris, W. P., Mostaghel, E. A., Nelson, P. S., & Montgomery, B. (2009c). Androgen deprivation therapy: progress in understanding mechanisms of resistance and optimizing androgen depletion. *Nature Clinical Practice. Urology*, *6*(2), 76–85. https://doi.org/10.1038/NCPURO1296

He, L., He, X., Lim, L. P., De Stanchina, E., Xuan, Z., Liang, Y., Xue, W., Zender, L., Magnus, J., Ridzon, D., Jackson, A. L., Linsley, P. S., Chen, C., Lowe, S. W., Cleary, M. A., & Hannon, G. J. (2007). A microRNA component of the p53 tumour suppressor network. *Nature*, *447*(7148), 1130–1134. https://doi.org/10.1038/NATURE05939

Heidenreich, A., Aus, G., Bolla, M., Joniau, S., Matveev, V. B., Schmid, H. P., & Zattoni, F. (2008). EAU Guidelines on Prostate Cancer. *European Urology*, *53*(1), 68–80. https://doi.org/10.1016/J.EURURO.2007.09.002

Herbert, K. M., Pimienta, G., DeGregorio, S. J., Alexandrov, A., & Steitz, J. A. (2013). Phosphorylation of DGCR8 Increases Its Intracellular Stability and Induces a Progrowth miRNA Profile. *Cell Reports*, *5*(4), 1070–1081. https://doi.org/10.1016/j.celrep.2013.10.017

Hermida, M. A., Dinesh Kumar, J., & Leslie, N. R. (2017a). GSK3 and its interactions with the PI3K/AKT/mTOR signalling network. *Advances in Biological Regulation*, *65*, 5–15. https://doi.org/10.1016/J.JBIOR.2017.06.003

Hermida, M. A., Dinesh Kumar, J., & Leslie, N. R. (2017b). GSK3 and its interactions with the PI3K/AKT/mTOR signalling network. *Advances in Biological Regulation*, *65*, 5–15. https://doi.org/10.1016/J.JBIOR.2017.06.003

Hermida, M. A., Dinesh Kumar, J., & Leslie, N. R. (2017c). GSK3 and its interactions with the PI3K/AKT/mTOR signalling network. *Advances in Biological Regulation*, *65*, 5–15. https://doi.org/10.1016/J.JBIOR.2017.06.003

Hong, D. S., Kang, Y. K., Borad, M., Sachdev, J., Ejadi, S., Lim, H. Y., Brenner, A. J., Park, K., Lee, J. L., Kim, T. Y., Shin, S., Becerra, C. R., Falchook, G., Stoudemire, J., Martin, D., Kelnar, K., Peltier, H., Bonato, V., Bader, A. G., … Beg, M. S. (2020). Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours. *British Journal of Cancer*, *122*(11), 1630–1637. https://doi.org/10.1038/S41416-020-0802-1

Horoszewicz, J., Leong, S., Chu, T., Zl, W., Friedman, M., Papsidero, L., Kim, U., Chai, L., Kakati, S., Arya, S., & Sandberg, A. (1980). The LNCaP cell line--a new model for studies on human prostatic carcinoma. *Progress in Clinical and Biological Research*.

Hu, H. Y., Yan, Z., Xu, Y., Hu, H., Menzel, C., Zhou, Y. H., Chen, W., & Khaitovich, P. (2009). Sequence features associated with microRNA strand selection in

humans and flies. *BMC Genomics*, *10*(1), 413. https://doi.org/10.1186/1471-2164-10-413/FIGURES/4

Hu, R., Dunn, T. A., Wei, S., Isharwal, S., Veltri, R. W., Humphreys, E., Han, M., Partin, A. W., Vessella, R. L., Isaacs, W. B., Bova, G. S., & Luo, J. (2009a). Ligand-Independent Androgen Receptor Variants Derived from Splicing of Cryptic Exons Signify Hormone-Refractory Prostate Cancer. *Cancer Research*, *69*(1), 16–22. https://doi.org/10.1158/0008-5472.CAN-08-2764

Hu, R., Dunn, T. A., Wei, S., Isharwal, S., Veltri, R. W., Humphreys, E., Han, M., Partin, A. W., Vessella, R. L., Isaacs, W. B., Bova, G. S., & Luo, J. (2009b). Ligand-Independent Androgen Receptor Variants Derived from Splicing of Cryptic Exons Signify Hormone-Refractory Prostate Cancer. *Cancer Research*, *69*(1), 16–22. https://doi.org/10.1158/0008-5472.CAN-08-2764

Hu, R., Lu, C., Mostaghel, E. A., Yegnasubramanian, S., Gurel, M., Tannahill, C., Edwards, J., Isaacs, W. B., Nelson, P. S., Bluemn, E., Plymate, S. R., & Luo, J. (2012). Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. *Cancer Research*, *72*(14), 3457–3462. https://doi.org/10.1158/0008-5472.CAN-11-3892/650230/AM/DISTINCT-TRANSCRIPTIONAL-PROGRAMS-MEDIATED-BY-THE

Huang, C. Y., Fong, Y. C., Lee, C. Y., Chen, M. Y., Tsai, H. C., Hsu, H. C., & Tang, C. H. (2009a). CCL5 increases lung cancer migration via PI3K, Akt and NF-kappaB pathways. *Biochemical Pharmacology*, *77*(5), 794–803. https://doi.org/10.1016/J.BCP.2008.11.014

Huang, C. Y., Fong, Y. C., Lee, C. Y., Chen, M. Y., Tsai, H. C., Hsu, H. C., & Tang, C. H. (2009b). CCL5 increases lung cancer migration via PI3K, Akt and NF-κB pathways. *Biochemical Pharmacology*, *77*(5), 794–803. https://doi.org/10.1016/J.BCP.2008.11.014

Huang, R., Wang, S., Wang, N., Zheng, Y., Zhou, J., Yang, B., Wang, X., Zhang, J., Guo, L., Wang, S., Chen, Z., Wang, Z., & Xiang, S. (2020). CCL5 derived from tumor-associated macrophages promotes prostate cancer stem cells and metastasis via activating β-catenin/STAT3 signaling. *Cell Death & Disease 2020 11:4*, *11*(4), 1–20. https://doi.org/10.1038/s41419-020-2435-y

Hughes, C. E., & Nibbs, R. J. B. (2018). A guide to chemokines and their receptors. *The FEBS Journal*, *285*(16), 2944–2971. https://doi.org/10.1111/FEBS.14466

Imamura, Y., & Sadar, M. D. (2016). Androgen receptor targeted therapies in castration-resistant prostate cancer: Bench to clinic. *International Journal of Urology*, *23*(8), 654–665. https://doi.org/10.1111/IJU.13137

Iwata, T., Schultz, D., Hicks, J., Hubbard, G. K., Mutton, L. N., Lotan, T. L., Bethel, C., Lotz, M. T., Yegnasubramanian, S., Nelson, W. G., Dang, C. V., Xu, M. M., Anele, U., Koh, C. M., Bieberich, C. J., & De Marzo, A. M. (2010). MYC

Overexpression Induces Prostatic Intraepithelial Neoplasia and Loss of Nkx3.1 in Mouse Luminal Epithelial Cells. *PLOS ONE*, *5*(2), e9427. https://doi.org/10.1371/JOURNAL.PONE.0009427

Jalava, S. E., Urbanucci, A., Latonen, L., Waltering, K. K., Sahu, B., Jänne, O. A., Seppälä, J., Lähdesmäki, H., Tammela, T. L. J., & Visakorpi, T. (2012). Androgen-regulated miR-32 targets BTG2 and is overexpressed in castration-resistant prostate cancer. *Oncogene*, *31*(41), 4460–4471. https://doi.org/10.1038/ONC.2011.624

Jiang, Y., Duan, Y., & Zhou, H. (2015). MicroRNA-27a directly targets KRAS to inhibit cell proliferation in esophageal squamous cell carcinoma. *Oncology Letters*, *9*(1), 471–477. https://doi.org/10.3892/OL.2014.2701

Jiao, X., Nawab, O., Patel, T., Kossenkov, A. V., Halama, N., Jaeger, D., & Pestell, R. G. (2019). Recent advances targeting CCR5 for cancer and its role in immuno-oncology. *Cancer Research*, *79*(19), 4801–4807. https://doi.org/10.1158/0008-5472.CAN-19-1167/653842/AM/RECENT-ADVANCES-TARGETING-CCR5-FOR-CANCER-AND-ITS

Jiao, X., Velasco-Velazquez, M. A., Wang, M., Li, Z., Rui, H., Peck, A. R., Korkola, J. E., Chen, X., Xu, S., DuHadaway, J. B., Guerrero-Rodriguez, S., Addya, S., Sicoli, D., Mu, Z., Zhang, G., Stucky, A., Zhang, X., Cristofanilli, M., Fatatis, A., … Pestell, R. G. (2018). CCR5 Governs DNA damage repair and breast cancer stem cell expansion. *Cancer Research*, *78*(7), 1657–1671. https://doi.org/10.1158/0008-5472.CAN-17-0915/652843/AM/CCR5-GOVERNS-DNA-DAMAGE-AND-BREAST-CANCER-STEM

Johnson, G. L., & Lapadat, R. (2002). Mitogen-Activated Protein Kinase Pathways Mediated by ERK, JNK, and p38 Protein Kinases. *Science*, *298*(5600), 1911–1912. https://doi.org/10.1126/SCIENCE.1072682

Kaighn, M. E., Narayan, K. S., Ohnuki, Y., Lechner, J. F., & Jones, L. W. (1979). Establishment and characterization of a human prostatic carcinoma cell line (PC-3). *Investigative Urology*, *17*(1), 16–23. https://europepmc.org/article/med/447482

Kale, J., Osterlund, E. J., & Andrews, D. W. (2017). BCL-2 family proteins: changing partners in the dance towards death. *Cell Death & Differentiation 2018 25:1*, *25*(1), 65–80. https://doi.org/10.1038/cdd.2017.186

Karantanos, T., Corn, P. G., & Thompson, T. C. (2013). Prostate cancer progression after androgen deprivation therapy: mechanisms of castrate resistance and novel therapeutic approaches. *Oncogene 2013 32:49*, *32*(49), 5501–5511. https://doi.org/10.1038/onc.2013.206

Karatas, O. F., Guzel, E., Suer, I., Ekici, I. D., Caskurlu, T., Creighton, C. J., Ittmann, M., & Ozen, M. (2014). miR-1 and miR-133b Are Differentially Expressed in Patients with Recurrent Prostate Cancer. *PLoS ONE*, *9*(6). https://doi.org/10.1371/JOURNAL.PONE.0098675

Kasper, S., Sheppard, P. C., Yan, Y., Pettigrew, N., Borowsky, A. D., Prins, G. S., Dodd, J. G., Duckworth, M. L., & Matusik, R. J. (1998). Development, progression, and androgen-dependence of prostate tumors in probasin-large T antigen transgenic mice: a model for prostate cancer. *Laboratory Investigation; a Journal of Technical Methods and Pathology*, *78*(6).

Kato, T., Fujita, Y., Nakane, K., Mizutani, K., Terazawa, R., Ehara, H., Kanimoto, Y., Kojima, T., Nozawa, Y., Deguchi, T., & Ito, M. (2013). CCR1/CCL5 interaction promotes invasion of taxane-resistant PC3 prostate cancer cells by increasing secretion of MMPs 2/9 and by activating ERK and Rac signaling. *Cytokine*, *64*(1), 251–257. https://doi.org/10.1016/J.CYTO.2013.06.313

Kawamata, T., & Tomari, Y. (2010). Making RISC. *Trends in Biochemical Sciences*, *35*(7), 368–376. https://doi.org/10.1016/j.tibs.2010.03.009

Keller, E. T., Fu, Z., Yeung, K., & Brennan, M. (2004). Raf kinase inhibitor protein: a prostate cancer metastasis suppressor gene. *Cancer Letters*, *207*(2), 131–137. https://doi.org/10.1016/J.CANLET.2004.02.006

Kinkade, C. W., Castillo-Martin, M., Puzio-Kuter, A., Yan, J., Foster, T. H., Gao, H., Sun, Y., Ouyang, X., Gerald, W. L., Cordon-Cardo, C., & Abate-Shen, C. (2008a). Targeting AKT/mTOR and ERK MAPK signaling inhibits hormone-refractory prostate cancer in a preclinical mouse model. *The Journal of Clinical Investigation*, *118*(9), 3051–3064. https://doi.org/10.1172/JCI34764

Kinkade, C. W., Castillo-Martin, M., Puzio-Kuter, A., Yan, J., Foster, T. H., Gao, H., Sun, Y., Ouyang, X., Gerald, W. L., Cordon-Cardo, C., & Abate-Shen, C. (2008b). Targeting AKT/mTOR and ERK MAPK signaling inhibits hormone-refractory prostate cancer in a preclinical mouse model. *The Journal of Clinical Investigation*, *118*(9), 3051–3064. https://doi.org/10.1172/JCI34764

Klezovitch, O., Chevillet, J., Mirosevich, J., Roberts, R. L., Matusik, R. J., & Vasioukhin, V. (2004). Hepsin promotes prostate cancer progression and metastasis. *Cancer Cell*, *6*(2), 185–195. https://doi.org/10.1016/J.CCR.2004.07.008

Ko, C. J., Huang, C. C., Lin, H. Y., Juan, C. P., Lan, S. W., Shyu, H. Y., Wu, S. R., Hsiao, P. W., Huang, H. P., Shun, C. T., & Lee, M. S. (2015). Androgen-induced TMPRSS2 activates matriptase and promotes extracellular matrix degradation, prostate cancer cell invasion, tumor growth, and metastasis. *Cancer Research*, *75*(14), 2949–2960. https://doi.org/10.1158/0008-5472.CAN-14-3297/651718/AM/ANDROGEN-INDUCED-TMPRSS2-ACTIVATES-MATRIPTASE-AND

Kong, L., Wu, Q., Zhao, L., Ye, J., Li, N., & Yang, H. (2019). Effect of microRNA-27a-5p on apoptosis and inflammatory response of pancreatic acinar cells in acute pancreatitis by targeting PTEN. *Journal of Cellular Biochemistry*, *120*(9), 15844–15850. https://doi.org/10.1002/JCB.28855

Kong, L.-Y., Xue, M., Zhang, Q.-C., Su, C.-F., Kong, L.-Y., Xue, M., Zhang, Q.-C., & Su, C.-F. (2017). In vivo and in vitro effects of microRNA-27a on proliferation, migration and invasion of breast cancer cells through targeting of SFRP1 gene via Wnt/β-catenin signaling pathway. *Oncotarget*, *8*(9), 15507–15519. https://doi.org/10.18632/ONCOTARGET.14662

König, J. E., Senge, T., Allhoff, E. P., & König, W. (2004). Analysis of the inflammatory network in benign prostate hyperplasia and prostate cancer. *The Prostate*, *58*(2), 121–129. https://doi.org/10.1002/PROS.10317

Kranjc, M. K., Novak, M., Pestell, R. G., & Lah, T. T. (2019). Cytokine CCL5 and receptor CCR5 axis in glioblastoma multiforme. *Radiology and Oncology*, *53*(4), 397–406. https://doi.org/10.2478/RAON-2019-0057

Ku, A., Fredsøe, J., Sørensen, K. D., Borre, M., Evander, M., Laurell, T., Lilja, H., & Ceder, Y. (2021). High-Throughput and Automated Acoustic Trapping of Extracellular Vesicles to Identify microRNAs With Diagnostic Potential for Prostate Cancer. *Frontiers in Oncology*, *11*, 631021. https://doi.org/10.3389/FONC.2021.631021/BIBTEX

Kumar-Sinha, C., Kalyana-Sundaram, S., & Chinnaiyan, A. M. (2015). Landscape of gene fusions in epithelial cancers: seq and ye shall find. *Genome Medicine 2015 7:1*, *7*(1), 1–18. https://doi.org/10.1186/S13073-015-0252-1

Laoukili, J., Kooistra, M. R. H., Brás, A., Kauw, J., Kerkhoven, R. M., Morrison, A., Clevers, H., & Medema, R. H. (2005). FoxM1 is required for execution of the mitotic programme and chromosome stability. *Nature Cell Biology 2005 7:2*, *7*(2), 126–136. https://doi.org/10.1038/ncb1217

Lardizabal, J., Ding, J., Delwar, Z., Rennie, P. S., & Jia, W. (2018). A TRAMP-derived orthotopic prostate syngeneic (TOPS) cancer model for investigating anti-tumor treatments. *The Prostate*, *78*(6), 457–468. https://doi.org/10.1002/PROS.23490

Lau, N. C., Lim, L. P., Weinstein, E. G., & Bartel, D. P. (2001). An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. *Science*, *294*(5543), 858–862. https://doi.org/10.1126/SCIENCE.1065062/SUPPL_FILE/31.HTML

Lee, R. C., Feinbaum, R. L., & Ambros, V. (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*, *75*(5), 843–854. https://doi.org/10.1016/0092-8674(93)90529-Y

Lee, S. H., Johnson, D., Luong, R., & Sun, Z. (2015). Crosstalking between Androgen and PI3K/AKT Signaling Pathways in Prostate Cancer Cells. *Journal of Biological Chemistry*, *290*(5), 2759–2768. https://doi.org/10.1074/JBC.M114.607846

Li, E., Liu, L., Li, F., Luo, L., Zhao, S., Wang, J., Kang, R., Luo, J., & Zhao, Z. (2017). PSCA promotes prostate cancer proliferation and cell-cycle progression by up-

regulating c-Myc. *The Prostate*, *77*(16), 1563–1572. https://doi.org/10.1002/PROS.23432

Li, M., Han, Y., Zhou, H., Li, X., Lin, C., Zhang, E., Chi, X., Hu, J., & Xu, H. (2018). Transmembrane protein 170B is a novel breast tumorigenesis suppressor gene that inhibits the Wnt/β-catenin pathway. *Cell Death & Disease 2018 9:2*, *9*(2), 1–15. https://doi.org/10.1038/s41419-017-0128-y

Li, Q., Liu, L., Zhang, Q., Liu, S., Ge, D., & You, Z. (2014). Interleukin-17 Indirectly Promotes M2 Macrophage Differentiation through Stimulation of COX-2/PGE2 Pathway in the Cancer Cells. *Cancer Research and Treatment*, *46*(3), 297–306. https://doi.org/10.4143/CRT.2014.46.3.297

Li, S., Li, J., Fei, B. Y., Shao, D., Pan, Y., Mo, Z. H., Sun, B. Z., Zhang, D., Zheng, X., Zhang, M., Zhang, X. W., & Chen, L. (2015). MiR-27a promotes hepatocellular carcinoma cell proliferation through suppression of its target gene peroxisome proliferator-activated receptor γ. *Chinese Medical Journal*, *128*(7), 941–947. https://doi.org/10.4103/0366-6999.154302

Li, T., Li, R. S., Li, Y. H., Zhong, S., Chen, Y. Y., Zhang, C. M., Hu, M. M., & Shen, Z. J. (2012). miR-21 as an independent biochemical recurrence predictor and potential therapeutic target for prostate cancer. *The Journal of Urology*, *187*(4), 1466–1472. https://doi.org/10.1016/J.JURO.2011.11.082

Li, X., Liu, X., Xu, W., Zhou, P., Gao, P., Jiang, S., Lobie, P. E., & Zhu, T. (2013). C-MYC-regulated miR-23a/24-2/27a cluster promotes mammary carcinoma cell invasion and hepatic metastasis by targeting sprouty2. *Journal of Biological Chemistry*, *288*(25), 18121–18133. https://doi.org/10.1074/jbc.M113.478560

Li, X., Xu, M., Ding, L., Tang, J., & Tang, J.-H. (2019). MiR-27a: A Novel Biomarker and Potential Therapeutic Target in Tumors. *Journal of Cancer*, *10*(12), 2836–2848. https://doi.org/10.7150/jca.31361

Li, Y., Cai, B., Shen, L., Dong, Y., Lu, Q., Sun, S., Liu, S., Ma, S., Ma, P. X., & Chen, J. (2017). MiRNA-29b suppresses tumor growth through simultaneously inhibiting angiogenesis and tumorigenesis by targeting Akt3. *Cancer Letters*, *397*, 111–119. https://doi.org/10.1016/J.CANLET.2017.03.032

Li, Z., Hu, S., Wang, J., Cai, J., Xiao, L., Yu, L., & Wang, Z. (2010). MiR-27a modulates MDR1/P-glycoprotein expression by targeting HIPK2 in human ovarian cancer cells. *Gynecologic Oncology*, *119*(1), 125–130. https://doi.org/10.1016/J.YGYNO.2010.06.004

Liang, T., Yu, J. F., Liu, C., & Guo, L. (2014). An Exploration of Evolution, Maturation, Expression and Function Relationships in Mir-23∼27∼24 Cluster. *PLOS ONE*, *9*(8), e106223. https://doi.org/10.1371/JOURNAL.PONE.0106223

Liao, R. S., Ma, S., Miao, L., Li, R., Yin, Y., & Raj, G. V. (2013). Androgen receptor-mediated non-genomic regulation of prostate cancer cell proliferation.

Translational Andrology and Urology, *2*(3), 18796–18196. https://doi.org/10.3978/J.ISSN.2223-4683.2013.09.07

Lin, H. K., Yeh, S., Kang, H. Y., & Chang, C. (2001). Akt suppresses androgen-induced apoptosis by phosphorylating and inhibiting androgen receptor. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(13), 7200–7205. https://doi.org/10.1073/PNAS.121173298

Lin, J., Song, T., Li, C., & Mao, W. (2020). GSK-3β in DNA repair, apoptosis, and resistance of chemotherapy, radiotherapy of cancer. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1867*(5), 118659. https://doi.org/10.1016/J.BBAMCR.2020.118659

Lin, S. C., Kao, C. Y., Lee, H. J., Creighton, C. J., Ittmann, M. M., Tsai, S. J., Tsai, S. Y., & Tsai, M. J. (2016). Dysregulation of miRNAs-COUP-TFII-FOXM1-CENPF axis contributes to the metastasis of prostate cancer. *Nature Communications 2016 7:1*, *7*(1), 1–14. https://doi.org/10.1038/ncomms11418

Lin, S., & Gregory, R. I. (2015). MicroRNA biogenesis pathways in cancer. *Nature Reviews Cancer 2015 15:6*, *15*(6), 321–333. https://doi.org/10.1038/nrc3932

Lin, Y., Fukuchi, J., Hiipakka, R. A., Kokontis, J. M., & Xiang, J. (2007). Up-regulation of Bcl-2 is required for the progression of prostate cancer cells from an androgen-dependent to an androgen-independent growth stage. *Cell Research 2007 17:6*, *17*(6), 531–536. https://doi.org/10.1038/cr.2007.12

Ling, H., Fabbri, M., & Calin, G. A. (2013a). MicroRNAs and other non-coding RNAs as targets for anticancer drug development. *Nature Reviews. Drug Discovery*, *12*(11), 847–865. https://doi.org/10.1038/NRD4140

Ling, H., Fabbri, M., & Calin, G. A. (2013b). MicroRNAs and other non-coding RNAs as targets for anticancer drug development. *Nature Reviews. Drug Discovery*, *12*(11), 847–865. https://doi.org/10.1038/NRD4140

Liu, G., Cao, P., Chen, H., Yuan, W., Wang, J., & Tang, X. (2013). MiR-27a Regulates Apoptosis in Nucleus Pulposus Cells by Targeting PI3K. *PLOS ONE*, *8*(9), e75251. https://doi.org/10.1371/JOURNAL.PONE.0075251

Liu, J., Stevens, P. D., & Gao, T. (2011). mTOR-Dependent Regulation of PHLPP Expression Controls the Rapamycin Sensitivity in Cancer Cells. *The Journal of Biological Chemistry*, *286*(8), 6510. https://doi.org/10.1074/JBC.M110.183087

López-Cotarelo, P., Gómez-Moreira, C., Criado-García, O., Sánchez, L., & Rodríguez-Fernández, J. L. (2017). Beyond Chemoattraction: Multifunctionality of Chemokine Receptors in Leukocytes. *Trends in Immunology*, *38*(12), 927–941. https://doi.org/10.1016/J.IT.2017.08.004

Lu, C., & Luo, J. (2013). Decoding the androgen receptor splice variants. *Translational Andrology and Urology*, *2*(3), 17886–17186. https://doi.org/10.3978/J.ISSN.2223-4683.2013.09.08

Lu, J., Getz, G., Miska, E. A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebert, B. L., Mak, R. H., Ferrando, A. A., Downing, J. R., Jacks, T., Horvitz, H. R., & Golub, T. R. (2005). MicroRNA expression profiles classify human cancers. *Nature 2005 435:7043*, *435*(7043), 834–838. https://doi.org/10.1038/nature03702

Lu, S., Liu, M., Epner, D. E., Tsai, S. Y., & Tsai, M. J. (1999). Androgen regulation of the cyclin-dependent kinase inhibitor p21 gene through an androgen response element in the proximal promoter. *Molecular Endocrinology (Baltimore, Md.)*, *13*(3), 376–384. https://doi.org/10.1210/MEND.13.3.0254

Lytle, J. R., Yario, T. A., & Steitz, J. A. (2007). Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5′ UTR as in the 3′ UTR. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(23), 9667–9672. https://doi.org/10.1073/PNAS.0703820104/ASSET/FE5E1B1A-4CB5-46C3-A962-CE39B959C1A4/ASSETS/GRAPHIC/ZPQ0240766020005.JPEG

M., J. R. (2018). Functions and epigenetic aspects of miR-15/16: Possible future cancer therapeutics. *Gene Reports*, *12*, 149–164. https://doi.org/10.1016/J.GENREP.2018.06.012

Madden, S. K., de Araujo, A. D., Gerhardt, M., Fairlie, D. P., & Mason, J. M. (2021). Taking the Myc out of cancer: toward therapeutic strategies to directly inhibit c-Myc. *Molecular Cancer 2021 20:1*, *20*(1), 1–18. https://doi.org/10.1186/S12943-020-01291-6

Maroulakou, I. G., Anver, M., Garrett, L., & Green, J. E. (1994). Prostate and mammary adenocarcinoma in transgenic mice carrying a rat C3(1) simian virus 40 large tumor antigen fusion gene. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(23), 11236–11240. https://doi.org/10.1073/PNAS.91.23.11236

Masumori, N., Tsuchiya, K., Tu, W. H., Lee, C., Kasper, S., Tsukamoto, T., Shappell, S. B., & Matusik, R. J. (2004). An allograft model of androgen independent prostatic neuroendocrine carcinoma derived from a large probasin promoter-t antigen transgenic mouse line. *Journal of Urology*, *171*(1), 439–442. https://doi.org/10.1097/01.ju.0000099826.63103.94

Mateo, J., Fizazi, K., Gillessen, S., Heidenreich, A., Perez-Lopez, R., Oyen, W. J. G., Shore, N., Smith, M., Sweeney, C., Tombal, B., Tomlins, S. A., & de Bono, J. S. (2019). Managing Nonmetastatic Castration-resistant Prostate Cancer. *European Urology*, *75*(2), 285–293. https://doi.org/10.1016/J.EURURO.2018.07.035

McCubrey, J. A., Steelman, L. S., Chappell, W. H., Abrams, S. L., Wong, E. W. T., Chang, F., Lehmann, B., Terrian, D. M., Milella, M., Tafuri, A., Stivala, F., Libra, M., Basecke, J., Evangelisti, C., Martelli, A. M., & Franklin, R. A. (2007). Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug

resistance. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1773*(8), 1263–1284. https://doi.org/10.1016/J.BBAMCR.2006.10.001

Mediwala, S. N., Sun, H., Szafran, A. T., Hartig, S. M., Sonpavde, G., Hayes, T. G., Thiagarajan, P., Mancini, M. A., & Marcelli, M. (2013). The activity of the androgen receptor variant AR-V7 is regulated by FOXO1 in a PTEN-PI3K-AKT-dependent way. *The Prostate*, *73*(3), 267–277. https://doi.org/10.1002/PROS.22566

Mellinger, G. T. (1977). Prognosis of prostatic carcinoma. *Recent Results in Cancer Research, no.60*, 61–72. https://doi.org/10.1007/978-3-642-81095-4_6/COVER

Mellman, I., Coukos, G., & Dranoff, G. (2011). Cancer immunotherapy comes of age. *Nature*, *480*(7378), 480–489. https://doi.org/10.1038/NATURE10673

Mendoza, M. C., Er, E. E., & Blenis, J. (2011). The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. *Trends in Biochemical Sciences*, *36*(6), 320–328. https://doi.org/10.1016/J.TIBS.2011.03.006

Mertens-Talcott, S. U., Chintharlapalli, S., Li, X., & Safe, S. (2007). The Oncogenic microRNA-27a Targets Genes That Regulate Specificity Protein Transcription Factors and the G2-M Checkpoint in MDA-MB-231 Breast Cancer Cells. *Cancer Research*, *67*(22), 11001–11011. https://doi.org/10.1158/0008-5472.CAN-07-2416

Meyer, N., & Penn, L. Z. (2008). Reflecting on 25 years with MYC. *Nature Reviews Cancer 2008 8:12*, *8*(12), 976–990. https://doi.org/10.1038/nrc2231

Mickey, D. D., Stone, K. R., Wunderli, H., Mickey, G. H., & Paulson, D. F. (1980). Characterization of a human prostate adenocarcinoma cell line (DU 145) as a monolayer culture and as a solid tumor in athymic mice. *Progress in Clinical and Biological Research*, *37*, 67–84. https://europepmc.org/article/MED/7384095

Mitchell, P. S., Parkin, R. K., Kroh, E. M., Fritz, B. R., Wyman, S. K., Pogosova-Agadjanyan, E. L., Peterson, A., Noteboom, J., O'Briant, K. C., Allen, A., Lin, D. W., Urban, N., Drescher, C. W., Knudsen, B. S., Stirewalt, D. L., Gentleman, R., Vessella, R. L., Nelson, P. S., Martin, D. B., & Tewari, M. (2008). Circulating microRNAs as stable blood-based markers for cancer detection. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(30), 10513. https://doi.org/10.1073/PNAS.0804549105

Mitsiades, N., Sung, C. C., Schultz, N., Danila, D. C., He, B., Eedunuri, V. K., Fleisher, M., Sander, C., Sawyers, C. L., & Scher, H. I. (2012). Distinct patterns of dysregulated expression of enzymes involved in androgen synthesis and metabolism in metastatic prostate cancer tumors. *Cancer Research*, *72*(23), 6142–6152. https://doi.org/10.1158/0008-5472.CAN-12-1335

Mo, W., Zhang, J., Li, X., Meng, D., Gao, Y., Yang, S., Wan, X., Zhou, C., Guo, F., Huang, Y., Amente, S., Avvedimento, E. V., Xie, Y., & Li, Y. (2013). Identification of Novel AR-Targeted MicroRNAs Mediating Androgen Signalling

through Critical Pathways to Regulate Cell Viability in Prostate Cancer. *PLOS ONE*, *8*(2), e56592. https://doi.org/10.1371/JOURNAL.PONE.0056592

Mohler, J. L., Gregory, C. W., Ford, H., Kim, D., Weaver, C. M., Petrusz, P., Wilson, E. M., & French, F. S. (2004). The Androgen Axis in Recurrent Prostate Cancer. *Clinical Cancer Research*, *10*(2), 440–448. https://doi.org/10.1158/1078-0432.CCR-1146-03

Molina, A., & Belldegrun, A. (2011). Novel Therapeutic Strategies for Castration Resistant Prostate Cancer: Inhibition of Persistent Androgen Production and Androgen Receptor Mediated Signaling. *The Journal of Urology*, *185*(3), 787–794. https://doi.org/10.1016/J.JURO.2010.10.042

Mossmann, D., Park, S., & Hall, M. N. (2018). mTOR signalling and cellular metabolism are mutual determinants in cancer. *Nature Reviews Cancer 2018 18:12*, *18*(12), 744–757. https://doi.org/10.1038/s41568-018-0074-8

Mulholland, D. J., Kobayashi, N., Ruscetti, M., Zhi, A., Tran, L. M., Huang, J., Gleave, M., & Wu, H. (2012a). Pten loss and RAS/MAPK activation cooperate to promote EMT and metastasis initiated from prostate cancer stem/progenitor cells. *Cancer Research*, *72*(7), 1878–1889. https://doi.org/10.1158/0008-5472.CAN-11-3132/650317/AM/PTEN-LOSS-AND-RAS-MAPK-ACTIVATION-COOPERATE-TO

Mulholland, D. J., Kobayashi, N., Ruscetti, M., Zhi, A., Tran, L. M., Huang, J., Gleave, M., & Wu, H. (2012b). Pten loss and RAS/MAPK activation cooperate to promote EMT and metastasis initiated from prostate cancer stem/progenitor cells. *Cancer Research*, *72*(7), 1878–1889. https://doi.org/10.1158/0008-5472.CAN-11-3132/650317/AM/PTEN-LOSS-AND-RAS-MAPK-ACTIVATION-COOPERATE-TO

Mulholland, D. J., Tran, L. M., Li, Y., Cai, H., Morim, A., Wang, S., Plaisier, S., Garraway, I. P., Huang, J., Graeber, T. G., & Wu, H. (2011a). Cell Autonomous Role of PTEN in Regulating Castration-Resistant Prostate Cancer Growth. *Cancer Cell*, *19*(6), 792–804. https://doi.org/10.1016/J.CCR.2011.05.006

Mulholland, D. J., Tran, L. M., Li, Y., Cai, H., Morim, A., Wang, S., Plaisier, S., Garraway, I. P., Huang, J., Graeber, T. G., & Wu, H. (2011b). Cell autonomous role of PTEN in regulating castration-resistant prostate cancer growth. *Cancer Cell*, *19*(6), 792–804. https://doi.org/10.1016/J.CCR.2011.05.006

Murooka, T. T., Rahbar, R., & Fish, E. N. (2009). CCL5 promotes proliferation of MCF-7 cells through mTOR-dependent mRNA translation. *Biochemical and Biophysical Research Communications*, *387*(2), 381–386. https://doi.org/10.1016/J.BBRC.2009.07.035

Nam, R. K., Wallis, C. J. D., Amemiya, Y., Benatar, T., & Seth, A. (2018). Identification of a Novel MicroRNA Panel Associated with Metastasis Following

Radical Prostatectomy for Prostate Cancer. *Anticancer Research*, *38*(9), 5027–5034. https://doi.org/10.21873/ANTICANRES.12821

Newton, A. C., & Trotman, L. C. (2014). Turning Off AKT: PHLPP as a Drug Target. *Annual Review of Pharmacology and Toxicology*, *54*, 537. https://doi.org/10.1146/ANNUREV-PHARMTOX-011112-140338

Ng, E. K. O., Tsang, W. P., Ng, S. S. M., Jin, H. C., Yu, J., Li, J. J., Röcken, C., Ebert, M. P. A., Kwok, T. T., & Sung, J. J. Y. (2009). MicroRNA-143 targets DNA methyltransferases 3A in colorectal cancer. *British Journal of Cancer*, *101*(4), 699–706. https://doi.org/10.1038/SJ.BJC.6605195

Nguyen, T. V. V., Yao, M., & Pike, C. J. (2005). Androgens activate mitogen-activated protein kinase signaling: role in neuroprotection. *Journal of Neurochemistry*, *94*(6), 1639–1651. https://doi.org/10.1111/J.1471-4159.2005.03318.X

Nickols, N. G., Nazarian, R., Zhao, S. G., Tan, V., Uzunangelov, V., Xia, Z., Baertsch, R., Neeman, E., Gao, A. C., Thomas, G. V., Howard, L., De Hoedt, A. M., Stuart, J., Goldstein, T., Chi, K., Gleave, M. E., Graff, J. N., Beer, T. M., Drake, J. M., … Rettig, M. B. (2019a). MEK-ERK signaling is a therapeutic target in metastatic castration resistant prostate cancer. *Prostate Cancer and Prostatic Diseases*, *22*(4), 531–538. https://doi.org/10.1038/S41391-019-0134-5

Nickols, N. G., Nazarian, R., Zhao, S. G., Tan, V., Uzunangelov, V., Xia, Z., Baertsch, R., Neeman, E., Gao, A. C., Thomas, G. V., Howard, L., De Hoedt, A. M., Stuart, J., Goldstein, T., Chi, K., Gleave, M. E., Graff, J. N., Beer, T. M., Drake, J. M., … Rettig, M. B. (2019b). MEK-ERK signaling is a therapeutic target in metastatic castration resistant prostate cancer. *Prostate Cancer and Prostatic Diseases 2019 22:4*, *22*(4), 531–538. https://doi.org/10.1038/s41391-019-0134-5

Nomiyama, H., Osada, N., & Yoshie, O. (2013). Systematic classification of vertebrate chemokines based on conserved synteny and evolutionary history. *Genes to Cells*, *18*(1), 1–16. https://doi.org/10.1111/GTC.12013

Okada, C., Yamashita, E., Lee, S. J., Shibata, S., Katahira, J., Nakagawa, A., Yoneda, Y., & Tsukihara, T. (2009). A high-Resolution structure of the pre-microrna nuclear export machinery. *Science*, *326*(5957), 1275–1279. https://doi.org/10.1126/SCIENCE.1178705/SUPPL_FILE/OKADA.SOM.PDF

Okamura, K., Liu, N., & Lai, E. C. (2009). Distinct Mechanisms for MicroRNA Strand Selection by Drosophila Argonautes. *Molecular Cell*, *36*(3), 431–444. https://doi.org/10.1016/j.molcel.2009.09.027

Onishi, R. M., & Gaffen, S. L. (2010). Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. *Immunology*, *129*(3), 311–321. https://doi.org/10.1111/J.1365-2567.2009.03240.X

Oppermann, M. (2004a). Chemokine receptor CCR5: Insights into structure, function, and regulation. *Cellular Signalling*, *16*(11), 1201–1210. https://doi.org/10.1016/j.cellsig.2004.04.007

Oppermann, M. (2004b). Chemokine receptor CCR5: Insights into structure, function, and regulation. *Cellular Signalling*, *16*(11), 1201–1210. https://doi.org/10.1016/j.cellsig.2004.04.007

Owen, K. L., Brockwell, N. K., & Parker, B. S. (2019). JAK-STAT Signaling: A Double-Edged Sword of Immune Regulation and Cancer Progression. *Cancers 2019, Vol. 11, Page 2002*, *11*(12), 2002. https://doi.org/10.3390/CANCERS11122002

Paroo, Z., Ye, X., Chen, S., & Liu, Q. (2009). Phosphorylation of the Human MicroRNA-Generating Complex Mediates MAPK/Erk Signaling. *Cell*, *139*(1), 112–122. https://doi.org/10.1016/j.cell.2009.06.044

Patel, B. J., Pantuck, A. J., Zisman, A., Tsui, K. H., Paik, S. H., Caliliw, R., Sheriff, S., Wu, L., DeKernion, J. B., Tso, C. L., & Belldegrun, A. S. (2000). CL1-GFP: AN ANDROGEN INDEPENDENT METASTATIC TUMOR MODEL FOR PROSTATE CANCER. *The Journal of Urology*, *164*(4), 1420–1425. https://doi.org/10.1016/S0022-5347(05)67210-2

Peltier, H. J., Kelnar, K., & Bader, A. G. (2016). Effects of MRX34, a liposomal miR-34 mimic, on target gene expression in human white blood cells (hWBCs): qRT-PCR results from a first-in-human trial of microRNA cancer therapy. *Annals of Oncology*, *27*, vi531. https://doi.org/10.1093/ANNONC/MDW392.16

Peterziel, H., Mink, S., Schonert, A., Becker, M., Klocker, H., & Cato, A. C. B. (1999). Rapid signalling by androgen receptor in prostate cancer cells. *Oncogene 1999 18:46*, *18*(46), 6322–6329. https://doi.org/10.1038/sj.onc.1203032

Peti, W., & Page, R. (2013). Molecular basis of MAP kinase regulation. *Protein Science*, *22*(12), 1698–1710. https://doi.org/10.1002/PRO.2374

Pflug, B., Reiter, R., & Nelson, J. (1999). Caveolin expression is decreased following androgen deprivation in human prostate cancer cell lines. *The Prostate*. https://doi.org/10.1002/(SICI)1097-0045(19990901)40:4

Pillai, R. S., Bhattacharyya, S. N., Artus, C. G., Zoller, T., Cougot, N., Basyuk, E., Bertrand, E., & Filipowicz, W. (2005). Molecular biology: Inhibition of translational initiation by let-7 microRNA in human cells. *Science*, *309*(5740), 1573–1576. https://doi.org/10.1126/SCIENCE.1115079/SUPPL_FILE/PILLAI.SOM.REV1.PDF

Pilling, A. B., Hwang, C., Ford, H., & System, H. (2019a). Targeting prosurvival BCL2 signaling through Akt blockade sensitizes castration-resistant prostate cancer cells

to enzalutamide. *The Prostate*, *79*(11), 1347–1359. https://doi.org/10.1002/PROS.23843

Pilling, A. B., Hwang, C., Ford, H., & System, H. (2019b). Targeting prosurvival BCL2 signaling through Akt blockade sensitizes castration-resistant prostate cancer cells to enzalutamide. *The Prostate*, *79*(11), 1347–1359. https://doi.org/10.1002/PROS.23843

Prigol, A. N., Rode, M. P., Silva, A. H., Cisilotto, J., & Creczynski-Pasa, T. B. (2021). Pro-angiogenic effect of PC-3 exosomes in endothelial cells in vitro. *Cellular Signalling*, *87*, 110126. https://doi.org/10.1016/J.CELLSIG.2021.110126

Qin, J., Wu, S. P., Creighton, C. J., Dai, F., Xie, X., Cheng, C. M., Frolov, A., Ayala, G., Lin, X., Feng, X. H., Ittmann, M. M., Tsai, S. J., Tsai, M. J., & Tsai, S. Y. (2013). COUP-TFII inhibits TGF-β-induced growth barrier to promote prostate tumorigenesis. *Nature*, *493*(7431), 236–240. https://doi.org/10.1038/NATURE11674

Rao, A., & Herr, D. R. (2017). G protein-coupled receptor GPR19 regulates E-cadherin expression and invasion of breast cancer cells. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1864*(7), 1318–1327. https://doi.org/10.1016/J.BBAMCR.2017.05.001

Raver-Shapira, N., Marciano, E., Meiri, E., Spector, Y., Rosenfeld, N., Moskovits, N., Bentwich, Z., & Oren, M. (2007). Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. *Molecular Cell*, *26*(5), 731–743. https://doi.org/10.1016/J.MOLCEL.2007.05.017

Regis, S., Caliendo, F., Dondero, A., Casu, B., Romano, F., Loiacono, F., Moretta, A., Bottino, C., & Castriconi, R. (2017). TGF-ß1 downregulates the expression of CX3CR1 by inducing miR-27a-5p in primary human NK cells. *Frontiers in Immunology*, *8*(JUL), 264860. https://doi.org/10.3389/FIMMU.2017.00868/BIBTEX

Rishabh, K., Khadilkar, S., Kumar, A., Kalra, I., Kumar, A. P., & Kunnumakkara, A. B. (2021a). MicroRNAs as Modulators of Oral Tumorigenesis-A Focused Review. *International Journal of Molecular Sciences*, *22*(5), 1–32. https://doi.org/10.3390/IJMS22052561

Rishabh, K., Khadilkar, S., Kumar, A., Kalra, I., Kumar, A. P., & Kunnumakkara, A. B. (2021b). MicroRNAs as Modulators of Oral Tumorigenesis-A Focused Review. *International Journal of Molecular Sciences*, *22*(5), 1–32. https://doi.org/10.3390/IJMS22052561

Robinson, D., Van Allen, E. M., Wu, Y. M., Schultz, N., Lonigro, R. J., Mosquera, J. M., Montgomery, B., Taplin, M. E., Pritchard, C. C., Attard, G., Beltran, H., Abida, W., Bradley, R. K., Vinson, J., Cao, X., Vats, P., Kunju, L. P., Hussain, M., Feng, F. Y., … Chinnaiyan, A. M. (2015a). Integrative Clinical Genomics of

Advanced Prostate Cancer. *Cell*, *161*(5), 1215–1228. https://doi.org/10.1016/J.CELL.2015.05.001

Robinson, D., Van Allen, E. M., Wu, Y. M., Schultz, N., Lonigro, R. J., Mosquera, J. M., Montgomery, B., Taplin, M. E., Pritchard, C. C., Attard, G., Beltran, H., Abida, W., Bradley, R. K., Vinson, J., Cao, X., Vats, P., Kunju, L. P., Hussain, M., Feng, F. Y., … Chinnaiyan, A. M. (2015b). Integrative clinical genomics of advanced prostate cancer. *Cell*, *161*(5), 1215–1228. https://doi.org/10.1016/J.CELL.2015.05.001

Rodríguez-Berriguete, G., Prieto, A., Fraile, B., Bouraoui, Y., De Bethencourt, F. R., Martínez-Onsurbe, P., Olmedilla, G., Paniagua, R., & Royuela, M. (2010). Relationship between IL-6/ERK and NF-κB: a study in normal and pathological human prostate gland. *European Cytokine Network*, *21*(4). https://doi.org/10.1684/ECN.2010.0211

Rubinfeld, H., & Seger, R. (2005). The ERK cascade. *Molecular Biotechnology 2005 31:2*, *31*(2), 151–174. https://doi.org/10.1385/MB:31:2:151

Sadrkhanloo, M., Paskeh, M. D. A., Hashemi, M., Raesi, R., Motahhary, M., Saghari, S., Sharifi, L., Bokaie, S., Mirzaei, S., Entezari, M., Aref, A. R., Salimimoghadam, S., Rashidi, M., Taheriazam, A., & Hushmandi, K. (2023). STAT3 signaling in prostate cancer progression and therapy resistance: An oncogenic pathway with diverse functions. *Biomedicine & Pharmacotherapy*, *158*, 114168. https://doi.org/10.1016/J.BIOPHA.2022.114168

Sailer, V., von Amsberg, G., Duensing, S., Kirfel, J., Lieb, V., Metzger, E., Offermann, A., Pantel, K., Schuele, R., Taubert, H., Wach, S., Perner, S., Werner, S., & Aigner, A. (2022a). Experimental in vitro, ex vivo and in vivo models in prostate cancer research. *Nature Reviews Urology 2022 20:3*, *20*(3), 158–178. https://doi.org/10.1038/s41585-022-00677-z

Sailer, V., von Amsberg, G., Duensing, S., Kirfel, J., Lieb, V., Metzger, E., Offermann, A., Pantel, K., Schuele, R., Taubert, H., Wach, S., Perner, S., Werner, S., & Aigner, A. (2022b). Experimental in vitro, ex vivo and in vivo models in prostate cancer research. *Nature Reviews Urology 2022 20:3*, *20*(3), 158–178. https://doi.org/10.1038/s41585-022-00677-z

Samatar, A. A., & Poulikakos, P. I. (2014). Targeting RAS–ERK signalling in cancer: promises and challenges. *Nature Reviews Drug Discovery 2014 13:12*, *13*(12), 928–942. https://doi.org/10.1038/nrd4281

Saranyutanon, S., Deshmukh, S. K., Dasgupta, S., Pai, S., Singh, S., & Singh, A. P. (2020). Cellular and Molecular Progression of Prostate Cancer: Models for Basic and Preclinical Research. *Cancers 2020, Vol. 12, Page 2651*, *12*(9), 2651. https://doi.org/10.3390/CANCERS12092651

Sastry, K. S. R., Karpova, Y., & Kulik, G. (2006). Epidermal Growth Factor Protects Prostate Cancer Cells from Apoptosis by Inducing BAD Phosphorylation via

Redundant Signaling Pathways. *Journal of Biological Chemistry*, *281*(37), 27367–27377. https://doi.org/10.1074/JBC.M511485200

Sax, M. J., Gasch, C., Athota, V. R., Freeman, R., Rasighaemi, P., Westcott, D. E., Day, C. J., Nikolic, I., Elsworth, B., Wei, M., Rogers, K., Swarbrick, A., Mittal, V., Pouliot, N., & Mellick, A. S. (2016). Cancer cell CCL5 mediates bone marrow independent angiogenesis in breast cancer. *Oncotarget*, *7*(51), 85437. https://doi.org/10.18632/ONCOTARGET.13387

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods 2012 9:7*, *9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Sekhoacha, M., Riet, K., Motloung, P., Gumenku, L., Adegoke, A., & Mashele, S. (2022a). Prostate Cancer Review: Genetics, Diagnosis, Treatment Options, and Alternative Approaches. *Molecules 2022, Vol. 27, Page 5730*, *27*(17), 5730. https://doi.org/10.3390/MOLECULES27175730

Sekhoacha, M., Riet, K., Motloung, P., Gumenku, L., Adegoke, A., & Mashele, S. (2022b). Prostate Cancer Review: Genetics, Diagnosis, Treatment Options, and Alternative Approaches. *Molecules*, *27*(17). https://doi.org/10.3390/MOLECULES27175730

Shafi, A. A., Yen, A. E., & Weigel, N. L. (2013). Androgen receptors in hormone-dependent and castration-resistant prostate cancer. *Pharmacology & Therapeutics*, *140*(3), 223–238. https://doi.org/10.1016/J.PHARMTHERA.2013.07.003

Sharma, V., Kumar, L., Mohanty, S. K., Maikhuri, J. P., Rajender, S., & Gupta, G. (2016). Sensitization of androgen refractory prostate cancer cells to anti-androgens through re-expression of epigenetically repressed androgen receptor – Synergistic action of quercetin and curcumin. *Molecular and Cellular Endocrinology*, *431*, 12–23. https://doi.org/10.1016/J.MCE.2016.04.024

Sicoli, D., Jiao, X., Ju, X., Velasco-Velazquez, M., Ertel, A., Addya, S., Li, Z., Andò, S., Fatatis, A., Paudyal, B., Cristofanilli, M., Thakur, M. L., Lisanti, M. P., & Pestell, R. G. (2014). CCR5 receptor antagonists block metastasis to bone of v-Src-oncogene-transformed metastatic prostate cancer cell lines. *Cancer Research*, *74*(23), 7103. https://doi.org/10.1158/0008-5472.CAN-14-0612

Simões, V. L., Alves, M. G., Martins, A. D., Dias, T. R., Rato, L., Socorro, S., & Oliveira, P. F. (2013). Regulation of apoptotic signaling pathways by 5α-dihydrotestosterone and 17β-estradiol in immature rat Sertoli cells. *The Journal of Steroid Biochemistry and Molecular Biology*, *135*(1), 15–23. https://doi.org/10.1016/J.JSBMB.2012.11.019

Singh, R., & Mo, Y. Y. (2013). Role of microRNAs in breast cancer. *Cancer Biology & Therapy*, *14*(3), 201–212. https://doi.org/10.4161/CBT.23296

Soares-Silva, M., Diniz, F. F., Gomes, G. N., & Bahia, D. (2016). The mitogen-activated protein kinase (MAPK) pathway: Role in immune evasion by trypanosomatids. *Frontiers in Microbiology*, *7*(FEB), 179112. https://doi.org/10.3389/FMICB.2016.00183/BIBTEX

Soliman, L., De Souza, A., Srinivasan, P., Danish, M., Bertone, P., El-Deiry, W. S., & Carneiro, B. A. (2021a). The Role of BCL-2 Proteins in the Development of Castration-resistant Prostate Cancer and Emerging Therapeutic Strategies. *American Journal of Clinical Oncology: Cancer Clinical Trials*, *44*(7), 374–382. https://doi.org/10.1097/COC.0000000000000829

Soliman, L., De Souza, A., Srinivasan, P., Danish, M., Bertone, P., El-Deiry, W. S., & Carneiro, B. A. (2021b). The Role of BCL-2 Proteins in the Development of Castration-resistant Prostate Cancer and Emerging Therapeutic Strategies. *American Journal of Clinical Oncology: Cancer Clinical Trials*, *44*(7), 374–382. https://doi.org/10.1097/COC.0000000000000829

Soria, G., & Ben-Baruch, A. (2008). The inflammatory chemokines CCL2 and CCL5 in breast cancer. *Cancer Letters*, *267*(2), 271–285. https://doi.org/10.1016/J.CANLET.2008.03.018

Spans, L., Helsen, C., Clinckemalie, L., Van Den Broeck, T., Prekovic, S., Joniau, S., Lerut, E., & Claessens, F. (2014). Comparative Genomic and Transcriptomic Analyses of LNCaP and C4-2B Prostate Cancer Cell Lines. *PLOS ONE*, *9*(2), e90002. https://doi.org/10.1371/JOURNAL.PONE.0090002

Stahlhut, C., & Slack, F. J. (2015). Combinatorial Action of MicroRNAs let-7 and miR-34 Effectively Synergizes with Erlotinib to Suppress Non-small Cell Lung Cancer Cell Proliferation. *Cell Cycle*, *14*(13), 2171–2180. https://doi.org/10.1080/15384101.2014.1003008

Stelloo, S., Nevedomskaya, E., Kim, Y., Schuurman, K., Valle-Encinas, E., Lobo, J., Krijgsman, O., Peeper, D. S., Chang, S. L., Feng, F. Y. C., Wessels, L. F. A., Henrique, R., Jerónimo, C., Bergman, A. M., & Zwart, W. (2018). Integrative epigenetic taxonomy of primary prostate cancer. *Nature Communications 2018 9:1*, *9*(1), 1–12. https://doi.org/10.1038/s41467-018-07270-2

Stone, K. R., Mickey, D. D., Wunderli, H., Mickey, G. H., & Paulson, D. F. (1978). Isolation of a human prostate carcinoma cell line (DU 145). *International Journal of Cancer*, *21*(3), 274–281. https://doi.org/10.1002/IJC.2910210305

Suarez-Arnedo, A., Figueroa, F. T., Clavijo, C., Arbeláez, P., Cruz, J. C., & Muñoz-Camargo, C. (2020). An image J plugin for the high throughput image analysis of in vitro scratch wound healing assays. *PloS One*, *15*(7). https://doi.org/10.1371/JOURNAL.PONE.0232565

Suarez-Carmona, M., Chaorentong, P., Kather, J. N., Rothenheber, R., Ahmed, A., Berthel, A., Heinzelmann, A., Moraleda, R., Valous, N. A., Kosaloglu, Z., Eurich, R., Wolf, J., Grauling-Halama, S., Hundemer, M., Lasitschka, F., Klupp, F.,

Kahlert, C., Ulrich, A., Schneider, M., … Halama, N. (2019). CCR5 status and metastatic progression in colorectal cancer. *OncoImmunology*, *8*(9). https://doi.org/10.1080/2162402X.2019.1626193

Suzuki, A., De La Pompa, J. L., Stambolic, V., Elia, A. J., Sasaki, T., Del Barco Barrantes, I., Ho, A., Wakeham, A., Itie, A., Khoo, W., Fukumoto, M., & Mak, T. W. (1998). High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. *Current Biology*, *8*(21), 1169–1178. https://doi.org/10.1016/S0960-9822(07)00488-5

Tabara, H., Sarkissian, M., Kelly, W. G., Fleenor, J., Grishok, A., Timmons, L., Fire, A., & Mello, C. C. (1999). The rde-1 gene, RNA interference, and transposon silencing in C. elegans. *Cell*, *99*(2), 123–132. https://doi.org/10.1016/S0092-8674(00)81644-X

Tamaki, H., Harashima, N., Hiraki, M., Arichi, N., Nishimura, N., Shiina, H., Naora, K., Harada, M., Tamaki, H., Harashima, N., Hiraki, M., Arichi, N., Nishimura, N., Shiina, H., Naora, K., & Harada, M. (2014). Bcl-2 family inhibition sensitizes human prostate cancer cells to docetaxel and promotes unexpected apoptosis under caspase-9 inhibition. *Oncotarget*, *5*(22), 11399–11412. https://doi.org/10.18632/ONCOTARGET.2550

Tan, M. E., Li, J., Xu, H. E., Melcher, K., & Yong, E. L. (2015). Androgen receptor: structure, role in prostate cancer and drug discovery. *Acta Pharmacologica Sinica*, *36*(1), 3–23. https://doi.org/10.1038/APS.2014.18

Tang, Q., Wu, J. J., Zheng, F., Chen, Y. Q., & Hann, S. S. (2017). Emodin Increases Expression of Insulin-Like Growth Factor Binding Protein 1 through Activation of MEK/ERK/AMPKα and Interaction of PPARγ and Sp1 in Lung Cancer. *Cellular Physiology and Biochemistry*, *41*(1), 339–357. https://doi.org/10.1159/000456281

Tang, S., Xiang, T., Huang, S., Zhou, J., Wang, Z., Xie, R., Long, H., & Zhu, B. (2016). Ovarian cancer stem-like cells differentiate into endothelial cells and participate in tumor angiogenesis through autocrine CCL5 signaling. *Cancer Letters*, *376*(1), 137–147. https://doi.org/10.1016/J.CANLET.2016.03.034

Tang, X., Li, M., Tucker, L., & Ramratnam, B. (2011). Glycogen Synthase Kinase 3 Beta (GSK3β) Phosphorylates the RNAase III Enzyme Drosha at S300 and S302. *PLOS ONE*, *6*(6), e20391. https://doi.org/10.1371/JOURNAL.PONE.0020391

Tang, X., Zhang, Y., Tucker, L., & Ramratnam, B. (2010). Phosphorylation of the RNase III enzyme Drosha at Serine300 or Serine302 is required for its nuclear localization. *Nucleic Acids Research*, *38*(19), 6610–6619. https://doi.org/10.1093/NAR/GKQ547

Taylor, B. S., Schultz, N., Hieronymus, H., Gopalan, A., Xiao, Y., Carver, B. S., Arora, V. K., Kaushik, P., Cerami, E., Reva, B., Antipin, Y., Mitsiades, N., Landers, T., Dolgalev, I., Major, J. E., Wilson, M., Socci, N. D., Lash, A. E., Heguy, A., …

Gerald, W. L. (2010a). Integrative genomic profiling of human prostate cancer. *Cancer Cell*, *18*(1), 11–22. https://doi.org/10.1016/J.CCR.2010.05.026

Taylor, B. S., Schultz, N., Hieronymus, H., Gopalan, A., Xiao, Y., Carver, B. S., Arora, V. K., Kaushik, P., Cerami, E., Reva, B., Antipin, Y., Mitsiades, N., Landers, T., Dolgalev, I., Major, J. E., Wilson, M., Socci, N. D., Lash, A. E., Heguy, A., … Gerald, W. L. (2010b). Integrative Genomic Profiling of Human Prostate Cancer. *Cancer Cell*, *18*(1), 11–22. https://doi.org/10.1016/J.CCR.2010.05.026

Testa, J. R., Zhou, J. Y., Bell, D. W., & Yen, T. J. (1994). Chromosomal localization of the genes encoding the kinetochore proteins CENPE and CENPF to human chromosomes 4q24-->q25 and 1q32-->q41, respectively, by fluorescence in situ hybridization. *Genomics*, *23*(3), 691–693. https://doi.org/10.1006/GENO.1994.1558

Testa, U., Castelli, G., & Pelosi, E. (2019a). Cellular and Molecular Mechanisms Underlying Prostate Cancer Development: Therapeutic Implications. *Medicines 2019, Vol. 6, Page 82*, *6*(3), 82. https://doi.org/10.3390/MEDICINES6030082

Testa, U., Castelli, G., & Pelosi, E. (2019b). Cellular and Molecular Mechanisms Underlying Prostate Cancer Development: Therapeutic Implications. *Medicines 2019, Vol. 6, Page 82*, *6*(3), 82. https://doi.org/10.3390/MEDICINES6030082

Thakur, N., Sorrentino, A., Heldin, C. H., & Landström, M. (2009). TGF-β uses the E3-ligase TRAF6 to turn on the kinase TAK1 to kill prostate cancer cells. *Http://Dx.Doi.Org/10.2217/14796694.5.1.1*, *5*(1), 1–3. https://doi.org/10.2217/14796694.5.1.1

Tong, D. (2021). Unravelling the molecular mechanisms of prostate cancer evolution from genotype to phenotype. *Critical Reviews in Oncology/Hematology*, *163*, 103370. https://doi.org/10.1016/J.CRITREVONC.2021.103370

Toren, P., Kim, S., Cordonnier, T., Crafter, C., Davies, B. R., Fazli, L., Gleave, M. E., & Zoubeidi, A. (2015). Combination AZD5363 with Enzalutamide Significantly Delays Enzalutamide-resistant Prostate Cancer in Preclinical Models. *European Urology*, *67*(6), 986–990. https://doi.org/10.1016/J.EURURO.2014.08.006

Treiber, T., Treiber, N., Plessmann, U., Harlander, S., Daiß, J. L., Eichner, N., Lehmann, G., Schall, K., Urlaub, H., & Meister, G. (2017). A Compendium of RNA-Binding Proteins that Regulate MicroRNA Biogenesis. *Molecular Cell*, *66*(2), 270-284.e13. https://doi.org/10.1016/J.MOLCEL.2017.03.014

Udi, J., Schüler, J., Wider, D., Ihorst, G., Catusse, J., Waldschmidt, J., Schnerch, D., Follo, M., Wäsch, R., & Engelhardt, M. (2013). Potent in vitro and in vivo activity of sorafenib in multiple myeloma: induction of cell death, CD138-downregulation and inhibition of migration through actin depolymerization. *British Journal of Haematology*, *161*(1), 104–116. https://doi.org/10.1111/BJH.12226

Vaday, G. G., Peehl, D. M., Kadam, P. A., & Lawrence, D. M. (2006). Expression of CCL5 (RANTES) and CCR5 in prostate cancer. *The Prostate*, *66*(2), 124–134. https://doi.org/10.1002/PROS.20306

van den Bergh, R. C. N., Roemeling, S., Roobol, M. J., Aus, G., Hugosson, J., Rannikko, A. S., Tammela, T. L., Bangma, C. H., & Schröder, F. H. (2009). Outcomes of Men with Screen-Detected Prostate Cancer Eligible for Active Surveillance Who Were Managed Expectantly. *European Urology*, *55*(1), 1–8. https://doi.org/10.1016/J.EURURO.2008.09.007

van Dessel, L. F., van Riet, J., Smits, M., Zhu, Y., Hamberg, P., van der Heijden, M. S., Bergman, A. M., van Oort, I. M., de Wit, R., Voest, E. E., Steeghs, N., Yamaguchi, T. N., Livingstone, J., Boutros, P. C., Martens, J. W. M., Sleijfer, S., Cuppen, E., Zwart, W., van de Werken, H. J. G., … Lolkema, M. P. (2019). The genomic landscape of metastatic castration-resistant prostate cancers reveals multiple distinct genotypes with potential clinical impact. *Nature Communications 2019 10:1*, *10*(1), 1–13. https://doi.org/10.1038/s41467-019-13084-7

van Zandwijk, N., Pavlakis, N., Kao, S. C., Linton, A., Boyer, M. J., Clarke, S., Huynh, Y., Chrzanowska, A., Fulham, M. J., Bailey, D. L., Cooper, W. A., Kritharides, L., Ridley, L., Pattison, S. T., MacDiarmid, J., Brahmbhatt, H., & Reid, G. (2017). Safety and activity of microRNA-loaded minicells in patients with recurrent malignant pleural mesothelioma: a first-in-man, phase 1, open-label, dose-escalation study. *The Lancet. Oncology*, *18*(10), 1386–1396. https://doi.org/10.1016/S1470-2045(17)30621-6

Vangelista, L., & Vento, S. (2018). The Expanding Therapeutic Perspective of CCR5 Blockade. *Frontiers in Immunology*, *8*(JAN). https://doi.org/10.3389/FIMMU.2017.01981

Velasco-Velázquez, M., Jiao, X., De La Fuente, M., Pestell, T. G., Ertel, A., Lisanti, M. P., & Pestell, R. G. (2012). CCR5 antagonist blocks metastasis of basal breast cancer cells. *Cancer Research*, *72*(15), 3839–3850. https://doi.org/10.1158/0008-5472.CAN-11-3917

Villarino, A. V., Kanno, Y., & O'Shea, J. J. (2017a). Mechanisms and consequences of Jak–STAT signaling in the immune system. *Nature Immunology 2017 18:4*, *18*(4), 374–384. https://doi.org/10.1038/ni.3691

Villarino, A. V., Kanno, Y., & O'Shea, J. J. (2017b). Mechanisms and consequences of Jak–STAT signaling in the immune system. *Nature Immunology 2017 18:4*, *18*(4), 374–384. https://doi.org/10.1038/ni.3691

Wan, X., Huang, W., Yang, S., Zhang, Y., Zhang, P., Kong, Z., Li, T., Wu, H., Jing, F., & Li, Y. (2016). Androgen-induced miR-27A acted as a tumor suppressor by targeting MAP2K4 and mediated prostate cancer progression. *The International Journal of Biochemistry & Cell Biology*, *79*, 249–260. https://doi.org/10.1016/J.BIOCEL.2016.08.043

Wang, J., Kobayashi, T., Floc'h, N., Kinkade, C. W., Aytes, A., Dankort, D., Lefebvre, C., Mitrofanova, A., Cardiff, R. D., McMahon, M., Califano, A., Shen, M. M., & Abate-Shen, C. (2012). B-raf activation cooperates with PTEN loss to drive c-Myc expression in advanced prostate cancer. *Cancer Research*, *72*(18), 4765–4776. https://doi.org/10.1158/0008-5472.CAN-12-0820

Wang, S. W., Wu, H. H., Liu, S. C., Wang, P. C., Ou, W. C., Chou, W. Y., Shen, Y. S., & Tang, C. H. (2012a). CCL5 and CCR5 interaction promotes cell motility in human osteosarcoma. *PloS One*, *7*(4). https://doi.org/10.1371/JOURNAL.PONE.0035101

Wang, S. W., Wu, H. H., Liu, S. C., Wang, P. C., Ou, W. C., Chou, W. Y., Shen, Y. S., & Tang, C. H. (2012b). CCL5 and CCR5 Interaction Promotes Cell Motility in Human Osteosarcoma. *PLOS ONE*, *7*(4), e35101. https://doi.org/10.1371/JOURNAL.PONE.0035101

Wang, Z., Wang, Y., Zhang, J., Hu, Q., Zhi, F., Zhang, S., Mao, D., Zhang, Y., & Liang, H. (2017). Significance of the TMPRSS2: ERG gene fusion in prostate cancer. *Molecular Medicine Reports*, *16*(4), 5450–5458. https://doi.org/10.3892/MMR.2017.7281/HTML

Wani, N. A., Nasser, M. W., Ahirwar, D. K., Zhao, H., Miao, Z., Shilo, K., & Ganju, R. K. (2014). C-X-C motif chemokine 12/C-X-C chemokine receptor type 7 signaling regulates breast cancer growth and metastasis by modulating the tumor microenvironment. *Breast Cancer Research : BCR*, *16*(3). https://doi.org/10.1186/BCR3665

Wee, Z. N., Li, Z., Lee, P. L., Lee, S. T., Lim, Y. P., & Yu, Q. (2014). EZH2-Mediated Inactivation of IFN-γ-JAK-STAT1 Signaling Is an Effective Therapeutic Target in MYC-Driven Prostate Cancer. *Cell Reports*, *8*(1), 204–216. https://doi.org/10.1016/J.CELREP.2014.05.045

Wightman, B., Ha, I., & Ruvkun, G. (1993). Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. *Cell*, *75*(5), 855–862. https://doi.org/10.1016/0092-8674(93)90530-4

Wu, F., Li, J., Guo, N., Wang, X. H., & Liao, Y. Q. (2017). MiRNA-27a promotes the proliferation and invasion of human gastric cancer MGC803 cells by targeting SFRP1 via Wnt/β-catenin signaling pathway. *American Journal of Cancer Research*, *7*(3), 405. /pmc/articles/PMC5385632/

Wu, H., Ye, C., Ramirez, D., & Manjunath, N. (2009). Alternative Processing of Primary microRNA Transcripts by Drosha Generates 5′ End Variation of Mature microRNA. *PLOS ONE*, *4*(10), e7566. https://doi.org/10.1371/JOURNAL.PONE.0007566

Wu, X., Bhayani, M. K., Dodge, C. T., Nicoloso, M. S., Chen, Y., Yan, X., Adachi, M., Thomas, L., Galer, C. E., Jiffar, T., Pickering, C. R., Kupferman, M. E., Myers, J. N., Calin, G. A., & Lai, S. Y. (2013). Coordinated targeting of the EGFR signaling

axis by microRNA-27a*. *Oncotarget*, *4*(9), 1388–1398. https://doi.org/10.18632/ONCOTARGET.1239

Xiang, P., Jin, S., Yang, Y., Sheng, J., He, Q., Song, Y., Yu, W., Hu, S., & Jin, J. (2019). Infiltrating CD4+ T cells attenuate chemotherapy sensitivity in prostate cancer via CCL5 signaling. *The Prostate*, *79*(9), 1018–1031. https://doi.org/10.1002/PROS.23810

Xu, Q., Tong, J. L., Zhang, C. P., Xiao, Q., Lin, X. L., & Xiao, X. Y. (2017). miR-27a induced by colon cancer cells in HLECs promotes lymphangiogenesis by targeting SMAD4. *PLOS ONE*, *12*(10), e0186718. https://doi.org/10.1371/JOURNAL.PONE.0186718

Yan, Y., Sheppard, P. C., Kasper, S., Lin, L., Hoare, S., Kapoor, A., Dodd, J. G., Duckworth, M. L., & Matusik, R. J. (1997). *Large Fragment of the Probasin Promoter Targets High Levels of Transgene Expression to the Prostate of Transgenic Mice*. https://doi.org/10.1002/(SICI)1097-0045(19970701)32:2

Yang, Y., Hou, H., Haller, E. M., Nicosia, S. V., & Bai, W. (2005). Suppression of FOXO1 activity by FHL2 through SIRT1-mediated deacetylation. *The EMBO Journal*, *24*(5), 1021–1032. https://doi.org/10.1038/SJ.EMBOJ.7600570

Yeh, C. R., Slavin, S., Da, J., Hsu, I., Luo, J., Xiao, G. Q., Ding, J., Chou, F. J., & Yeh, S. (2016). Estrogen receptor α in cancer associated fibroblasts suppresses prostate cancer invasion via reducing CCL5, IL6 and macrophage infiltration in the tumor microenvironment. *Molecular Cancer*, *15*(1). https://doi.org/10.1186/S12943-015-0488-9

Yu, Z., Chen, S., Sowalsky, A. G., Voznesensky, O. S., Mostaghel, E. A., Nelson, P. S., Cai, C., & Balk, S. P. (2014). Rapid induction of androgen receptor splice variants by androgen deprivation in prostate cancer. *Clinical Cancer Research*, *20*(6), 1590–1600. https://doi.org/10.1158/1078-0432.CCR-13-1863/442147/AM/RAPID-INDUCTION-OF-ANDROGEN-RECEPTOR-SPLICE

Zedan, A. H., Hansen, T. F., Assenholt, J., Pleckaitis, M., Madsen, J. S., & Osther, P. J. S. (2018). microRNA expression in tumour tissue and plasma in patients with newly diagnosed metastatic prostate cancer. *Tumour Biology : The Journal of the International Society for Oncodevelopmental Biology and Medicine*, *40*(5). https://doi.org/10.1177/1010428318775864

Zeller, K. I., Zhao, X. D., Lee, C. W. H., Kuo, P. C., Yao, F., Yustein, J. T., Hong, S. O., Orlov, Y. L., Shahab, A., How, C. Y., Fu, Y. T., Weng, Z., Kuznetsov, V. A., Sung, W. K., Ruan, Y., Dang, C. V., & Wei, C. L. (2006). Global mapping of c-Myc binding sites and target gene networks in human B cells. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(47), 17834–17839. https://doi.org/10.1073/PNAS.0604129103/SUPPL_FILE/04129TABLE8.XLS

Zeng, Z., Lan, T., Wei, Y., & Wei, X. (2022). CCL5/CCR5 axis in human diseases and related treatments. *Genes & Diseases*, *9*(1), 12. https://doi.org/10.1016/J.GENDIS.2021.08.004

Zhang, F., Arnatt, C. K., Haney, K. M., Fang, H. C., Bajacan, J. E., Richardson, A. C., Ware, J. L., & Zhang, Y. (2012). Structure activity relationship studies of natural product chemokine receptor CCR5 antagonist anibamine toward the development of novel anti prostate cancer agents. *European Journal of Medicinal Chemistry*, *55*, 395–408. https://doi.org/10.1016/J.EJMECH.2012.07.049

Zhang, J., Cao, Z., Yang, G., You, L., Zhang, T., & Zhao, Y. (2019). MicroRNA-27a (miR-27a) in Solid Tumors: A Review Based on Mechanisms and Clinical Observations. *Frontiers in Oncology*, *9*, 484245. https://doi.org/10.3389/FONC.2019.00893/BIBTEX

Zhang, L., Zhang, X. W., Liu, C. H., Lu, K., Huang, Y. Q., Wang, Y. D., Xing, L., Zhang, L. J., Liu, N., Jiang, H., Sun, C., Yang, Y., Chen, S. Q., Chen, M., & Xu, B. (2016). miRNA-30a functions as a tumor suppressor by downregulating cyclin E2 expression in castration-resistant prostate cancer. *Molecular Medicine Reports*, *14*(3), 2077–2084. https://doi.org/10.3892/MMR.2016.5469

Zhang, Q., Liu, S., Zhang, Q., Xiong, Z., Wang, A. R., Myers, L., Melamed, J., Tang, W. W., & You, Z. (2014). Interleukin-17 promotes development of castration-resistant prostate cancer potentially through creating an immunotolerant and pro-angiogenic tumor microenvironment. *The Prostate*, *74*(8), 869–879. https://doi.org/10.1002/PROS.22805

Zhao, R., Bei, X., Yang, B., Wang, X., Jiang, C., Shi, F., Wang, X., Zhu, Y., Jing, Y., Han, B., Xia, S., & Jiang, Q. (2018). Endothelial cells promote metastasis of prostate cancer by enhancing autophagy. *Journal of Experimental & Clinical Cancer Research : CR*, *37*(1). https://doi.org/10.1186/S13046-018-0884-2

Zhou, B., Sun, C., Li, N., Shan, W., Lu, H., Guo, L., Guo, E., Xia, M., Weng, D., Meng, L., Hu, J., Ma, D., & Chen, G. (2016). Cisplatin-induced CCL5 secretion from CAFs promotes cisplatin-resistance in ovarian cancer via regulation of the STAT3 and PI3K/Akt signaling pathways. *International Journal of Oncology*, *48*(5), 2087–2097. https://doi.org/10.3892/IJO.2016.3442

Zhou, L., Liang, X., Zhang, L., Yang, L., Nagao, N., Wu, H., Liu, C., Lin, S., Cai, G., Liu, J., Zhou, L., Liang, X., Zhang, L., Yang, L., Nagao, N., Wu, H., Liu, C., Lin, S., Cai, G., & Liu, J. (2016). MiR-27a-3p functions as an oncogene in gastric cancer by targeting BTG2. *Oncotarget*, *7*(32), 51943–51954. https://doi.org/10.18632/ONCOTARGET.10460

Zhu, Y., Dalrymple, S. L., Coleman, I., Zheng, S. L., Xu, J., Hooper, J. E., Antonarakis, E. S., De Marzo, A. M., Meeker, A. K., Nelson, P. S., Isaacs, W. B., Denmeade, S. R., Luo, J., Brennen, W. N., & Isaacs, J. T. (2020). Role of androgen receptor splice variant-7 (AR-V7) in prostate cancer resistance to 2nd-generation androgen

receptor signaling inhibitors. *Oncogene 2020 39:45*, *39*(45), 6935–6949. https://doi.org/10.1038/s41388-020-01479-6

Zhu, Y., Liu, C., Nadiminty, N., Lou, W., Tummala, R., Evans, C. P., & Gao, A. C. (2013). Inhibition of ABCB1 expression overcomes acquired docetaxel resistance in prostate cancer. *Molecular Cancer Therapeutics*, *12*(9), 1829–1836. https://doi.org/10.1158/1535-7163.MCT-13-0208

Zoubeidi, A., Zardan, A., Wiedmann, R. M., Locke, J., Beraldi, E., Fazli, L., & Gleave, M. E. (2010). Hsp27 promotes insulin-like growth factor-I survival signaling in prostate cancer via p90Rsk-dependent phosphorylation and inactivation of BAD. *Cancer Research*, *70*(6), 2307–2317. https://doi.org/10.1158/0008-5472.CAN-09-3252

7. ETHICAL DECLARATION

The tumor samples used in the framework of this thesis were obtained from the *in vivo* prostate cancer model generated with the project titled " Atimik nude farelerde prostat kanseri modeli oluşturulması", which was approved by Bezmialem Vakıf University Experimental Animals Local Ethical Committee.

BEZMİÂLEM VAKIF ÜNİVERSİTESİ

T.C.
BEZMİÂLEM VAKIF ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURULU
KARAR METNİ

SAYI: 2021/262 22.11.2021
KONU: Sn. Vet. Hek. Mert ÇELİKTEN

Sayın, Vet. Hek. Mert ÇELİKTEN

"Atimik Nude Farelerde Prostat Kanseri Modeli Oluşturulması" başlıklı projenize ait başvurunuz 22.11.2021 tarihinde yapılan Yerel Etik Kurul toplantısında değerlendirilmiş ve onanmıştır.

Çalışılacak Hayvanın	Türü	Atimik Nude Fare
	Sayısı	42

Prof. Dr. Fahri AKBAŞ
Etik Kurul Başkanı

Doç. Dr. Birsen ELİBOL
Üye

Doç. Dr. Özgün Yusuf ÖZYILMAZ
Üye

Doç. Dr. Remzi DOĞAN
Üye

Doç. Dr. Ömer Faruk ÖZER
Üye

Dr. Öğr. Üye. Falemeh BAHADORİ
Üye

KATILMADI
Dr. Öğr. Üye. Ömer UYSAL
Üye

KATILMADI
Vet. Hek. Mert ÇELİKTEN
Üye

Şerife GÖNCÜ
Üye

Semiha NİŞANCI
Üye