Use of miRNA as a Biomarker in Prostate Cancer and New Approaches

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SUMMARY

Prostate cancer has been found to be the most life-threatening disease among men all over the world, given its wide occurrence and partially-successful therapies associated with a high rate of mortality. Thus, meticulous analysis of this cancer and its characteristics, including molecular biology, epigenetic mechanisms and markers during tumor development, may provide the scientists with valuable insights to design the therapeutic protocol with improved efficiency and low rate of failure and limited further side effects, such as infertility. Moreover, the risk associated with the current invasive procedures on prostate cancer patients has prompted researchers to invest effort in the discovery of being less-invasive and more advantageous procedures based on the patient's own physiological and anatomical characteristics. This paper reviews past and present studies on epigenetics and molecular markers of prostate cancer, as well as the designed therapies. Additionally, we present a future vision and prospect of the current treatments.

Keywords: CRISPR-Cas; epigenetic mechanisms; miRNA; prostate cancer.

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Introduction

Prostate cancer (PC), unlike many other cancers, such as lung cancer, has been posing a huge threat to men due to lack of its connection with carcinogenic chemicals and heterogeneity of prostate glands which has made it rather difficult to analyze the sole cause of cancer and eventually efficient precaution that arises from constraint causative factors.[1] PC has been in existence since ancient times; hence, the development of prostatectomy, a surgical means for PC treatment, has been achieved for more than a century.[2] PC is described as a heterogeneous type of cancer that exhibits various forms, from the slow-growing tumors to fast-growing tumors, which gradually turns out to be fatal. Years of studies and analysis have provided

a rather more convenient way for the diagnosis of PC. Prostate-specific antigen (PSA) blood test is performed to determine the presence of kallikrein-related serine protease, the enzyme which is normally found in prostate secretion, but found in the bloodstream due to functional and histological anomaly of the prostate, and helps to diagnose PC in early stages for further treatment.[3]

Human serine proteases are members of human tissue kallikrein (KLK) family, which contain 15 homologues serine proteases (KLK1-KLK15). This family is encoded by the largest clustered human genome located on chromosome 19 [4], out of which KLK2 and KLK3 are found only in prostate and KLK3 with 33-kDa weight are considered as the biomarker (PSA) in PC screening.[4,5] Discovery of PSA dates back to 1979 when it was found in prostatic tissues.[6] Apart

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from contributing to cancer diagnosis, KLK3 is the main modulator of sperm status in terms of semen liquefaction and coagulation.[7] Genetic polymorphism of KLK3 has also been shown to contribute to the high risk of male infertility as a degrading factor to semenogelin.[8] However, KLK3 activation and defragmentation are controlled by the KLK14 function, which was detected by analysis of KLK3 fragments invitro using recombinant KLK14.[9]

The cancer is initiated and developed in the prostate gland, part of the male reproductive system responsible for semen production in the male body and located inferior to the bladder.[10] Approximately 98% of the PCs are reported to be of glandular origin.[11] This organ is made of epithelial cell clusters arranged in the form of the basal layer and contains stem cells, transit-amplifying cells (TACs), and committed basal (CB) cells as the most dominant cell population in this structure.[12] Based on histological analysis of prostate organ, stem cells undergo division to form TA cells, which further divide by mitosis and differentiate into CB cells, the only cell type in prostate capable of DNA synthesis and proliferation, which are finally transformed into differentiating luminal cells in human.[13] PC was observed with a lack of or disruptive basal layer along with the disruption of the above-mentioned cell number present in the prostate gland.[9] Such an irregular growth of stem cells was first noticed in 1989.[13]

Stem cells characterization, collected by biopsy of patients, were carried out using CD44$^+$ $\alpha_2\beta$1hi CD133$^+$ markers as the phenotype, which unfortunately was not specific for only cancer stem cells (CSCs).[14] Additionally, PAP (prostatic acid phosphate) was found to not be fully special PC diagnosis since PAP was found to be elevated in benign prostatic hyperplasia (BPH) and other organ's malfunctions, such as the liver.[15]

This paper reviews past and present studies on epigenetics and molecular markers of PC, as well as the designed therapies. Additionally, we present a future vision and prospect of the current treatments.

miRNA and DNA Methylation in PC

Studies on cancer show that epigenetic mechanisms are effective above cancer prognosis. miRNAs have been demonstrated that play a role in epigenetic mechanisms, such as DNA methylation and histone deacetylation (HDAC).[16,17]

There are regions called CpG islands, which are located there high rate repetition cytosine and guanine base pairs in the human genome. CpG islands mostly take part in promotor regions of a gene. In these regions, methylation occurs when attaching methyl (-CH3) to cytosine's fifth carbon. Abnormal situations, such as hypomethylation and hypermethylation, cause fall into decay cell functions, such as cell differentiation, cell division, and cell proliferation.[18,19]

Recent studies related to miRNA show that miRNA genes are situated near to CpG islands. In conclusion, methylation of the CpG islands will affect the expression of miRNAs.[20-22]

We can denote miRNA-152 as a sample to relationship miRNA and DNA methylation. In normal conditions, miRNA-152 prevent the expression of DNA (cytosine-5)-methyltransferase 1 (DNTM1) by targeting 3' UTR region of the DNMT1 gene. When DNMT1 expression and DNA methylation are lower, the gene is an expression. Studies with cell lines of PC show that methylation depending on increase DNMT1 expression down-regulate miRNA-152. Thus, the gene expression that serves a function in cell differentiation, cell division, and cell proliferation is prevented.[23,24]

miRNA and Histone Deacetylation in PC

miRNA and HDAC relationship is affecting the regulatory roles of epigenetic mechanism.[25] Change of electrostatic force in histone tails affects their binding to DNA. To illustrate the positive charge of lysine, amino acid in the amino tail is neutralized with acetylation of histone protein. Consequently, region binding to DNA of the histone protein is free, and the region is clarified to transcription factors, which causes expression in the region.[26] Two enzyme family function in here. One of them is acetyltransferase (HAT). The other is a histone deacetylase (HDAC). While HAT enzymes are acetylating the region, HDAC enzymes deacetylate the same region. This reversible situation identifies whether it will be an expression of the region. The breakdown of equilibrium between two enzymes brings about the malignity of the cell.[27] Table 1 shows HDAC classification.[28]

We can denote miRNA-449a as a sample to relationship miRNA and HDAC. In normal conditions, when expression miRNA-449, its down-regulate HDAC1, which brings on activation of p27/kip1 transcription, which causes cell proliferation. Studies with cell lines of PC show that down-regulation of miRNA-449a cause to up-regulation of HDAC1. Because of the HDCA1, up-regulation activation of p27/kip1 transcription causes cell proliferation out of control.[29]

Genetic Propensity and Markers of PC

Various genome-wide association studies (GWASs) implicate single-nucleotide polymorphism (SNP)

Aslan et al.
Use of miRNA as a Biomarker in Prostate Cancer and New Approaches

101

Table 1 HDAC classification [28]

Group	Class	Name	Cellular localization	Localization in body
Classic (Zinc-dependent)	Class I	HDAC1	Nucleus	Everywhere
		HDAC2		
		HDAC3		
		HDAC8		
	Class II	HDAC4	Nucleus/Cytoplasma	Tissue-spesific
		HDAC5		
		HDAC7		
		HDAC9		
	Class IIb	HDAC6	Cytoplasma	Tissue-spesific
		HDAC10		
	Class IV	HDAC11	Nucleus/Cytoplasma	Tissue-spesific
NAD-dependent	Class III	SIRT(I-7)	Nucleus/Cytoplasma	

NAD: Nicotinamide adenine dinucleotide; SIRT: Sirtuin

Table 2 Single nucleotide polymorphism region with PC [31]

Chromosomal region	Alternative alleles	Associated allele
17q12	T, C	T
17q12	G, A	G
17q12	A, C	C
17q24.3	G, T	G
17q24.3	C, T	T
17q24.3	A, G	A
17q24.3	A, G	A
8q24 (region 1)	C, A	A
8q24 (region 1)	G, A	A
8q24 (region 1)	A, C	C
8q24 (region 1)	C, T	T
8q24 (region 1)	G, T	T
8q24 (region 2)	A, C	C
8q24 (region 2)	C, A	A
8q24 (region 3)	G, T	G
8q24 (region 3)	C, T	T

to be responsible for PC initiation.[30,31] (Table 2). Although previous studies showed specific mutations on chromosome 15 and 19 to be responsible for aggressive PC [32,33], another genome analysis study has reported some genes with a mutation in PC (Table 3). Unlike most of the cancer types, various analyses indicate no specific oncogene connected to PC [68] although some oncogene exhibit overexpression in some cancers observed during anti-androgen therapy. C-ERB-B2 oncogene, located on chromosome 17, appears to be elevated in hormone-refractory PC in response to anti-androgen therapy.[69] Moreover, EZH2 mRNA has shown a significant peak due to EZH2 protein encoded by EZH2 oncogene, which acts as a repressor of transcription and causes cell proliferation in PC.[70] Additionally, retinoblastoma (RB) proteins seem to be reduced in malignant PC, which caused tumor cell proliferation.[71]

P53, also known as TP53, which protects against tumorgenesis, is observed to be increased in malignant and untreated PC, rather than primary or treated ones. [72] Some studies have tested wild type (WT) P53 with mutated P53 using DU145 cells (human prostate cancer cell lines) in response to chemotherapeutic drugs and observed that P53 increased tumor cells' susceptibility to drugs which could indicate high survival rate of prostatic cancer cells during chemotherapies when P53 is silenced or non-functional.[73] On the account of tumour suppressor genes being silenced resulting in PC cell's resistance, the other factor, over activation of androgen receptor (AR), plays significant role in development of castrate-resistant prostate cancer [74] due to hypermethylation of CpG islands (sequence of 5'-CG-3') [75] on AR genes and proven to be not related to androgen level which was observed during anti-androgen therapy in PC patients.[76,77]

miRNAs as Biomarkers in PC
microRNAs, also known as miRNAs, are small, non-coding endogenous RNA molecules that are found in eukaryotes, as well as some viruses. They have been found to play a key role in RNA regulations and diagnosis of various diseases on account of their circulation in body fluids.[78] Recent studies on miRNA transcripts have demonstrated their significance as

Table 3   Common genetic aberrations in PC

Gene	Genomic alterations	Chromosomal region
APC	Deletion	5q22.2 [33]
AR	Amplification/mutations/splicing variants	Xq12 [34,35]
ATM	Deletion/mutation	11q22.3 [36,37]
BRCA1	Deletion/mutation	17q21.31 [38]
BRCA2	Deletion/mutation	13q13.1 [39]
CHD1	Deletion	5q21.1 [40,41,42]
ERF	Deletion/mutation	19q13.2 [43,44]
ERG	Fusion/deletion	21q22.2 [45]
ETS2	Deletion	21q22.2 [33]
ETVs	Fusion/deletion	NA [46]
EZH2	Mutation	14q21.1 [47]
FOXA1	Mutation	14q21.1 [40,48]
IDH1	Mutation	2q34 [49]
KMT2A (MLL1)	Mutation/deletion	11q23.3 [50]
KMT2C (MLL3)	Mutation/deletion	7q36.1 [39]
KMT2D (MLL2)	Mutation/deletion	12q13.12 [51]
KDM1A (LSD1 [lysine-specific demetthylase 1])	Mutation/deletion	1p36.12 [52]
KDM3A (JMKD1A)	Mutation/deletion	2p11.2, Xp11.3 [53]
KDM6A (UTX)		
MYCN	Amplification	2p24.3 [54,55,56]
NCOR1	Deletion/mutation	17p11.2 [57]
NCOR2	Deletion/mutation	12q24.31 [58]
NKX3-1	Deletion	8p21.2 [59,60]
PTEN	Deletion/mutation	10q23.31 [33,40,61]
RB1	Deletion/mutation	13q14.2 [51,62]
SMAD4	Deletion/mutation	18q21.2 [63,64]
SPOP	Mutation	17q21.33 [40,65,66]
TP53	Deletion/mutation	17p13.1 [40,51,67]

PC: Prostate cancer; APC: Adenomatous polyposis coli; AR: Androgen receptor; ATM: ATM serine/threonine kinase; BRCA1: Breast cancer type 1 susceptibility protein; BRCA2: Breast cancer type 2 susceptibility protein; CADH1 E-Cadherin; CHD1: Chromodomain Helicase DNA Binding Protein 1; ERF: erythroblast transformation-specific 2 (ETS2) repressor factor; ERG: ETS-related gene; ETV1: ETS variant transcription factor 1; EZH2: Enhancer of zeste homolog 2; FOXA1: forkhead boxA1; IDH1: Isocitrate dehydrogenase 1; IDH1: Isocitrate dehydrogenase 1; KMT2A (MLL1): Lysine (K)-Specific Methyltransferase 2A; KMT2C (MLL3): Lysine (K)-Specific Methyltransferase 2C; KMT2D(MLL2): Lysine (K)-Specific Methyltransferase 2D; KDM1A (LSD1): lysine (K)-specific demethylase 1A; KDM3A (JMKD1A): lysine (K)-specific demethylase 3A; KDM6A (UTX): lysine (K)-specific demethylase 6A; MYCN: N-mycproto-oncogene protein; NCOR1: Nuclear receptor corepressor 1; NCOR2: Nuclear receptor corepressor 2; NX3-1: Homeobox protein Nkx-3.1; PTEN: phosphatase and tensin homolog; RB1: Retinoblastoma1; SMAD4: SMAD family member 4; SPOP: Speckle-type POZ protein; TP53: tumor protein p53

PC biomarkers. MicroRNA-21, also known as miR-21, mammalian miRNA encoded by the MIR21 gene located on chromosome 17, has been observed to suppress PTEN (tumor suppressor gene) in various cancers, such as colorectal and breast cancer, [79,80] but no indication of its direct effects on PC. Yang et al. conducted an experiment on the effects of miR-21 on PC cells using PC-3 cell lines, transfecting the cells with miR-21. They observed that in the presence of matured miR-21, which is generally conserved in mammalian cells, PC cells evade immune defence due to suppression of PTEN by miR-21, which results in cell growth and proliferation and eventually metastasis.[81] Based on the above-mentioned anal-

ysis, miR-21 has been introduced as a PC biomarker, which needs further studies and possible epigenetic influence on cancer cells.

Up-regulation of miR-21 has been found to be correlated with down-regulation of miR-15 and mirR-16 as well as overexpression of TGF-β signalling due to suppression of SMAD7 (Mothers against decapentaplegic homolog 7 leads to degradation of TGF-β and inhibits hedgehog signalling), which leads to AR hyper-activation result in the bone lesion in PC.[82] Moreover, several studies showed the functional influence of miR-221 and miR-222 on tumorigenesis and cancer progression in various cancers, including PC.[83-86] In-vivo and in-vitro studies of PC3 cells

Aslan et al.
Use of miRNA as a Biomarker in Prostate Cancer and New Approaches

103

have shown that reduction of cancer cell proliferation occurs when PC cells are induced with miR-221 and miR-222 inhibitors, which implicates miR-221/222 increasing expression in PC and its metastasis.[87]

Other miRNAs expressed rather significant volume changes in response to tumurgenesis. To demonstrate, miR-152-3p in LNCaP and PC3 were analyzed *in vitro*. The result indicated a low miR-152-3p level of tumour cells compared to healthy control cells. However, the same analysis experimented with the effects of high-levelmiR-152-3p mimic on tumour cells, which resulted in a great deal of proliferation and metastasis presenting external effects of the above-mentioned miRNA on tumurogenesis.[88] In addition to miRNA's direct effect on tumour progression, their function harbours inactivation or reduction of tumursupressors, such as tetraspanin, particularly tetraspanin CD4, which is found to suppress tumor progression and metastasis. In-vitro studies of miR-518f-5p on PC3 cells proved their down-regulating effects on CD4 leading to cancer progression and metastasis.[89] Table 4 shows that the role of miRNAs in PC.

In PC cancer patients, cfDNA (cell-free DNA) is released from apoptotic cells as nucleosomes from both healthy and diseased tissue that includes tumor cells, as well as microbial nucleic acids from systemic infections.[107] The regional chromosomal ploidy heterogeneity detected in cfDNA is an early indicator of progressive genomic instability. The following result indicated that cfDNA in serum could be used as a remarkable biomarker to distinguish PC from benign prostatic hypertrophy and prostatitis.[108]

Prostate Cancer Therapy

Over the past few decades, PC has attracted the attention of researchers working in this area considering its mortality rate in men, specifically in developed countries. Therefore, efforts have increased towards an efficient means of therapy.[109,110]

Apoptosis as a Cancer Therapy (Apoptosis as Therapeutic means in PC?)

One of the major observations on cancer cells is their evasion from apoptosis. Studies demonstrate the presence of Fas and FasL (Fas ligand) mediating apoptosis in PC cells. However, resistance to such apoptosis was observed.[109,110] Various in-vitro studies on PC cells show an increase in FADD (Fas-associated protein with death domain) and eventual apoptosis using 5-Azacitine combined with BCLT (bicalutamide) to guide the cancer cells to death path and block further progress and metastasis.[76]

Table 4   The role of miRNAs in PC

miRNA	Roles in pathogenesis	Type
miR-145 [90,91]	Apoptosis, Metastasis	Dysregulation tumor supressor
Let7c [92]	Apoptosis, cell proliferation, cell cycle	Dysregulation tumor supressor
miR-125b [93,94] (p53 and PUMA targeted by miR-125b)	Apoptosis, tumor stage and perineural invasion status	Dysregulation tumor supressor
miR-221/222 [92] (3′ UTR of ARH1 targeted by miR-222)	Metastasis, cell cycle	Dysregulation tumor supressor
miR-21 [95]	Invasion, metastasis, apoptosis	Dysregulation tumor supressor
miR-106a (cluster resides at Xq26.2 chromosomal localization) [92]	Cell cycle	Dysregulation tumor supressor
miR-27a (cluster resides at chromosome 19) [96]	Cell proliferation	Upregulations result in the downregulation of prohibitin
miR-34c (localized at chromosome 11q23) [97,98,99]	Metastasis	Dysregulated tumor supressor
miR-205 [100]		Downregulation
miR-29 [101]	targets DNA methyl transferases either directly or indirectly	Silenced tumor supressor genes
miR-221 [102]	Tumor progression and metastasis	Downregulation
miR-203 [103]	metastasis	Regulates the expressipn of prometastatic genes
miR-375, miR-9, miR-141, miR-200b, miR-516a-39 [104]	Potential roles in prognosis?	Potential roles in prognosis?
miR-26a, miR-195, let7 [105,106]	Potential roles in prognosis?	Potential roles in prognosis?

PC: Prostate cancer

Genetic Material into a Target Cell as Cancer Therapy

Gene therapy, defined as the introduction of genetic material into a target cell for therapeutic benefit, is a very promising treatment for many diseases, including cancer. To date, more than 2000 clinical trials employing gene transfer have taken place, and in general, many vehicles or vectors have been established as safe.[111,112] Various techniques and approaches are employed to target the altered genes in PC, such as using siRNA (small interfering RNA), antisense oligonucleotides and plasmid DNA, along with chemotherapy to achieve PC apoptosis and overcome multidrug resistance conditions that arise from various drug-therapies.[113,114]

Re-activation of Tumor Growth as a Cancer Therapy

In prostate tumours, re-activation of tumor growth has been observed in receptor-dependent androgen state, which may induce apoptosis of these cells by pro-apoptotic over-expressing tumor necrosis factor (TNF).[115] At the same time silence anti-apoptotic c-FLIP, allowing the development of a pro-apoptotic therapy which acts downstream and independently of androgen receptor (AR) signalling.[115] Analysis of PC cells has shown that the cells that express resistance towards castration-therapy also express a lower degree of polyphosphate-4-phosphatase type II (INPP4B), whose function includes potential tumor suppressor and a cascade activation which results in the negative control of phosphatidylinositol 3-kinase (PI3K) pathway leading to inhibition of cell malignant transformation. To prove the following findings, the PC cell line was transfected with the INPP4B gene, which evidently demonstrated a significant reduction in cancer cell viability and in a decrease in pAKT levels that down-regulates the PI3K pathway.[116] In addition to previous findings, some research experimented effects of an RNA-aptamer by transferring it into LNCaP and PC-3 and observed expression of genes that are observed phenotypically in normal prostate cells leading PC cells towards the apoptosis path and anti-proliferation mechanism and an eventually positive prognosis.[117]

Gene Editing and Activation Tumor suppressor Genes with Silencing RNA

Short interfering or silencing RNA (siRNA) was found to enhance the gene editing and activation of tumor suppressor genes and suppression of anti-apoptotic proteins in PC therapy. In a study, they transfected PC cell lines with siRNA molecules, treated with atecol-lagen, which facilitates the absorption of siRNA into tumor cells. The aim of this experiment was to reduce expression anti-apoptotic protein Bcl-xL, which is over expressed in PC both *in vitro* and *in vivo*.[118] Moreover, viruses have been found to express a rather efficient tool as vectors in PC therapy for the purpose of gene editing. For instance, baculoviruses are tested to deliver genes in a variety of tissues and cells, including benign prostate and PC cells line. Moreover, they can introduce therapeutic genes into dividing or quiescent cells, indicating that it might also act in tumor stem cells of PC.[119]

Adenoviral Vector- mediated Gene Therapy

Some other studies observed the effects of adenoviral vector- mediated gene therapy followed by radiotherapy, which was applied to 178-2 BMA and TSUPr1 cells *in vitro* to observe the colony formation of cells and their apoptosis process which led to tumor control with higher efficiency.[120]

Arafat et al. experimented the same combined (adenovirus and radiotherapy) or sole effect of using other types of adenoviruses as a vector (TRA-8 or Ad TRAIL (adenoviral encoding TRAIL)) in Human PC cell lines (LNCaP, PC-3 and DU-145) *in vitro* and colony-forming assay and RT-PCR were employed to determine cells' growth and genetic changes. In addition to *in vitro* studies, *in vivo* study using murine injected subcutaneously with PC cells was carried out which presented higher efficiency and lower cancer cell survival in combination therapy (vector combined with radiotherapy) rather than single treatment only Evaluated by elevation of BAX protein (responsible for apoptosis) and eventually apoptosis of cancer cells.[121]

miRNAs as Therapeutic Means in PC

As previously discussed, miRNAs play a vital role in PC recognition and diagnosis. However, their function is far more than just a biomarker. Various studies analyzed different miRNAs' effects in the treatment of PC in-vitro, *in vivo* and in silico.[122] The function of mature miRNA is achieved by seed sequence (short sequence located upstream of 5` end of miRNA) in miRNA, which binds to the complementary motif in UTR (untranslated region) of targeted mRNA. For miRNA to be a target of therapy, they must present adequate stability inside and outside the cells, as well as show pharmacokinetic factors which would be appropriate to cell and environmental condition *in vitro* and *in vivo*. Different approaches have been designed and considered to use miRNA in PC therapy by either

Aslan et al.
Use of miRNA as a Biomarker in Prostate Cancer and New Approaches

105

using complementary oligonucleotides targeting and preventing the function of specific miRNA [123,124] or transfecting PC cells with multiple miRNA-specific complementary sequences harboured in vector.[125]

ASOs (antisense oligonucleotides) via liposomal complexes and SMIR (small molecule inhibitors of miRNAs), which are designed based on the function of other molecules used for other disease therapies. They are used to target miRNAs. miRNAs have the ability to inhibit function other miRNAs that express the same see sequence, which is not seen with ASOs and is considered as an advantage of using miRNAs over antisense oligonucleotides. The SMIR approach aims to find compounds that bind and subsequently decrease the levels of mature miRNAs; however, such compounds can principally function to prevent transcription.[126]

Scientists designed other types of miRNA, known as miRNA sponge, which consists of numbers of binding sites (4-10) containing antisense or mismatched nucleotides, which will result in cleavage by argonaute RNA-induced silencing complex (RISC) when completely base-paired. The efficacy of miRNA sponge lies in two factors as follows: the binding site affinity and the ratio of miRNA sponge concentration to the ratio of mRNA targets.[126] PC cells were transfected with sponge miRNAs using plasmid *in vitro* and viral vector *in vivo* and a reporter gene for monitoring the functional changes and their actions on target miRNAs.[127] Some groups tested various sponge miRNAs and found that most of them, specifically pseudogenes, function as ceRNAs. They compete with target mRNAs for miRNA binding, making them endogenous sponges. The Phosphatase and tensin homolog (PTEN) pseudogene was the first demonstrated example, but since then, other pseudogenes and long non-coding RNAs have been shown to be valid ceRNAs.[128,129] However, various pseudogenes demonstrated particularly different functions in cancer cells. For instance, NANOGP8 pseudogene is responsible for malignancy and metastasis and PC, and research studies indicate that the knock-out or deactivation of this pseudogene can benefit from the positive prognosis of PC therapy greatly.[130]

Studies have reported miR-185 as a tumor suppressor factor, was observed to be down-regulated in PC. Therefore, studies have been carried out to present miR-185 as a therapeutic mean for PA. miR-185, used along with bromodomain 8 isoform 2 (BRD8 ISO2) on PC3 and LNCaP, inhibited expression of androgen receptors (AR) directly by binding to the 3'- UTR region of AR mRNA.[131] Among several known miRNAs,

the let-7 family is proved to play a significant rule in PC progress by regulating CSCs. BR-DIM upregulates the expression of the let-7 family, consequently downregulating the expression of EZH2 both in PC cell lines and human PC cells in the tissue. These results suggest that BR-DIM could serve as a novel agent for the inhibition of PC progression and recurrence.[132]

In addition to miRNA therapy, immunotherapy has expressed quite impressive results in cancer therapy that include vaccine therapy, which was administered in 2012 using the DNA fusion vaccine in PC cases. The designed vaccine consisted of the domain (DOM) from fragment C of tetanus toxin linked to an HLA-A2-binding epitope from prostate-specific membrane antigen (PSMA) and induced DOM-specific CD4 and PSMA-specific CD8 T cells, generating anti-PSMA responses in most of the individuals suffering from PC.[133] 3D cell culture, as an *in vitro* condition, was proposed to be beneficiary in PA. In 2015, scientists designed a 3D cell culture model of PC bone metastasis in which collagen-based scaffolds were employed for a more efficient physiological microenvironment and helped to deliver siRNA to PC cells (PC3), which result in the alternative for PC bone metastases treatment. [134] Other researchers used aptamers along with 3D cultures as an efficient therapeutic way for PC. In 2016, scientists used eight aptamers with 3D cell culture for PC therapy.[117,135]

Studies about preventing activation of HDAC enzymes with HDAC inhibitors are carried out. HDAC inhibitors precluding activation of the HDAC enzyme may change gen expression. Recent cancer studies showed that inhibition of HDAC enzymes activate inactive G2 checkpoint starts apoptosis. Cell stimulated for apoptosis is stopped in G1 and G2 phases. Thus, cell proliferation is blocked.[136-140]

miRNA expression has an important role in HDAC enzyme functions. Studies showed that with HDAC inhibitors affected the miRNA expression levels.(Table 5).[141]

Current Studies in PC: CRISPR-Cas System (CRISPR-Cas as Therapeutic Means in PC)

CRISPR-Cas is a system that prokaryotes (84% archaea and 45% bacteria) ensure gaining adaptive immunity against viruses.[145] This system is composed of clustered regularly interspaced short palindromic repeats (CRISPR) and cas gene that is consist of similar sequence to gene families many nuclease and helicase.[146]

The system works via RNA. Therefore, this system is called an immune system via RNA.[147] When bacte-

Table 5   miRNA expression affecting from HDACi and their mechanisms

HDACi	Cell Type	MiRNA expression	Mechanism
MGCD0103 (0.5, 1, 2.5 IM)	Prostate cancer (Panc1)	miRNA-141, 203, 200a, 200b, 200c (up regulation)	H3 ve H4 acetylation H3, H4 H3K9 acetylation and H3K4me3 metylation (upregülation) [142]
TSA (300 nM); 5-azaCdR (1IM)	Prostate cancer (PC-3, LNCaP, DU145, 22Rv1, LAPC-4, VCa)	miRNA-9-5p, 9-3p, 27a, 32, 33a, 34a, 132, 149, 183, 188, 192, 193b, 194, 203, 215, 218, 370, 375, 376a, 449a, 487b, 512-3p, 512-5p, 513a, 515-5p, 517a, 517b, 517c, 518b, 520f, 526a, 572, 601, 629, 630, 638, 663, 765	CpG island methylation [143]
TSA (0.3 IM); Genistein (25 IM); 5-aza (5 IM)	Prostate cancer (PC3)	MiRNA-145	Not reported [144]

HDACi: Histone deacetylase inhibitor

Table 6   Studies regarding PC benefited from the system of the CRISPR-Cas

Title of the Paper	Method
N-Myc promotes therapeutic resistance development of neuroendocrine prostate cancer by differentially regulating miR-421/ATM pathway [151]	CRISPR-Cas9 was used to knock out.
CRISPR/Cas9 offers a new tool for studying the role of chromatin architecture in disease pathogenesis [152]	CRISPR-Cas9 was used to base editing.
Generation of PTEN knockout (-/-) murine prostate cancer cells using the CRISPR/Cas9 system and comprehensive gene expression profiling. [153]	CRISPR-Cas9 was used to knock out.
FOXA1 knock-out via CRISPR/Cas9 altered Casp-9, Bax, CCND1, CDK4, and fibronectin expressions in LNCaP cells [154]	CRISPR-Cas9 was used to knock out.
A Somatically Acquired Enhancer of the Androgen Receptor Is a Noncoding Driver in Advanced Prostate Cancer. [155]	CRISPR-Cas9 was used to create insertions and deletions
Organelle-Derived Acetyl-CoA Promotes Prostate Cancer Cell Survival, Migration, and Metastasis via Activation of Calmodulin Kinase II [156]	CRISPR-Cas9 was used to create deletions.
Interleukin-30/IL27p28 Shapes Prostate Cancer Stem-like Cell Behavior and Is Critical for Tumor Onset and Metastasization [157]	CRISPR-Cas9 was used to knock out.
Knocking-out of HIF1α gene by CRISPR/cas9 inhibits proliferation and invasiveness of prostate cancer DU145 cells [158]	CRISPR-Cas9 was used to knock out.
CRISPR/Cas9 targeting of the androgen receptor suppresses the growth of LNCaP human prostate cancer cells [159]	Androgen genes were targeted in androgen-positive prostate cancer cells with CRISPR-Cas9 system.
Store-operated calcium entry is dispensable for the activation of the ERK1/2 pathway in prostate cancer cells. [160]	CRISPR-Cas9 was used to knock out.
Genome-wide CRISPR screen identifies HNRNPL as a prostate cancer dependency regulating RNA splicing. [161]	CRISPR-Cas9 was used to knock out.
Protecting PTEN in the Nucleus. [162]	CRISPR-Cas9 was used to knock out.
Targeted delivery of CRISPR/Cas9 to prostate cancer by modified gRNA using a flexible aptamer-cationic liposome. [163]	CRISPR-Cas9 was used to knock out.
RNase L is a negative regulator of cell migration [164]	CRISPR-Cas9 was used to knock out.
CRISPR/Cas9-mediated gene knockout of NANOG and NANOGP8 decreases the malignant potential of prostate cancer cells. [130]	CRISPR-Cas9 was used to knock out.

Aslan et al.
Use of miRNA as a Biomarker in Prostate Cancer and New Approaches

107

riophage infects bacteria, bacteria recognize the PAM (protospacer adjacent motif) sequence that belongs to bacteriophage via RNA. When bacteriophage infects bacteria, bacteria recognize the PAM sequence that belongs to bacteriophage via RNA. Consequently, the PAM region is cut by a cas enzyme showing endonuclease property. Information of the cutted region is attached above-mentioned among palindromic repeats. Thus, bacterium gains immunity memory against that virus. Pathogen property of the virus disappears with cutting the PAM region.[145,148]

The double-strand break occurs in target DNA. Because there is no compensation for this situation, the virus loses its function. Scientists have developed applications, such as gene silencing [with double-strand break] (knock out) and gene splice [with a donor constituting homology to target DNA sequence] (knock-in) using this property of the system. Thus, the CRISPR-Cas system is an inspiration for diagnosis and treatment in hereditary diseases, such as cancer. Consequently, change of arrays, such as insertion and deletion, in the gene region which they want to work is possible.[149,150] In Table 6, studies of PC benefited from the system of CRISPR-Cas are shown.

Conclusion

Current statistics show that approximately one in nine men is diagnosed with PC during their lifetime. Although PC can be a severe disease, luckily, most men diagnosed with PC do not die of PC. More than 2.9 million men in the United States who have been diagnosed with PC at some point are still alive today.

Genetic mutations are known to give rise to cancer. The epigenetic mechanisms which increase importance day by day and affect the structure of DNA play an important role in the pathogenesis of cancer.

As we understand the epigenetic mechanisms of PC better, we better modelling, which becomes a success in treatment will be.

miRNAs play a role in the epigenetic mechanism of cancer. When considering studies, we are clearly seen an important role in the therapeutic applications for PC.

Studies on the pathogenesis of the disease can be summarized in two steps as follows:
a. Modelling to better understand the pathogenesis of the disease.
b. Design of the therapeutic studies with different options for the treatment of the disease in consideration of the modelling.

The information which we have on the effects of epigenetic mechanisms involved in the progress of cancer is not yet at the desired level.

In particular, the use of miRNAs as a biomarker for therapies that are developed based on affecting epigenetic mechanisms is far away from the desired level. Thus, cohort studies that include genomic and epigenetic analyses are needed.

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Aslan et al.
Use of miRNA as a Biomarker in Prostate Cancer and New Approaches

109

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111

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Aslan et al.
Use of miRNA as a Biomarker in Prostate Cancer and New Approaches

113

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