Tumor Biol.
DOI 10.1007/s13277-016-4900-x

REVIEW

Missing link between microRNA and prostate cancer

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Received: 17 November 2015 / Accepted: 20 January 2016

Abstract MicroRNAs are the non-coding RNAs which regulate endogenous gene expression in animal and plant cells. Alterations in the level of micro-ribonucleic acids (miRNAs) involving the deletions, overexpression, mutations, epigenetic silencing, or dysregulation of transcription factors that target specific miRNAs may culminate in various diseases including cancer. Recent findings demonstrate the role of miRNAs in prostate cancer. Numerous discoveries of miRNAs have marked the research and development surrounding prostate cancer management, diagnosis, and therapy which has made prediction easy, but the effective treatment strategy remains a mystery. This review seeks to draw a link between miRNA and prostate cancer through an understanding of the numerous signaling pathways that these miRNAs control, which may prove to be helpful in identifying therapeutically interesting molecular targets.

Keywords Prostate cancer (PCa) · miRNA · Signaling · Apoptosis · Biomarker

Introduction

Progressive accumulation of mutations in the critical genes that control cell growth and differentiation results in an uncontrolled somatic cell proliferation, gradually leading to cancer [1]. Chromosomal instability, epigenetic silencing, and modulation in the expression of tumor suppressor genes, individually or together, may further advance cancer [2]. DNA accumulates the changes that activate proto-oncogenes, inactivating tumor suppressor genes and consequently resulting in genetic instability. As per cancer statistics 2015, prostate cancer is responsible for high mortality rate among the men ranking second to lung cancer. Globally, 20 % of the male population is diagnosed with prostate cancer during their lifetime with 3 % resulting in death [3]. Androgen stimulates and controls the development of androgen receptors important in male secondary sex characters importantly testosterone. Prostate cancer occurs as a consequence of the shifting of equilibrium from normal to abnormal prostate level. It may be due to old age, a family history of the disease, or race. Screening for prostate cancer with prostate-specific antigen provides platforms for detection but owing to pitfalls; it did not help in reducing the mortality rates. Further research in this field revealed various micro-ribonucleic acids (miRNAs) to be efficacious in combating cancer. miRNA regulation is dynamic in nature, and its complexity increased by the fact that a single miRNA can regulate a number of target genes. The current scenario in the research area faces the problem of choosing miRNA intended for specific targets based on computational or experimental methods. In this direction, various computer programs such as miRanda, PicTar, and TargetScan have been designed to decipher miRNA target by analyzing different parameters to predict the probability of a functional miRNA: messenger RNA (mRNA) interaction [4]. Computational programs predict various target genes having false positives, and due to the focus on conserved targets, many non-conserved targets are missed [5]. Thus, it leads to an incorrect inference about the target in miRNA. miRNAs are strongly involved in the signaling of various anomalies, also

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Published online: 28 January 2016

suppressing them effectively. To learn more about the therapeutic potency, miRNAs have been subjected to clinical trials and are currently in a different phase of the trials discussed later. The different review concludes the individual performance of miRNA in cancer [6], whereas some focus on the role of miRNA in the different types of cancers [7], but this study considered the signaling pathways responsible for the prostate cancer (PCa). Major signaling pathways targeted by miRNAs in prostate cancer are apoptosis, cell invasion and migration transforming growth factor-β, (TGF-β) pathway, extracellular signal-regulated kinases (ERKs), phosphatidylinositol-3-kinase (PI3K)/Akt/mTOR, cell cycle, and epithelial-mesenchymal transition (EMT).

Regulation of prostate development

The prostate gland is part of the male accessory reproductive system which constitutes 20 % of the semen. Any deviation from the normal level of prostate culminates in prostate cancer. Androgen plays a significant role in the development of prostate gland in that it binds to the androgen receptor (AR), an important step in prostate development. This in turn activates androgen and the related transcriptional complex initiating a cascade of events leading to the differentiation of luminal epithelial cells and regulating the transcription of genes necessary for prostate function. Binding of androgen to the AR in the hypothalamus stimulates luteinizing hormone-releasing hormone (LHRH) which, in turn, interacts with LHRH receptor in the pituitary stimulating the release of luteinizing hormones. This unfolds the events resulting in testosterone production from the precursor molecule cholesterol. In the prostate, in the presence of 5α-reductase enzyme, testosterone gets converted to dihydrotestosterone (DHT), which in combining with AR enters the cytoplasm, translocates to the nucleus, regulating cell survivability. DHT produced by 5-α reductase as the free testosterone enters the prostate cell. Subsequently, DHT binds to AR and induces dissociation of heat shock proteins (HSPs) and receptor phosphorylation. This phosphorylation event culminates in AR dimerization and binding of androgen response elements to the target gene in the promoter region. ARA70 acts as co-activator factor and co-repressor respectively bind to the AR complex and enhance the transcription process. Prostate cancer growth results as a consequence of imbalance in the ratio of cell proliferation to cell death, in particular, gene expression. Therefore, to maintain the balance, androgen stimulates growth and inhibits cell death by apoptosis. In PCa, mechanism undergoing AR signaling remains unclear, although it modulates the expression of proteins in the cell cycle regulation, survival, and growth [8]. Perpetual research in the field of drug discovery and research has given rise to repressors that are designed to inhibit the PCa. Among different inhibitors, antiandrogen is important which blocks appropriate receptors, competing for the binding site. Luteinizing hormone is produced by the pituitary in response to luteinizing hormone (LH). Ligand binding to the progesterone, estrogen, and androgen receptor impedes the binding of dihydrotestosterone and testosterone, thereby, inhibiting the production of LHRH. Therefore, antiandrogens block the LH release, increasing testosterone level by a normal feedback loop. Prolonged exposure to LHRH agonists downregulates the LHRH receptor inhibiting testosterone whereas LHRH antagonists inhibit the LHRH receptor and decrease the production of LH and testosterone. Castration by surgical or chemical means removes the testicles and decreases testosterone production. Adrenal androgen production was inhibited by drugs that prevent the conversion of cholesterol to adrenal androgen in the adrenal glands (Fig. 1) [9].

miRNA and its expression

A miRNA is a non-coding RNA participating critically in regulating the endogenous gene expression expressed in animal and plant cells. Various research groups have experimentally demonstrated the stability of miRNA on boiling, pH, storage, freeze-thaw cycles, and protection from ribonuclease in serum and plasma, and highly conserve in nature among the species [10]. miRNAs regulate the translation by degrading or impairing the process of translation in the target mRNA. Mechanistically, miRNAs control the expression quantitatively or qualitatively by altering the expression level by sequence recognition within the miRNA or its target, thereby, controlling biological processes involved in the development, differentiation, apoptosis, and metabolism. This feature of miRNA has brought it under the spotlight of molecular research and in the forefront of the treatment of life-altering human diseases. Structurally, primary transcripts of miRNAs (pri-miRNAs) are generated by RNA polymerases II/III and sequentially processed by enzymes Drosha and Dicer, to produce initial ~70-nt-long intermediate hairpin structures (pre-miRNAs) to 18–24-nt-long mature miRNAs. Sequentially, gene regulation in miRNAs is expressed in a particular manner followed by the incorporation of multi-protein complex RNA-induced silencing complex (RISC). miRNA silences the target mRNA either by cleaving or repressing the process of translation in which single strand miRNA binds to the target sequence in the 3′-prime untranslated regions (UTRs) of the mRNA (Fig. 2). The 3′ UTR contains important regulatory elements for the expression of polyadenylation signals and RNA binding protein motifs. In mammals, mRNAs are the targets of miRNA, and 3′ UTRs have potential to acquire or eliminate miRNA target sites [11]. Therefore, evolution favored short 3′ UTRs to avoid miRNA target sites and escaped miRNA-mediated translational repression and selected long 3′ UTRs for functioning

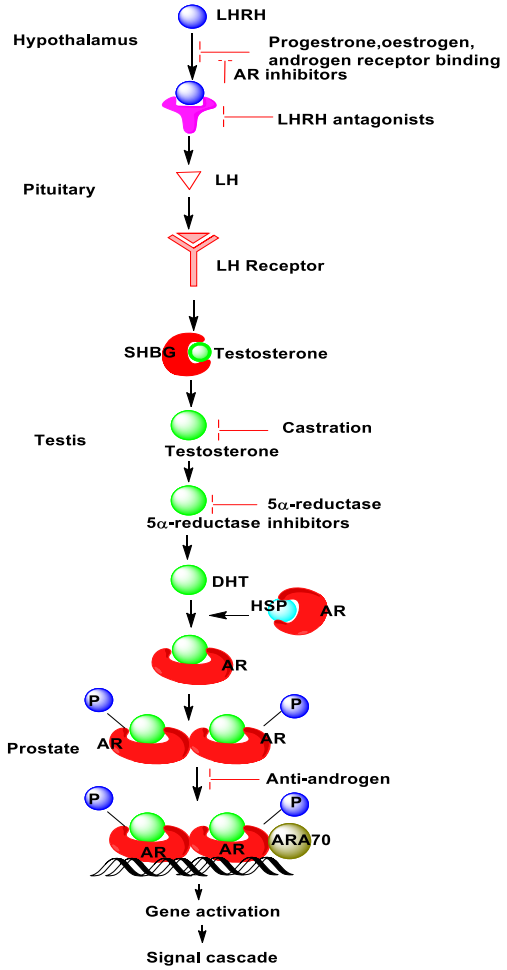

Fig. 1 The action of androgen and mechanism of regulation of production: The pituitary produces the luteinizing hormone in response to the LHRH synthesized in the hypothalamus. Ligand binding to progesterone, estrogen, and androgen receptor impedes the binding of dihydrotestosterone and testosterone, thus, inhibiting the production of LHRH. Antiandrogens block the LH release and increase the testosterone level in a normal feedback loop. Prolonged exposure to LHRH agonists such as histrelin and leuprolid downregulates the LHRH receptor inhibiting testosterone whereas LHRH antagonists such as degarelix inhibit the LHRH receptor and decrease the production of LH and testosterone. Castration by surgical or chemical means also decreases testosterone production. 5-α reductase irreversibly converts testosterone into dihydrotestosterone (DHT) but the blocking of 5-α reductase action prevents this conversion. Subsequently, DHT bound to the androgen receptor in the cytosol and transported into the nucleus where it served as a transcription for prostatic function. On the other hand, adrenal androgen production is inhibited by drugs that prevent the conversion of cholesterol to adrenal androgen in the adrenal glands

[12]. In animals, repression in translation results from the imperfect complementarity of the target mRNA, whereas, perfect complementarity induces the degradation of the target mRNA as commonly found in plants. Downregulation of target genes by miRNAs is reflected at the protein level, although mRNA levels remain unaffected indicating regulation to occur at the translational level [13].

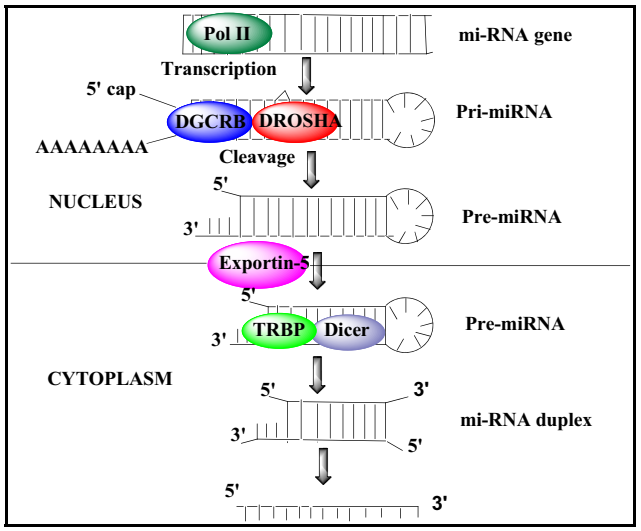

Fig. 2 miRNA processing from pri-miRNA to mature miRNA stage. Structurally, primary transcripts of miRNAs (pri-miRNAs) are generated by RNA polymerases II/III in the nucleus that were sequentially processed by enzymes Drosha and Dicer to produce initial ~70-nt long intermediate hairpin structures (pre-miRNAs) to 18–24-nt long mature miRNAs in the cytoplasm. Sequentially, gene regulation in miRNAs is expressed in a particular manner followed by the incorporation of multi-protein complex RISC (RNA-induced silencing complex). miRNA silences the target mRNA either by cleavage or repressing the process of translation in which single strand miRNA binds to the target sequence in the 3′-prime untranslated regions of the mRNA

This distinguishing feature makes them plausible candidates for exploring therapeutic values of miRNA [14]. The significance of miRNA was fully grasped when miRNA deletion was detected as the leading cause of leukemia as well as other cancers. Thus, lately, miRNA is being regarded a new broad-spectrum tumor suppressor gene acting as a beacon for research in diagnostic and therapeutic perspectives.

Micro-ribonucleic acids (miRNAs) prove to be the cornerstone of cancer therapy. Determination of the function of miRNA depends on the availability of target mRNAs since each miRNA portrays different function in various tissues. Extracellular miRNAs were reported to be highly stable, cell-free form isolated from body fluids including serum, plasma, tears, breast milk, urine, saliva, and semen and detected in a small volume by real-time PCR [15]. These findings indicate that miRNAs are packed in different forms particularly RNase-resistant lipid vesicles, such as exosomes and apoptotic bodies bound by RNA-binding proteins with high-density lipoprotein [16]. Iguchi research group reported that the miRNA secretions were triggered by the elevation of ceramide and sphingolipid, whose synthesis is firmly regulated by neutral sphingomyelinase 2 (nSMase2) [17].

miRNA expressions are known to be temporally or spatially regulated, with an even slight aberration in their functioning leading to life-threatening abnormalities. This result from the mutation arises in the seed region of miRNA. It causes Burkitt

lymphoma, colorectal cancer, lung cancer, breast cancer, papillary, thyroid carcinoma, hepatocellular carcinoma, and glioblastoma. It critically plays a role in neurodegeneration although the mechanism is still in its infancy. Expressions of miRNA were analyzed in tumors with respect to diagnosis, staging, progression, prognosis, and response to treatment in tumors. From the study conducted by Sekeres and his research group, it was concluded that incidence and death rates for African-American men were almost twice in comparison to Caucasian men [18]. These differences were attributed to an elevation in the expression of different genes mainly those involved in hypermethylation under which different genes in normal or premalignant areas were predisposed a full-blown malignancy.

Members of the miR-34 family including three homologous miRNAs expressed at two different loci of the chromosome (miR-34a, miR-34b, and miR-34c) are implicated in a various facets of neural development [19]. miR-9, miR-124a, miR-125b, miR-128, miR-132, and miR-219 abundantly in the fetal hippocampus are regulated differently in the aged brain. Similarly, an alteration in specific miRNA complexity arises in Alzheimer hippocampus in Alzheimer's disease (AD) [20]. miR-29a, miR-29b-1, and miR-9 have been known to play a vital role in regulating BACE expression as well as an essential role in AD [21]. The molecular alterations caused by variation in the gene expression are more in the malignant cell than normal cells. Parkinson's disease characterized by Lewy bodies or protein inclusions and progressive loss of dopaminergic neurons in the midbrain showed decreased expression levels of miR-34b and miR-34c in the brain areas with variable neuropathological affectation in PD. Moreover, deregulation in the action of miR-34b/c directly targets p53 triggering downstream transcriptome alterations underlying mitochondrial dysfunction and oxidative stress in PD. This gives the comprehensible picture of the progression and role of miRNA in AD and PD [22].

Combined approach of computational and experimental methods is used to identify the specific miRNA target. Various computer programs such as miRanda, PicTar, and TargetScan prove useful in elucidating miRNA targets and predicting miRNA-mRNA interaction [4]. Most of these programs identify 3′UTR of target sites within mRNA. Unfortunately, these criteria predict numerous target genes that contain conserved targets in which many non-conserved targets are missed giving many false positives and remain a problem [5].

Relationship between miRNA and prostate cancer

A prostate-specific antigen (PSA), a member of kallikrein-related peptidase family, is secreted by the epithelial cells of the prostate gland. Present in a small amount in the male serum, any fluctuations in normal level indicate prostate-related diseases. The presence of PSA in serum has of late proved to be a milestone biomarker for the diagnosis and treatment of prostate cancer. Clinical biopsy reports indicated more than 50 % of male population to have increased PSA level, highlighting increased cancer risk. Due to the risk of over-diagnosis and overtreatment as most cancers diagnosed, United States Preventive Services Task Force (USPSTF) recommends against screening using the PSA testing. The USPSTF concludes that the potential benefits of testing do not outweigh the expected harms. Lack of sensitivity of elevated serum PSA as in malignant prostate cancer renders the diagnosis and the subsequent treatment difficult. Moreover, Schroder research group reported that PSA testing gives the false-negative rate of approximately 15 % [23]. In the light of these reports, there is an urgent need to validate PSA as an efficient biomarker to accelerate efficacious drug development pathway. Clinical results obtained during diagnosis, progression, and prognosis have indicated the role played by miRNA expression in human cancers. The link between miRNAs and cancer was first highlighted by Calin and his research group [24] that miR-16 and miR-15 are located in a region on chromosome 13 and were deleted in over 65 % of chronic lymphocytic leukemia (CLL) patients. The systematic profiling detailing the miRNA expression in prostate cancer was published in 2007. Firstly reported in 2007, both upregulation and downregulation in benign and metastatic cancer were shown [25]. Numerous miRNAs were reported which downregulate or upregulate genes indulged in cancer signaling (Tables 1 and 2).

The literature highlighted 26 among 286 possible miR-125b target genes that are significantly enhanced in prostate cancer which impact the biology and clinical behavior of prostate cancer. SEL1L, a protein has been identified to play a major role in breast and pancreatic cancer aggressiveness [81], and histochemistry immune studies have shown it to be upregulated in the prostate cancer. Worth noting is the fact that several genes influencing the transcription factor have been seen to be involved and upregulated such as EIF4EBP1, RPL29, MGC16063, and PAPB. In PCa, miR-21 targets both programmed cell death (PDCNA) and phosphatase and tensin homolog (PTEN). Some of the miRNA-based therapeutics have been subjected to clinical trials such as let-7 which in the present moment is in preclinical development whereas miR-34 have undergone phase 1 clinical trial in patients with liver cancer and displayed antineoplastic activity in mouse cancer models including melanoma, lymphoma, lung, prostate, and pancreatic cancer [82]. SPC3649, a miR-122 antagonist, designed to block the production of HCV in hepatocytes has undergone phase 2 clinical trials successfully [83]. Fifty-three surgical specimens from men who undergo radical prostatectomy exhibited differential expression in relation to prognostic factors and biochemical recurrence of PCa. Microarray

Table 1 Targeted genes downregulated by miRNA

S. no.	miRNA	Target gene (downregulation)	Function	References
1	miR-296	HMGA-1	Translation	[26]
2	miR-1	PTK 9, exportin-6, F-actin, ERK, and AKT	Gene expression, cell cycle, mitosis, DNA replication/repair	[27, 28]
3	miR-34a	p53, CD44, BCL2, SIRT 1, E2F1	Apoptosis, drug resistance	[29]
4	miR-330	ROCK 1, E2F1	Apoptosis	[30]
5	miR-146a	BUB1, ROCK1, SPI1	Motility	[31, 32]
6	miR-101	EZH2	Metastasis	[33]
7	miR-15a-16 cluster	CCND1, WNT3a, BCL2	Cell cycle regulation, apoptosis, proliferation	[34]
8	miR-143, miR-145	E-cadherin, fibronectin, EMT, MYO6, ERK5	Cell migration, proliferation	[35]
9	miR-145	MYO6, BNIP3L-> AIFM1, CCNA2, TNFSF10	Cell migration, apoptosis, cell cycle control	[36]
10	miR-205	HIF-1	Metastasis, EMT	[37]
11	miR-331-3P	ERBB-2	Control cell cycle, signal transduction	[38]
12	miR-449a	HDAC-1, cell cycle arrest	Gene expression	[39, 40]
13	miRNA-218	TPD52	Apoptosis	[41]
14	miR-188-5p	LAPTM4B	Proliferation, migration, invasion	[42]
15	miR-1, miR-133	Purine nucleoside phosphorylase (PNP)	Metabolism	[43]
16	miR-29b	Ncadherin, Twist, Snail expression, MMP2, Mcl-1, collagens	Epithelial-mesenchymal transition	[44, 45]
17	miR-34a/b/c	HuR, SIRT1, Bcl2, PSA, Notch-1	Apoptosis, growth, self-renewal, paclitaxel resistance	
18	mir-34a	LEF1	Cell proliferation and invasion (Wnt signaling pathway)	[46]
19	miR-185, miR-342	AR, CDC6, SREBP-1/2, FASN, HMGCR	Growth, cell cycle, invasive, migration, tumorigenicity, apoptosis	[47]
20	miR-31	AR, MCM2, EXO1, E2F1, E2F2, FOXM1	AR pathway, cell cycle, growth	[48]
21	Let-7b/c/d/g	AR, c-Myc, PBX3	AR pathway, radioresistance, growth	[49]
22	miR-199a	MMP9, Smad4, mTOR	Apoptosis	[50, 51]
23	miR-143	KRAS, ERK5	Cell cycle	[52]
24	miR-203	SIP1, EMT, SNAI2, TGF-β	Epithelial-mesenchymal transition	[53, 54]
25	miR-200a, miR-200b, miR-200c, miR-141, and miR-429	TGF-β-mediated EMT, E-cadherin	Cell migration, invasion	[55]
26	miR-940	MEIN1, EMT	Migration, invasion, anchorage-independent growth	[56]
27	miR-133b	CDC2L5 PTPRK, RB1CC1, CPNE3	Cell proliferation, apoptosis	[57]
28	MiR-144	PTEN, IRS1, Wnt, EZH2, FOXO3a	Cell proliferation, self-renewal, invasion	[58–61]
29	miR-421, miR-24	ATM, H2QAX	Cell death	[62, 63]
30	miR-210	HIF-1	Radio resistance	[64]
31	miR-128	BMI-1, NANOG, and TGFBR1	Tumor suppression	[65]
32	miR-145	DNA methyltransferase 3b	Crosstalk	[66]

Table 2 Targeted genes upregulated by miRNA

S. no.	miRNA	Target gene (upregulation)	Related function	References
1	miR-21	PDCD 4, TPM1, MARCKS, P53, BAK 1, PTEN, AKT, androgen pathway	Mobility, invasion, apoptosis, mTOR pathway, androgen independence	[67, 68]
2	miR-125b	P53, PUMA, P27, BAK1, EIF4EBP1	Apoptosis proliferation	[69]
3	miR-1247-5p	MYCBP2	Suppression	[70]
4	miR-145	SENP1	Cell cycle arrest	[71]
5	miR-20a	E2F1-3	Apoptosis	[72]
6	miR-24	FAF1	Apoptosis	[73]
7	miR-32	BTG2, PIK3IP1	Apoptosis	[74]
8	miR-99a/125b-2	NCOR2, IGF1R, BAK1, BBC3, p53	Apoptosis, growth, Her2-AR pathway	[75]
9	miR-23b/-27b	Rac1 activity	Invasion, migration	[76]
10	miR-19a	SUZ12, RAB13, SC4MOL, PSAP, ABCA1	AR association	[57]
11	miR-23a, 23b	MAPK, JAK/STAT	Cell proliferation and neoplastic transformation	[77]
12	miR-153	PTEN, Akt, FOXO, Bcl2, Mcl-1	Cell cycle	[78, 79]
13	miR-223-3p	SEPT6	Tumor suppressor	[80]

analysis showed miR-21 to decrease the expression of RECK resulting in an increased expression of matrix metalloproteinase-9 (MMP9) and tumor cell invasion [84]. In another study, in vivo intratumoral delivery of miR-23b reduced tumor mass in animals that were attributed to the suppression of proto-oncogenic pathways in neoplastic cells in PCa [85].

Gaur and research group demonstrated the microenvironment role in the tumor progression especially in miRNA-145 which inhibited IGF-1 and cell viability in PCa. Activation of the IGF-1/1R signaling pathway induces crosstalk leading to the c-MET activation that is a potent inhibitor as seen in the clinical trial for mCRPC. Controlling the multiple gene by miRNA-mediated gene regulation and effective delivery strategies for miRNAs enrich them with therapeutic efficacy. miR-34a inhibits c-MET, Axl, and c-MYC in PCa progression; however, miR-34a is not predicted to target IGF-1/1R pathway. miR-145, a tumor-suppressive miRNA, binds the 3′UTR of both IGF-1 and IGF-1R. Transfection of miR-145 does not inhibit IGF-1R but inhibits IGF-1 at both the protein and mRNA levels. As a result, inhibiting the constitutive phosphorylation of IGF-1R leads to decreased cell viability and the G1 arrest and thus inhibited PCa. Both miR-34a and miR-145 can target multiple genes and crosstalk therapy of miRNA proving effective control in PCa [29].

Role of diet in prostate cancer

A daily diet inclusive of various substrates and cofactors plays a very crucial part in epigenetics modeling [86]. Epigenetics is axiomatic in the consequential role it plays in the pathophysiology of prostate cancer, although the mechanism remains elusive. Diet epigenetically modifies the chromosomal packing bringing about heritable changes having the potential to control transcriptional machinery and gene expression. Ngo research group demonstrated the importance of dietary fat intake in delaying PCa when tested in batches of LAPC-4 xenografted SCID mice [87]. Despite this fact, lack of sufficient clinical evidence has limited the changes in diet plans of patients as a means to reduce prostate cancer risk. Studies conducted have failed to give a lucid picture regarding the impact of cholesterol and statin therapy, lycopene, selenium, vitamin E, calcium, and green tea on prostate cancer [88].

Stegeman and their team finds the genetic association between 2169 miRSNPs and Pca risk by large-scale analysis of 22,301 cases in European ancestry. Among them, 22 miRSNPs were found associated with risk of PCa mapped by genome-wide association studies (GWASs). This study concludes that the large association can recognize individual risk loci compared with the accepted statistical levels of genome-wide significance [89].

miRNA as a diagnostic biomarker in cancer

Cancer is a multi-factorial disease involving uncontrolled cell growth and proliferation. Dynamic nature and gene network in cancer renders the researchers with the challenging task of designing therapy. miRNAs are redundant in nature having a probability of targeting protein in the initiation and progression of cancer. Mature miRNA regulates gene expression by either perfect complementary binding or repressing the target sequence. Various studies have highlighted alteration in miRNA expression in human malignancies [90]. This factor further complexes cancer and efficiency of miRNA to

modulate functioning either by suppressing tumor or acting as oncogenes. The different process involved in transcription and translation further underlines the role of miRNA. In this, aberrant changes in miRNA resulting from alteration in miRNA copy number or epigenetic modification (gene silencing) alter the whole machinery. Brase and research group checked potentially the clinical relevance of 667 metastatic and localized circulating miRNAs in prostate cancer. The study concluded miRNA-375 and miRNA-141 to be the most pronounced markers for high-risk tumors [91]. Recently, out of 1205 human miRNAs experimented in PCa by the miRNA array, 73 were observed to be downregulated and 10 to be unregulated. Furthermore, it showed miR-29b-1, miR-200a, miR-370, and miR-31 to be modulated and potentially targeted to dicer that has a key role in carcinogenesis, migration, and behavior of castration-resistant PCa [92].

Early stage localized PCa requires androgen for stimulation and proliferation making androgen ablation a necessary requisite for the treatment of prostate cancer. On the contrary, other stories have shown PCa to progress even in the absence of androgen by developing resistance to hormone therapy. Differential expression of miR-125b in androgen-dependent and independent PCa has been reported in cases of benign and malignant prostate tissues. Androgen upregulated the miR-125b expression and downregulated the expression of Bak1 in PCa [93]. miR-146a, let-7C, miR-124, miR-34a, miR-34c, miR-148a, miR-31, miR-200b-3p, miR-185, and miR-205 downregulated androgen-independent prostate cancers (AIPCs), specifically miR-146a regulated ROCK1, EGFR, and MMP2 [31]. miR-616 induced the androgen-independent growth of PCa by suppressing the expression of tissue factor pathway inhibitor-2 (TFPI-2) [94]. miR-221/-222 upregulated 90 % of the AIPCs in the human tumor specimen [95].

miRNAs have been recognized and precisely involved in PCa progression and development. Mo and his research group through series of the experiment studying dynamic genome-wide expression identified AR to target miRNA by a time course microarray, thus, confirming the association of miRNA with androgen receptors. Furthermore, miR-19a has been seen to be directly upregulated by AR, which, in turn, repressed SUZ12 involved with histone methyltransferase activity, RAB13 found at cell-cell epithelial tight junctions, SC4MOL utilized in cholesterol synthesis pathway, PSAP, and ABCA1. Similarly, miR-27a is also upregulated by AR and repressing the proteins that interact with the conserved protein complex such as ABCA1 and PDS5B [96].

Androgen receptors are also found to upregulate miR-133b leading to the suppression of cyclin-dependent serine/threonine protein kinase (CDC2L5), protein tyrosine phosphatase receptor type K (PTPRK), a transcription factor termed RB1-inducible coiled-coil 1 (RB1CC1), forming a complex with p53 and another calcium-dependent membrane-binding protein (CPNE3; copine III) [57]. Studies revealed that the expression level of miRNA in PCa is remarkably different in the benign and malignant tumor. However, the generation of the miRNA is based on computationally based studies to identify and target miRNA. These bioinformatics tools used are not entirely precise and reliable to identify the target. Another problem for identifying a specific target is that mRNA contains multiple binding sites and can target multiple sites. To rely on the computationally based result, experimental validation of miRNA and target is required. To find the missing link between miRNA and PCa, there is a need to divulge numerous interlinking mechanisms and signaling pathways of miRNA that contribute towards tumor progression via methylation, apoptosis, cell migration and invasion via TGFβ1 pathway, AKT/mTOR signaling, and epithelial-mesenchymal transition signaling. Different miRNAs are expressed in cells under particular conditions, their expression being modulated by altering adaptor molecules involved in the cellular signaling.

DNA methylation is a crucial phenomenon in the regulation of gene expression and chromatin organization within the cells. In cancer, DNA methylation pattern in CpG islands is altered via histone modifications (H3K4me2/3, H4K16Ac, H3K9me2/3, H3K27me3, H4K20me3), chromatin-modifying enzymes (G9a, EZH2, hMOF, SUV4-20H), and aberrant DNA methylation [97]. DNA methylation has been observed to downregulate the expression of miRs such as miR-127, miR-124a, and miR-34 [98]. miR-200c and miR-141 lost the ability to express PC-3 cells without any effect in LnCaP and DU145 cells [99]. Furthermore, an association between p53 and methylation in miRNA regulation has been observed in which methylation downregulates miR-34 which is directly transactivated by p53 as seen in the case of microRNA-145 [100]. In this case, PCa expression by the miRNA was downregulated by DNA methylation in the promoter region and p53 gene mutation, and the target sequences were methylated by DNA methyltransferases inferred by the abundant CpG dinucleotides [101]. Along the same lines, Sachdeva et al. reported p53 response to the promoter sequence of miR-145 and its involvement (p53) in the functioning of breast and colon cancer cell lines [102]. Re-expression of miR-132 in PC-3 cells induced cell detachment followed by cell death in addition to a downregulation in methylation. Heparin-binding epidermal growth factor and TALIN2 (pro-survival proteins) were confirmed to target directly miR-132 demonstrating the antimetastatic role in PCa as concluded by a study carried out by Formosa and his research group [57]. Recent reports have suggested regulation of migration and invasion enhancer 1 (MIEN1) to be a major role of DNA methylation mechanism and enhances the invasive potential of cancer cells. miRNA-940 post-transcriptionally downregulate MIEN1 consequently inhibiting cancer progression by dwindling the migration, invasion, anchorage-independent growth, and epithelial-to-mesenchymal transition. High

expression of MIEN1 in prostate and breast cancer and their control of the expression by miR-940 has made this miR a potential target for cancer therapy [56].

miRNA as tumor suppressor

miRNA controls the progression of cancer signaling by modulating the expression of tumor suppressor genes critical in oncogenic and tumor suppressor networks. It checks and modulates the regulation of PDCD4, TPM1, PTEN, p53, and HMGA2 gene in cancer, particularly in apoptosis. Gu and his research group revealed that the upregulation of miR-183 is associated with the advanced clinical stage, positive lymph node, deep stromal invasion, and distant metastasis in gastric cancer patients. miR-183 promotes the inhibition of apoptosis by enhancing cell migration and invasion. Mechanistically, the decrease in the expression of miR-183 and inhibition of miR-183 increased the expression of PDCD4 both at mRNA as well as protein levels [103]. RISC co-immunoprecipitation-based biochemical studies demonstrated that the inactivation of miR-21 by antisense oligonucleotides in androgen-independent PCa resulted in increased sensitivity to apoptosis, inhibiting cell motility and invasion. This may be attributed to the fact that miR-21 regulates PDCD4, TPM1, and MARCKS, which promote motility, apoptosis resistance, and invasion in prostate cancer [67]. Also, miR-21 downregulates PTEN by altering focal adhesion kinase phosphorylation and expression of MMP-2 and MMP-9. Thus, in human hepatocellular cancer cells (HCCs), the inhibition of miR-21 led to an increase in phosphatase and tensin homolog (PTEN) tumor suppressor expression, decreasing tumor cell proliferation, migration, and invasion [104].

miR-32 reduces the expression of BTG2 and PIK3IP1 participating in apoptosis in castration-resistant prostate cancer [74]. miR-185 and miR-342 inhibit SREBP-1 and 2 expressions, thus, controlling lipogenesis and cholesterogenesis and downregulating fatty acid synthase (FASN) and 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR). This, as a consequence, inhibited tumorigenicity, cell growth, migration, and invasion of prostate cancer cells [47].

Major signaling pathways

Cancer is complex and dynamic in nature, involving a variety of changes in structure and gene expression. Non-protein-coding RNA (miRNA) regulates cell growth, differentiation, apoptosis and is well documented in numerous diseases, but unable to find a direct link between cancer and miRNA. Numerous therapeutic effects and miR role as oncogene or tumor suppressor gene have opened the revolutionary field of research. In signaling, different miRNAs modulate different stages in the cell cycle. miRNAs interact with the apoptotic pathway in prostate cells and their dysregulation may promote apoptosis by regulating tumor suppressor genes. In apoptosis, the B cell lymphoma 2 (Bcl-2) family is regularly influenced by miR-125b whereas miR-221 and miR-222 modulated tumor necrosis factor. The downregulation in the expression level of miR-145 results in an enhancement in the process of cell proliferation, migration, and invasion in prostate cancer. miR-182 and miR-141/200a modulate the expression of certain genes involved in signaling networks, including PTEN/phosphatidylinositol-3-kinase (PI3K), TGF-β, and p53 pathways in prostate cancers.

Apoptosis

miRNA plays a significant role in the regulation of the cell processes that have gone awry in cancer, especially apoptosis. In this respect, miRNA critically regulates apoptosis in any event of tumorigenesis since cancer cells possess the innate ability to control miRNA in such way that enhances their probability of their survival and development. To achieve this, miRNA may upregulate or downregulate the protein expression in the pathway. The mechanism of miRNAs in apoptotic signaling is yet to be fully revealed, but several studies highlight its role in regulation. The unique morphology and biochemical changes in the cell gradually culminate in apoptosis and dysregulation in the process enhancing chemoresistance in prostate cancers [105].

miRNA is indulged in apoptosis process either intrinsic or extrinsic apoptotic pathways. Intrinsic pathways mainly target anti-apoptotic and pro-apoptotic factor of Bcl-2 (Fig. 3). The Bcl-2 family is marked overexpressed in different cancers featuring chromosomal translocation in Bcl-2 [106]. miR15a/161 downregulation has been reported in the several PCa specimens [107]. miR-34c silencing and downregulation of miR-34c activate PKC-ERK pathway upregulating Bcl2 and ameliorating ketamine-induced apoptosis in the hippocampus in Alzheimer's disease [108]. miR-125b upregulates the expression of apoptosis by targeting the BAK1 (pro-apoptotic member of Bcl-2 gene family) and eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1) [93]. This miRNA downregulates apoptosis by modulating bcl-1, Bcl-w, interleukin (IL)-6 and reducing the mitochondrial membrane potential promoting the cleavage of pro-caspase-3 [109].

Several studies have implicated miRNAs' role in the regulation of the extrinsic pathway of apoptosis. Numerous factors initiate signal directly by initiating apoptosis, and one of the important factors is tumor necrosis factor and its receptors. The tumor necrosis factor (TNF) family comprises Fas ligand (FasL), TNF-alpha (TNFα), TNF-related apoptosis-inducing ligand (TRAIL) cytokines involved in physiological

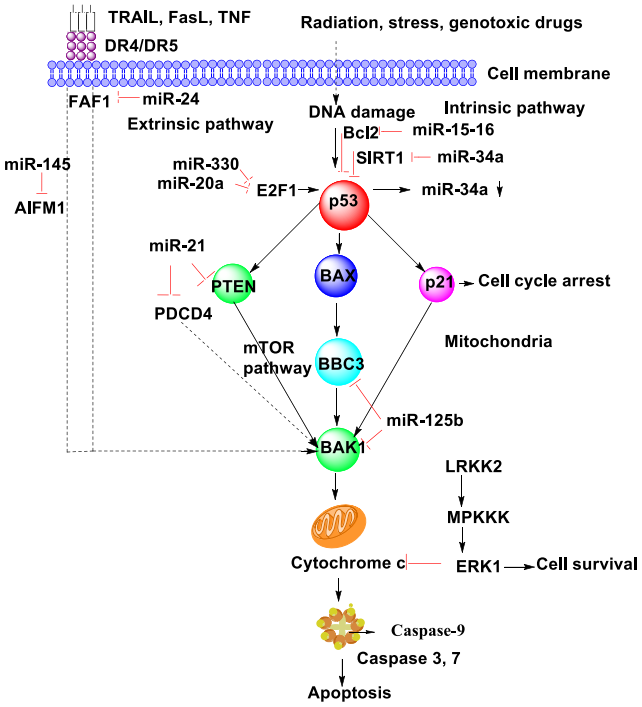

Fig. 3 miRNA influencing the apoptosis pathway. Different miRNAs modulate apoptotic pathways in PCa by controlling the expression of p53, p21, BAK1, and PTEN. In apoptosis, radiation or any stimulus causes cell damage by the process of apoptosis by miR-15-16, miR-34a, miR-330, miR-20a, miR-21, miR-145, and miR-125b. miR-15-16 inhibits the expression of Bcl2; miR-330 and miR-20 inhibit the expression E2F1, which modulates tumor suppressor p53 and subsequently the process of apoptosis. miR-21 inhibits PTEN and PDCD4 by modulating the expression of mTOR ultimately altering the expression of BAK1, also inhibited by miR-125b by inhibiting BBC3. On the other hand, miR-145 inhibits AIFM1 and controls the process of apoptosis

processes, systematic inflammation, tumor lysis, and apoptosis [110] (Fig. 3). Garofalo et al. (2008) identified miR-221 and miR-222 as regulators of TRAIL sensitivity in NSCLC. TRAIL-resistant (CALU-1) and TRAIL-sensitive (H460) cell lines were identified; differences in sensitivity to TRAIL were not related to differences in endogenous receptor levels, as receptor levels were demonstrated to be comparable to both cell lines. miRNA analysis indicated differential expression of seven miRNAs in TRAIL-resistant cells compared to TRAIL-sensitive H460 cells. Overexpression of two of these miRNAs, miR-221 and miR-222, in TRAIL-sensitive cells augmented resistance to TRAIL-induced cell death by approximately 40 %. On the other hand, the inhibition of these two miRNAs in TRAIL-resistant cells resulted in a TRAIL-sensitive phenotype and had a role in determining cell sensitivity to TRAIL [111]. Along with this, it also represses the protein product of the ink4a/ARF locus and $p14^{ARF}$ and modulates p53 network by hindering the downregulation of Mdm2, thereby affecting p53 and its target genes p21 and Puma to inhibit apoptosis [69]. Literature survey reports have established a link between miRNA and TRAIL-induced apoptosis. In this reports, small interfering RNA (siRNA) and microRNAs (miRNAs) target CDK4, PTGS1, ALG2, CLCN3, IRAK4, and MAP3K8 altered TRAIL-induced caspase-3 activation responses by Let-7c, mir-10a, mir-144, mir-150, mir-155, and mir-193 [112].

miRNA-330 induced the process of apoptosis in PCa cells through E2F1-mediated suppression of Akt phosphorylation and suppresses tumor growth [30]. Expression of another miRNA, miR-101, is decreased during cancer progression resulting in a simultaneous rise in the expression of epigenetic EZH2 (enhancer of zeste 2) attributed to the loss of genomic loci encoding miR-101. On the other hand, miR-31 downregulates EZH2 and targets anti-apoptotic protein E2F6, and subsequently PCa. This was further demonstrated by siRNA knockdown of EZH2 and DZNep histone methyltransferase contributing to silence the target gene in cancer cells in a study carried out by Varambally research group [33]. miR-31 downregulates EZH2 expression by targeting anti-apoptotic protein E2F6, thus, enhancing resistance to chemotherapy in apoptosis. This was further demonstrated by siRNA knockdown of EZH2 and DZNep (3-deazaneplanocin) treatment (an EZH2 inhibitor) in PCa that showed an enhancement in the miR-31 expression. Strategies involving the targeting of EZH2 and miR-31 in apoptosis improve cancer response to chemotherapy [48]. miR-34a suppresses c-Myc–Skp2–Miz1 transcriptional complex that activates and disrupts the assembly of RhoA which eventually represses cell migration and invasion [113]. Along with this, CD44, adhesion molecule biomarker of cancer was also inhibited by miR-34a. miR-20a, the member of the mir-17–92 cluster, modulates the translation of the E2F2 and E2F3 mRNAs in apoptosis [72] and gets upregulated by suppressing CX43 expression (a gap junction protein) during apoptosis [114]. miR-24 is known to suppress initiation and elongation in the translation of mRNA encoding the tumor suppressor p16 (INK4a) and target Fas-associated factor 1 (FAF1) in apoptosis [73].

PSA provokes prostate cancer cells by degrading the extracellular matrix components fibronectin and laminin. Also, PSA stimulates the release of IGF-I (insulin-like growth factor I) and TGF-β from their binding partners, thus are implicated in osteoblastic lesions [115]. miR-203 behaves as antimetastatic in PCa by acting in multiple steps of the metastatic cascade via the repression of a cohort of prometastatic targets [116]. miR-203 downregulated the expression of the E-box-binding transcription factor ZEB2, also known as Smad-interacting protein 1 (SIP1), regulating the expression of E-cadherin and EMT [53]. miR-203 regulates Smad4 mediator of TGF-β intracellular signaling and brings down the levels of miR-203 transcription factor E2F1 reported to be elevated in metastatic hormone-resistant PCa [117]. miR-1247-5p is overexpressed in castration-resistant prostate cancer as a consequence of targeting myc-binding protein 2 (MYCBP2) [70].

Cell invasion and migration

Cell migration and invasion form an important part of cancer progression. Cancer therapies target adhesion receptors or proteases effective in checking cancer progression [118]. In the same view, miRNA also plays a significant role in propagating metastasis by modulating the expression of various proteins in the concerned pathway. Christoffersen and his research group highlighted the role of miR-200 in olfactory systems which becomes important in neurodegenerative conditions [119]. Furthermore, many studies concluded miR-144 to be downregulated in hepatocellular carcinoma, lung cancer, as well as prostate cancer. miR-144 is also known to participate in zinc finger X-chromosomal protein (ZFX) pathway, important for its involvement in self-renewal and maintenance of both embryonic stem cells and hematopoietic stem cells, and in growth regulation of non-small cell lung cancer [61]. The ability of microRNA-144 to control cell proliferation, migration, and invasion in nasopharyngeal carcinoma was thought to be a consequence of PTEN repression. Mechanistically, miR-144 suppresses PTEN expression leading to increased pAkt and cyclin D1 expression, promoting G1-phase transition, subsequently inhibiting E-cadherin to promote migration and invasion [60]. Increased circulating level of miR-144 has been correlated with the downregulation of insulin receptor substrate 1 (IRS1) at both mRNA and protein levels in type 2 diabetes mellitus. This study has helped to conclude the participation of circulating miRNAs in controlling the expression of the corresponding targets and regulatory networks governing different stages of the insulin pathway [120]. miR-144 is also seen to be involved in regulating bladder cancer cell proliferation by Wnt signaling that regulates tumorigenesis and cancer progression by receiving and transmitting signals from outside the cell to inside. miR-144 downregulates the expression of EZH2 which is a consequence in the activation of Wnt/β-catenin signaling [59]. EZH2 is an enzymatic component of the polycomb repressive complex 2 expressed in breast and prostate cancers, catalyzing histone H3 trimethylation at lysine 27 (H3K27me3) and repressing the promoter region [121]. Forkhead box-O (FOXO3a) significantly inhibits the expression of β-catenin in PCa and also modulates transactivated microRNA-34b/c that initiates a cascade of events leading to the suppression of epithelial to mesenchymal transition in PCa. The miRNA suppresses β-catenin mRNA expression by targeting the untranslated regions (UTRs) of β-catenin. The complex of β-catenin with FOXO3a competes with T cell transcription factor (TCF) for interaction with β-catenin, thus, inhibiting β-catenin/TCF transcriptional activity and reducing the expression of β-catenin target genes. This suggests that FOXO3a modulates WNT/β-catenin signaling [58]. Recently, the downregulation of miR-188-5p has been reported in normal survival cells while the restoration of this miRNA significantly suppressed proliferation, migration, and invasion in vitro and also inhibits tumor growth and metastasis in vivo. The research group identified lysosomal protein transmembrane 4 beta (LAPTM4B) to be significantly overexpressed in PCa by inhibiting the PI3K/AKT signaling pathway and thus becoming a promising target for miR-188-5p [42].

TGF-β pathway

Members of the TGF-β pathway are known to be targeted by one or more miRNAs including miR-143, miR-145, miR-146a, and miR-199, reported in PCa signaling. The transforming growth factor-β (TGFβ1) family is a pleiotropic group of growth factors that transduce the signal through serine/threonine kinase receptors imperative in carcinogenesis and tumor progression. Research groups have been focussing primarily on the interaction of TGF superfamily intracellular signaling proteins with Smad-miRNA. A study performed by Davis research group laid the milestone in the field of Smad and miRNA crosstalk demonstrating the potential of TGF to bind with Smads. This is because the binding of the R-Smads-1/5 to Drosha in response to TGF and bone morphogenetic protein (BMP) stimulation promotes the Smad-4 independent cleavage of pri-miR-21 to pre-miR-21 [122]. Somatic mutations or epigenetic events leading to the loss of members in the TGF-β signaling result in G1 phase arrest, suppression of c-myc, and simultaneously stimulating cyclin-dependent kinase inhibitors including $p21^{WAF1}$ and $p15^{Ink4b}$. It is known to stimulate apoptosis by inducing death-associated protein kinase (DAP) in the TGF-β signaling pathway [123], at the same time playing an important role in tumorigenesis by its tumor suppressor/-promoting effect. The TGFβ1 portrays the different roles in different stages of cancer. In the initial stages, it behaves as a tumor suppressor inhibiting cell growth and inducing apoptosis, whereas, acting as a tumor promoter in the later stages enhancing the metastasizing ability of cancer [124]. Many of the human cancers, especially prostate cancer, have been reported to be devoid of TGFβ1 signaling pathway components such as TGFBR1, TGFBR2, SMAD2, and SMAD4 [125–127]. This was further confirmed by Davis-Dusenbery research group reporting miR-143 and miR-145 to be downregulated inTGFβ1 pathway of PCa [128]. Furthermore, various research group concluded that miR-143 targets Kirsten rat sarcoma viral oncogene homolog (KRAS), ELK1, myosin 6 (MYO6), B cell lymphoma 2 (Bcl-2), and extracellular signal-regulated kinase 5 (ERK5) were seen to be involved in the progression of cancer [129, 130].

miR-145 targets MYO6 (cancer-related cell migration) and fascin homolog1, an actin-bundling protein that controls cell proliferation, migration, and invasion in the progression of cancer [36]. This may be owed to the blocking action of miR-145 on Akt and ERK1/2 pathways in addition to hindering the TGFβ1 signaling [131]. The miR-146a expression is

increased in human dermal fibroblasts cells following TGF-β1 stimulation [132], but is downregulated in PCa in response to SPI1 gene activation, again, stimulating TGFβ1. Its variant miR-146b-5p regulates TGF-β signal transduction by repressing SMAD4 as in thyroid cancer [133]. On the contrary, the administration of TGFβ1 in the myeloid dendritic cell increases miR-146a expression, seen as an outcome of SPI1 expression [32]. miR-146a also inhibits angiogenesis and bone metastasis by suppressing both matrix metalloproteinase-2 (MMP2) and the expression of Rho-associated protein kinase 1 (ROCK1) [31]. miR-199a exhibited an inverse correlation with Smad4 expression in cancer, repressing Smad4 expression and blocking the canonical TGF-β transcriptional responses in different cell lines [50]. Thus, the loss of miR-199a and inhibition of mTOR eventually result in cancer. miR-199a downregulates the PCa by inhibiting MMP9 [51]. miR-199a normally inhibits c-Met that results in the loss of TGFβ1 signaling pathway [134]. In the future perspectives, the use of TGFβ1 as a therapeutic agent proves to be important in the signaling pathway, and the expression of miR-143, miR-145, miR-146a, and miR-199a might be a promising strategy for PCa therapy. Like miR-199a, miR-183 targets SMAD4, and Dkk-3, both having a crucial role in prostate cancer and Wnt signaling pathways [135]. TGFBR2 is also influenced by methylation in promoter region causing downregulation that is important in the breast, colon, and LNCaP prostate cancer cells. Moreover, coordinated action of miR-21 and androgen receptor (AR) in signaling inhibits TGFBR2 in PCa. miR-21/AR attenuates TGF-β-mediated Smad2/3 activation, cell growth inhibition, cell migration, and apoptosis in PCa [136].

ERK signaling

Extracellular signal-regulated kinases (ERKs) communicate and transmit signals from a receptor on the surface to the nucleus of the cell. Liu research group found that the overexpression of miR-21 in DU145 cells augmented the expression of HIF-1α and vascular endothelial growth factor (VEGF) and provoked tumor angiogenesis. Mechanistically, miR-21 activates Akt and (ERK) 1/2 and overexpressed miR-21 targets and inhibits tumor partially by inactivating AKT and ERK, increasing the expression of HIF-1 and VEGF. Furthermore, the enhancement of HIF-1α and VEGF expression regulates angiogenesis [68]. A persistent decrease in miR-143 was reported in cancer, resulting in the inhibition of KRAS and ERK5 proteins that act as a promoter of cell growth on tyrosine kinase activation [52]. Furthermore, an optimum level of miR-143 is necessary to prevent cancer as suppression or overexpression in the level of miR-143 and modulates the mitogen-activated protein kinase (MAPK) cascade via ERK5 pathway. miR-143 suppresses KRASV-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), a key molecule of EGFR/RAS/mitogen-activated protein kinase (MAPK) pathway [137]. Differential expression of miR375, miR378, and miR141 possesses therapeutic value when overexpressed in the serum of castration-resistant prostate cancer (CRPC) patients compared to serum from low-risk localized patients. Moreover, CRPC expression was significantly decreased by miR-146a that inhibits cell growth, colony formation, migration, tumorigenicity, and angiogenesis by repressing the expression of EGFR along with MMP2. Along with this, it also downregulates p-ERK expression critical for the pathogenesis of CRPC [31]. Other microRNAs such as miR-23a and miR-23b have also been reported to be indulged in MAPK and JAK/STAT signaling pathways in PCa [77].

PI3K/Akt/mTOR signaling

PI3K/Akt/mTOR is a core regulator of cell cycle proliferation, growth, survival, protein synthesis, and glucose metabolism [138]. PI3K is responsible for intercellular signaling regulating diverse cellular functions. Phosphorylation activates PI3K, and the further signaling activates Akt localizing it in the plasma membrane. Akt activates the various downstream proteins NF-KB, MDM2, mTOR inhibiting the expression of BAD, and GSK3β involved in signaling in the cell. Overall, this reduces apoptosis allowing cancer proliferation [139] (Fig. 4). In PCa, Akt plays a central role in the development of androgen-independent prostate cancer (Fig. 4). Akt activates mTOR that promotes translation through the phosphorylation of S6K1 and 4EBP1, which enhances the expression of AR-responsive genes [140]. The expression of mTOR is repressed by miR-100 and also by its other members—miR-99a and miR-99b. CD44 is a transmembrane receptor for hyaluronan playing critical roles in cell-cell and cell matrix adhesion, migration, signaling, and tumor metastasis that directly target miR-708 and serine/threonine kinase Akt2. miR-708 is believed to control CD44+ prostate cancer-initiating cells as concluded by a study performed by Saini and his group [141]. The serine/threonine kinase AKT2/PKBb is the member of the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway which is a key player in cellular proliferation, survival, migration, invasion, and metastasis. Any aberrations in the PI3K/Akt pathway lead to tumorigenesis associated with poor outcome in prostate cancer [142]. Rasheed research group identified GNA13 to be an important mediator in prostate cancer invasion and miR-182 and miR-200 members to regulate its expression post-transcriptionally. The post-transcriptional analysis showed miR-182 to inhibit the PI3K/Akt/mTOR pathway whereas miR-141/200a inhibited invasion in meningioma by indulging E-cadherin and Wnt signaling in PCa [143]. Overexpression of miR-1 represses colorectal carcinoma (CRC) cancer progression and modulating MAPK and PI3K/AKT pathways by the inhibition of ERK and Akt by targeting LIM and SH3 protein 1 (LASP1)

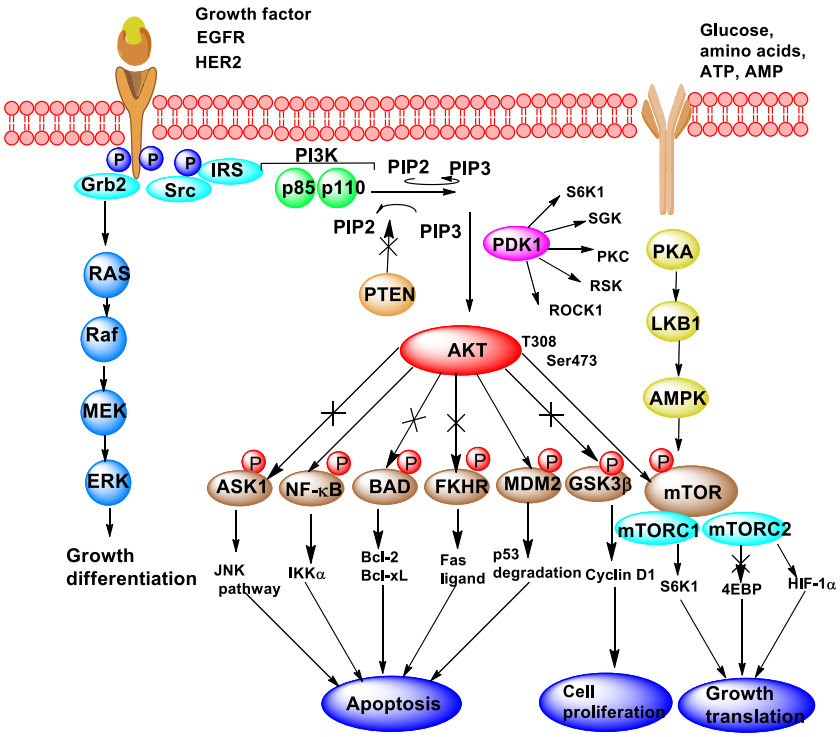

Fig. 4 Mechanism involved in the activation of PI3K/AKT/mTOR pathway and their downstream signaling involved in the pathway

in CRC progression [28]. Transfection with miR-331-3p reduced ERBB-2 mRNA expression blocking downstream PI3K/Akt signaling [38]. MicroRNAs miR-125b, miR-99a, miR-99b, and miR-100 were downregulated in PCa, decreasing the expression of prostate-specific antigen (PSA). Among these, micro-RNA-99 family post-transcriptionally regulates PSA by suppressing the levels of SMARCA5, which directly targets PSA. SMARCA5 is part of a chromatin-remodeling complex facilitated by ATP-dependent nucleosome remodeling and transcription initiation that belongs to the member of SWI/SNF family [144]. SMARCD1, another member of SWI/SNF family of proteins, interacts with the ligand-binding domain of AR in the FxxFF motif in an androgen-dependent manner and also inhibits the SMARCD1 expression in mRNA and protein levels [140].

Epithelial-mesenchymal transition signaling

The epithelial-mesenchymal transition (EMT) is a process involving the loss of epithelial cell properties such as cell polarity, cell-cell adhesion, gain migratory, and invasive property in the process of becoming mesenchymal stem cells. EMT is an obligatory developmental process such as wound healing in organ fibrosis and the initiation of metastasis for cancer progression and tightly regulated by miRNA. The miRNA participates with the E-cadherin transcriptional repressors ZEB1/δEF1, ZEB2/SIP1, and TGFβ2 signaling network that facilitate the maintenance of stable epithelial or mesenchymal states [145].

EMT is a critical step of cellular plasticity for cell detaching, migrating, invading, dispersing, and final residing in tumor cell invasion and metastasis. Loss or acquisition of epithelial and mesenchymal markers occurs as a result of disassembly of tight junction and loss of apical-basal polarity. E-cadherin and other connecting members enable cells to increase motility and invasiveness through the disruption of intercellular contacts. This results in an extracellular matrix (ECM)-induced stimulation of integrin signaling and formation of focal adhesion, together culminating in cell motility and invasion [146]. Loss of E-cadherin is also accompanied by a concomitant increase in the expression of mesenchymal-associated protein including vimentin, N-cadherin, fibronectin, and integrin, which confer a motile phenotype on the cancer cells by changing cell-matrix interactions and their architecture [147]. Mechanistically, signaling pathways such as TGF-β, Ras, Wnt, and Notch induce the epithelial-mesenchymal transition by repressing or inducing transcriptional factors. In turn, the stimulation of transcriptional factors such as ZEB1 and ZEB2 (zinc finger E-box-binding homeobox) hinders the expression of E-cadherin inducing cell migration and invasion in the process of activating the epithelial-mesenchymal transition [53, 148]. Transforming growth factor-β (TGF-β) has been noted to be the major inducer of EMT in the epithelial cells not only during the embryonic development but also in cancer progression. As a consequence of TGF-β-mediated EMT, the expression of members of the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) involved in the regulation of EMT was reduced

significantly. Inhibition of miR-200-induced mesenchymal-like spindle cell morphology increased ZEB1 expression and stimulated cell migration, whereas loss of miR-200 inhibited the E-cadherin expression in invasive breast cancer cell lines [55]. Initially, microRNA miR29b was found to inhibit MMP2, Mcl-1 (an anti-apoptotic Bcl-2 family member), and collagen expression and also modulated prostate cancer [45]. Further studies revealed their ability to regulate epithelial-mesenchymal transition signaling, thus, controlling the progression of PCa. On one hand, it enhanced the expression of E-cadherin and, on the other, downregulated the Ncadherin expression, Twist, and Snail expression in PCa [44]. Further research in the field led to the discovery of markers that remarkably improved specificity and sensitivity for early detection and prognostication of different abnormalities. miR-141 was one such stable marker detected in the plasma of PCa patients regulating the transition from epithelial to mesenchymal tissue. To determine the miR-141 response, longitudinal measurements of PSA, circulating tumor cells (CTCs), and lactate dehydrogenase (LDH) were employed where miR-141 is 78.9 % sensitive in predicting clinical outcome [149]. The expressions during metastasis were controlled by miR-143 and miR-145 and correlated with bone metastasis in primary PCa. Overexpression leads to the reduction in the ability of migration and invasion and their upregulation enhancing E-cadherin expression and reducing fibronectin, crucial in the regulation of EMT. Li research group demonstrated that miR-200b, miR-200c, let-7b, let-7c, let-7d, and let-7e expressions were significantly downregulated in gemcitabine-resistant cells having properties like elongated fibroblastoid morphology, lower expression of epithelial marker E-cadherin, and higher expression of mesenchymal markers identical to EMT [150]. TGF-β-induced E-box-binding transcription factor SNAI2 is known to promote EMT by repressing miR-203 which downregulates breast cancer by targeting SNAI2 and SNAI2, which directly binds to the miR-203 promoter region, inhibiting the transcription process. SNAI2 and miR-203 control EMT by inhibiting double negative feedback loop expression [54]. miR-99a/100~125b tricistrons converged and blocked combined TGF-β by targeting SMAD signal transducers. It downregulates APC/APC2 tumor suppressor gene and enhances Wnt signaling and thereby maintains a balance between Wnt and TGF-β signaling [75]. miR-34a is negatively correlated with migration and invasion of PCa cells through LEF1 [46], and miR-34a modulates the levels of lymphoid enhancer-binding factor-1 (LEF1) to regulate EMT in PCa cells. miR-205 down modulates miRNA in PCa cells by the stimulation of cancer-associated fibroblasts (CAFs) and suppresses the transcription with the involvement of HIF-1. It impairs the enhancement of cell invasion, acquisition of stem cell traits, tumorigenicity, and metastatic dissemination. Also, miR-205 also blocks tumor-driven activation of surrounding fibroblasts, by reducing pro-inflammatory cytokine secretion. This work highlights the role of miR-205 in the bidirectional interplay between PCa cells and fibroblasts [37].

Cell cycle

The cell cycle is regulated through multiple molecular pathways and checkpoints requiring the presence and activity of multiple proteins not expressed in the quiescent cell. The regulation of the levels of CDK and cyclin proteins is a central issue to the understanding of the cell cycle control. Literature highlighted the link of miRNAs and cell cycle regulation during the analysis of the antiproliferative potential of the mir-15a-16-1 cluster and identified in chronic lymphocytic leukemia (CLL) patients. This study infers that miRNA cluster is deleted or downregulated in about 70 % of CLL, prostate cancer, and gastric cancer cell line, suggesting an important role in tumor development [34, 151]. Ectopic miR-34a arrests the cell cycle at G1 phase regulating the process of apoptosis. c-Myc, a proto-oncogene, regulates cell proliferation and transformation both transcriptionally and non-transcriptionally, and any minute indiscretion causes human cancer [152]. Various reports have concluded the role of miR-153 upregulation in PCa brought about by the repression of the phosphatase and tensin homolog (PTEN) expression. The inhibition of the PTEN expression results in Akt kinase activation, at the same time, downregulating the transcriptional activity of Forkhead box-O (FOXO) 1. This combined event lead to the upregulation of the G1/S transitional promoter cyclin D1 and downregulation of the cyclin-dependent kinase (CDK) inhibitor p21 [78]. TNFSF10, also known as TRAIL, is an antitumor protein having the ability to induce apoptosis in cancer cell lines without altering the functioning of the normal cells. DNA fragmentation, expression of pro-phagocytic signals on the cell membrane, and cleavage of multiple intracellular proteins by caspases are the hallmarks of TRAIL-induced cell death [153]. Upregulation of pro-apoptotic genes TNFSF10 and IL-24 result from overexpressing miR-145 in PC-3 cells [154].

Overexpression of miR-1, a microRNA expressed mostly in the heart and skeletal muscle tissue, results in inhibition and downregulation of the cell cycle, mitosis, and DNA replication/repair. A gene set enrichment analysis revealed that miR-1 functions similar to histone deacetylase inhibitors. It also alters the cellular organization of F-actin inhibiting tumor cell invasion and filopodia formation [27]. Liu research group revealed that the inhibition of prostate cancer cell proliferation and clonal expansion in vitro and tumor regeneration in vivo is brought about by the overexpression of let-7 by inducing G2-M cell cycle arrest without senescence [155]. Also, miR-34b triggered the arrest in G0/G1 cell-cycle phase and apoptosis by directly impeding the Akt and inhibiting cell proliferation, colony formation, and migration/invasion in PCa. The different mesenchymal markers vimentin, ZO1, N-cadherin, and Snail showed lowering of expression with an enhancement

in the expression of E-cadherin, thus showing epithelial-to-mesenchymal transition and also providing the platform for the strategies of epigenetic regulation in PCa [156]. Further, the literature revealed that miR-449a can cause cell cycle arrest, apoptosis, and a senescent-like phenotype by hindering the HDAC-1 expression (histone deacetylase 1), frequently expressed in cancer [39]. Noonan and research group also identified miR-449a-mediated growth arrest dependent on the Rb protein. miR-449a was found to suppress phosphorylation by knockdown of cyclin D1 and target HDAC1 [40]. miR-744 and miR-1186 have been seen to induce cyclin B1 and stimulate cell cycle progression resulting in chromosomal instability. Mechanistically, miRNA undergoes interaction with the transcriptional apparatus to express numerous genes controlling the signaling cascade involved in cell proliferation, evading apoptosis, angiogenesis, and DNA damage repair in the PCa [157]. A study performed by Wang research group demonstrated that miR-145 expression was inversely related to the expression of small ubiquitin-like modifier (SUMO)-specific protease 1 (SENP1), but the transient level of miR-145 arrests cell cycle in PC-3 cells and an opposite effect was demonstrated when the miR-145 inhibitor was transfected [71].

Miscellaneous

The dynamic nature of miRNA makes it a target for numerous pathways. Jin research group demonstrated that miR-128 targets the stem cell regulatory factors BMI-1, NANOG, and TGFBR1 inversely controlling the expression. Among the miR-128 isoforms, miR-128-lo cells were found to possess higher clonal, clonogenic, and tumorigenic activities than miR-128-hi cells. [65]. Tumor suppressors miR1 and miR133a target the oncogenic function of purine nucleoside phosphorylase (PNP) in prostate cancer [43]. The introduction of miR-23b/-27b in metastatic castration-resistant PCa attenuated Rac1 activity without affecting total Rac1 levels by the enhancing cadherin level of expression. Inhibition of these miRs showed the complete opposite effect in androgen-dependent LNCaP cells [76]. One of the distinct characteristics of PCa is the overexpression of the small ubiquitin-like modifier (SUMO)-specific protease 1 (SENP1), and its upregulation results in cell proliferation. miR-145 expression was inversely related to the expression of SENP1, but the transient level of miR-145 arrests cell cycle in PC-3 cells, and an opposite effect was demonstrated when the miR-145 inhibitor was transfected [71]. Glutathione S-transferases (GSTs) play a crucial role as metabolic multi-gene detoxifying isozyme in the xenobiotics metabolism [158], both for endogenous, as well as exogenous cell substances. Silencing of GSTP1 marks the progression of human prostate cancer. GSTP1 can be potentially regulated by a group of microRNAs such as miR-133a/b, miR-144/144, miR-153-1/2, and miR-590-3p/5p [159]. miR-133b is involved actively in regulating death receptor-mediated apoptosis in PCa [160], its overexpression inhibiting tumor cell proliferation and inducing apoptosis in cancer [161]. Moreover, it is a target of the androgen receptor (AR) required for androgen-mediated stimulation in LNCaP cells and regulation of CDC2L5, PTPRK, RB1CC1, and CPNE3 in PCa [57]. miR-145 is upregulated, simultaneously downregulating DNMT3b, an epigenetic modification consequential in cancer initiation and progression especially in PC3 cells. This was further proved by a study which highlighted the combination of ionizing radiation and epigenetic regulation in prostate cancer therapy [66] to be a dangerous recipe in that such a correlation displayed crosstalk between miR-145 and DNMT3b via a double negative feedback loop.

miRNA in clinical trials

In recent times, numerous drugs have undergone and successfully passed the clinical trials. In the race to find clinically approved drugs, numerous companies such as MiRagen Therapeutics, Regulus Therapeutics, SantarisPharma, and Mirna Therapeutics have been carrying out the clinical trials on onco-miRs or tumor-suppressive miRs [162]. In a recent study, Mirna Therapeutics and Regulus Therapeutics targeted miR-34a as a tumor-suppressive miRNA in p53 and found it to be downregulated in the prostate, breast, lung, kidney, bladder, ovary, and skin cancer [163–165]. MRX34 is Mirna's lead product that entered clinical testing in 2013, mimicking the open-label Phase 1 clinical trial as in patients with primary liver cancer or solid cancers with liver involvement. This candidate was designed to deliver a mimic of miR-34 encapsulated in an innovative liposomal formulation called SMARTICLES and was found to be under-expressed in the patients with cervical cancer, ovarian cancer, glioblastoma, hepatocellular carcinoma (liver cancer), colon cancer, non-small cell lung cancer (NSCLC), and in cancer stem cells. The preclinical trials conducted revealed that intravenous delivery of MRX34 was greater than the 100-fold as compared to miR-34 levels in liver tumor cells. The main concern for the clinical trial was to establish the maximum level of dose tolerance, and for this purpose, Phase 2 dose has been recommended for future clinical trials [166]. miRNA-directed therapy 122 targeting hepatitis C (HCV) had gone under clinical trials after surmounting the toxicity issues and specificity. A study carried out by the researchers on chimpanzee model of chronic HCV infection demonstrated that systemic delivery of antimiR-122 could reduce HCV viral load with minimal toxicity. SantarisPharma conducted a phase IIa trial in human to ascertain safety and antiviral function using miravirsen, a locked nucleic acid-modified miR-122 antagonist. let-7 is known to suppress KRAS—an oncogene frequently mutated in lung cancer. KRAS 3′UTR polymorphism was predicted

for the monoclonal antibody cetuximab responsiveness in KRAS wild-type mCRC patients arising a need for validation in other clinical trials [167]. MicroRNA, miR-208, encoded by an intron of the alpha-myosin heavy chain (α-MHC) gene, stimulates cardiomyocyte hypertrophy, fibrosis, β-MHC expression in response to stress, and hypothyroidism. Knockout of this miR was resistant suggesting the role of an antagonist in chronic heart disease [168, 169]. Recently, Saini and his group identified novel miR-3607 and its role in directly repressing oncogenic SRC family kinases LYN and SRC in PCa [170]. miRNAs that are in different phase of clinical trials, since 2000 to the recent times, are represented in Table 3.

miRNA as therapy

Advancement and tailoring of appropriate molecular drugs are based on innovation and inculcation of new ideas [175]. Early detection and diagnosis by radical prostatectomy or radiation therapy give positive outcomes. Irrespective of massive development, researchers are unable to find a biomarker and pose a challenge to non-availably of an efficient biomarker for the detection in PCa. The prostate-specific antigen (PSA) is a most common research biomarker for mass screening but due to its limitation like low detection rate has restricted its use. A diagnostic, prognostic, and therapeutically adept molecule may help in predicting disease severity and establish effective therapy against PCa. Therefore, miRNA presents an appealing target for biomarker discovery. Due to its short sequences, it is resistant to the RNase degradation in both serum and tissues. miRs provide a fast fine-tuning and energy-saving mechanism for the post-transcriptional process. Therapies for the human disorder by RNA inhibition include drug preparation against mRNA concerned in the pathogenesis and direct targeting of non-coding RNAs in pathogenesis. The primary RNA inhibition agents used in clinical studies are antisense oligonucleotides (ASOs), ribozymes, DNAzymes, small interfering RNAs (siRNAs), short hairpinRNAs (shRNAs), and anti-miRNA agents such as ASOs-anti-miRNAs, locked nucleic acids (LNA)-anti-miRNAs, or antagomirs, class of bicyclic high-affinity RNA targeting miRNA.

The ligand-activated transcription factor, androgen receptor (AR), is involved in the process of development and progression of PCa. Treatment of PCa by androgen ablation therapy achieved success in 1960s. Later, it was reported that PCa in patients primarily respond to androgen ablation therapy, relapses with castration-resistant prostate cancer (CRPC) within 3 years [176]. The AR protein is a ligand-dependent transcription factor proving to be a novel therapeutic target for the effective management. The therapy comprises of the use of gonadotropin-releasing hormone (GnRH) agonists (suppressing the testicular androgen synthesis) and anti-androgens (inhibit the binding of androgen to the ligand binding domain of AR) [177]. Initially, therapies repressed AR activity and initiated tumor suppression but after a short period of 3 years, the expression of AR changed consequently in the development of CRPC. The basic mechanism behind AR-mediated CRPC included AR gene amplification, hypersensitivity of the AR to trace levels of circulating androgens, the promiscuous affinity of the AR due to the accumulation of numerous mutations, and finally, growth factor-activated aberrant pathways, which are exclusively mediated via AR molecular actions [178].

Strategy for targeting particular defect is linked to the pathogenesis of the disease that is directed by multi-step pathways, destructed by miRNA inhibitors. RNA has remained neglected as promising drug target owing to its high electronegative surface and flexibility in their structure. The secondary structures of miRNA display higher plausibility of drug delivery with increasing specificity. Formation of stem-loops and bulges in pre-miRNA might become the possible target for small molecules. These structural features may facilitate drug entry by the enlargement of the major groove, and partially disclosing the internal bases scattering the local electronegative distribution, and, providing specificity basis for structure-based drug design [179]. Discovery of the small

Table 3 Different miRNAs in clinical trials by different companies

S. no.	miRNA	Therapy	Phase	Company	Reference
1	miRNA-34a	Mimics	Phase I	Mirna Therapeutics	[163–165]
2	MRX34,	Mimics	Preclinical	Mirna Therapeutics	[166]
3	miR-122	HCV	Phase II	Regulus Therapeutics and SantarisPharma	[83, 169]
4	miR-146a	Mimics	Preclinical		[171]
5	miR-208	Chronic heart failure	Preclinical	MiRagen	[168]
6	miR-15/195	Post-MI remodeling	Preclinical	Therapeutics	[172]
7	let-7	Mimic	Preclinical		[167]
8	miR-21	HCC, cancer, fibrosis	Preclinical	Regulus Therapeutics	[173]
9	miR-33	Inhibitor	Preclinical	Therapeutics	[174]

molecule targeting miRNA facilitated the expression and achieved through computational methods to predict the correct 3-D structure of miRNA.

miRNA manipulation involves either direct silencing or reduction of tumor promoting. In vivo approaches mainly include genetically engineered animals and miRNA vector systems such as viral vectors, nanoparticle-based delivery, mimics, sponges, and antimiRs. There are three types of modified antimiRNA oligonucleotides: 2-OH residues of the ribose by 2′-O-methyl modified oligonucleotides, 2′-O-methoxyethyl, and locked nucleic acid [180]. In addition, conjugation with cholesterol may serve to improve target specificity. Sponges have been reported to silence miRNAs by targeting multiple binding sites and competing with the target mRNA for miRNA occupancy, thus, decreasing the binding and subsequent effect of miRNA [181].

miR agonists or antagonists may either restore or block the function of a given miR [182]. Synthesis of pre and anti-RNA molecules within the carrier vehicle is helpful for a patient administered systematically during cell signaling. This enables disease-specific targeting and, more importantly, is regarded as the future of oncology. Radiotherapy (RT) has proven worthwhile in the treatment of solid tumor in cancer. Irradiation is adept enough to target the cancerous lesions without affecting adjacent normal cells. Optimal use in term of time and dose has highlighted its efficacy, minimizing the risk of toxicity in the patients. Studies involving knockdown experiments with irradiation at multiple steps in miR biogenesis have evaluated its potential. miRNA represses DNA damage response (DDR) pathway and increases radiosensitivity. Especially, miR-421 and miR-24 downregulate ATM and H2AX, respectively, increasing induced genomic instability and cell death [62, 63]. Numerous studies have highlighted that hypoxia-induced miR-210 stabilizes the hypoxia-inducible factor-1 (HIF-1) complex, thus, augmenting radioresistance [64]. Issues in radioresistance in RT were reversed by synthetic miR made of oligonucleotides as tumor suppressor replacement therapies. Lung cancer xenograft using liposomal nanoparticles used in miR-200c mimics intracellular reactive oxygen species exhibiting sensitization with radiation [183]. Some miRNA regulates self-renewal potential in the cancer stem cells (CSC) affecting tumor radioresistance. In particular, miR-34a negatively regulates human primary tumor-derived CD44+ prostate CSCs and hampers the PCa progression [184]. Insufficient data and results after administering RT dose in PCa are still doubtful. To predict whether patients have increased the risk of normal tissue toxicity, researchers performed genomic analyses for single-nucleotide polymorphisms in normal cells. However, none of biomarker proved to be significant [185]. It is due to a different subpopulation of patients at risk of developing significant radiotoxicity and, therefore, to make it rational, needs radiation dose-volume metric [186]. Recently, it was observed that radiation enteropathy increases miR-210 expression and repressed by anti-fibrotic therapy [187]. miR-296 directly targets high-motility group At-hook gene 1 (HMGA1) protein (oncofetal protein) expressed in PCa and represses HMGA1 translation [26].

Delivery system

miRNAs are being heralded as an imperative biomarker, but the successful delivery process remains a challenge. Global miRNA suppression augments cellular transformation, and tumorigenesis is highlighting the pro-tumorigenic effects of miRNA loss-of-function. Successful drug delivery becomes important in protecting miRNA against serum nucleases, prolonging circulation time, avoiding renal clearance, and minimizing the non-specific interaction with non-target cells. In addition, it also enhances the accumulation of the miRNA drug in the target tissues facilitating their uptake by target cells and their subsequent release in the cells. These safety concerns are in the light of their successful delivery. Recombinant MS2 bacteriophages virus-like particles (VLPs) by virtue of their ability to pack specific exogenous RNA with *pac site* protect mRNA and microRNA from degradation. On the contrary, such protection is compromised on insertion of the cell-penetrating peptide (CPP) between amino acid residues 15 and 16 of the second subunit of the MS2 coat protein where the single-chain dimer improves the delivery system. They are nuclease resistant, non-replicative, non-infectious, and non-toxic. Pre-miR-146 was produced by combining the expression of MS2 (MS2 bacteriophage) virus-like particles (VLPs) and *Escherichia coli*, followed by conjugation of these particles with HIV-1 $Tat_{47–57}$ peptides. This system facilitated effective miRNA transfer into various cells [188]. Systemic administration of miR-26a using an adeno-associated virus (AAV) was observed to reduce hepatocellular carcinoma (HCC) and induce cell-cycle arrest associated with direct targeting of cyclins D2 and E2. Another delivery method comprises of the alternate coating of dopamine-modified alginate on miRNA to construct multiple layers on titanium surface using a layer by layer technique. This system exhibiting significant multi-layer enhancement in cell proliferation was evaluated by water contact angle tests and fluorescence microscopy [189].

Influenza A-based delivery system utilizes miR-155 to knock down SOCS1 mRNA, in turn, downregulating SOCS1 gene expression [190]. Another delivery system involving a nanoparticle-based biodegradable lipid rapidly eliminates lipids from plasma and tissues and substantially improved tolerability in tested preclinical tests. Different modifications in the structure by biodegradable linkers, ester linkages aid in rapid enzymatic cleavage facile for the design of lipid with cleavable groups within the hydrophobic lipid tails

[191]. Dual drug delivery systems for endogenous microRNA miR-34a and paclitaxel have been demonstrated recently for synergistic cancer therapy. Nanoparticle prepared by this combination use to treat murine B16F10-CD44^{+} melanoma prepared by cationic solid lipid nanoparticles (miSLNs-34a/PTX) [192].

siRNA delivery systems are also available which are based on lipoplexes prepared by cationic polymeric liposomes and pH-sensitive diblock copolymer. PEGylation reduces nonspecific interaction avoiding uptake by the cells of the reticuloendothelial system (RES), minimizing the kidney elimination, and increasing the circulation time [193]. Intravenous injection of cholesterol-conjugated siRNA (chol-siRNA) improved the efficacy in hepatocyte targeted by reversibly masking endosomolytic polymer [194]. Hao and research group reported the role of miR-15a and miR-16-1 during gene delivery in PCa in synergistically inducing selective cell death by second-generation APT (ATE–APT). Atelocollagen (ATE) and RNA aptamer (APT) A10-3.2-mediated siRNA delivery system can be used to silence endogenous genes concerned in PCa metastatic tumor cell growth [195]. The successful delivery system of siRNA as a therapeutic agent provides the platform to the researchers to work more in this field.

Limitations with miRNA

miRs have time and have again proven to be important mediators of tumorigenesis, disease progression, and metastasis in cancer. miRNAs are known to affect the protein synthesis drastically with even minute aberration resulting in the inception of numerous abnormalities. The prime requisite is for miRs to become successful "biomarkers" and replace the conventional biomarker like PSA [23], alphafetoprotein (AFP), and carcinoembryonic antigen (CEA). Biomarkers including CEA (colon cancer), AFP (HCC), and PSA are highly expressed in benign tumors. Although, androgen deprivation therapy (ADT) is employed to treat patients with aggressive prostate cancers, response to the treatment in such cases is often temporary, relapsing, and ultimately advancing androgen-independent prostate cancers (AIPCs). Taxanes (e.g., docetaxel) were indicated as standard treatments for AIPCs but only a handful of patients respond to docetaxel therapy and they also eventually become resistant. Another challenge facing the research community is the need to devise a mechanism that would maintain constant makeup of miRNA inside the body. U6 small nuclear RNA normalizes miRNA levels measured in tissues and body fluids but being unstable degraded in serum [196]. Therefore, to find stable miRNA, more research and validation become imperative. Secondly, impact and consequences of miRNA in the blood are poorly revealed. Another problem that persists in miRNA is the variation in the result of the analysis during different studies.

Conclusion

Literature survey strongly implicates the role of miRNA in cancer, especially prostate cancer. This review discusses the contribution of miRNA as biomarkers in various signaling pathways. The successful story of commercialization of products glorified its name. Mirna Therapeutics and Regulus Therapeutics product of miR-34a successfully undergone clinical trial as a tumor-suppressive miRNA for p53. MRX34 has passed phase 2 clinical trials, whereas, mir-122 proves successful against hepatitis C in clinical trials. Different agonists and antagonists were also proved worthy in the preparation of therapies in this direction. The strategies were adopted to design and synthesize active drugs and successfully deliver them with the help of bacteriophage and coating with dopamine. Although the underlying intricate mechanisms involving miRNA have been brought to light, there is still scope to delve deeper in understanding them as targets. Non-reproducibility of data results obtained from different clinical studies combined with non-reliability of miRNA target in the computational method are the major hurdles that need to be overcome to make treatment strategies involving miRNA a success.

Acknowledgments The authors acknowledge the Central University of Punjab, Bathinda for providing the necessary facilities to carry out the present work.

Compliance with ethical standards

Conflicts of interest None

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