Chapter 5
MicroRNAs as Regulators of Prostate Cancer Metastasis

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Abstract Prostate cancer causes significant morbidity in men and metastatic disease is a major cause of cancer related deaths. Prostate metastasis is controlled by various cellular intrinsic and extrinsic factors, which are often under the regulatory control of various metastasis-associated genes. Given the dynamic nature of metastatic cancer cells, the various factors controlling this process are themselves regulated by microRNAs which are small non-coding RNAs. Significant research work has shown differential microRNA expression in primary and metastatic prostate cancer suggesting their importance in prostate pathogenesis. We will review the roles of different microRNAs in controlling the various steps in prostate metastasis.

Keywords Metastasis · Prostate cancer · microRNAs · ECM · EMT

5.1 Introduction

Prostate cancer (PCa) is the second most commonly diagnosed cancer among men in the United States and is the third leading cause of cancer related deaths. Metastatic disease accounts for ~16.5% of deaths from PCa [76]. Despite improvement in early screening methods and development of effective therapies, the rates at which aggressive prostate cancers are diagnosed are showing an increasing trend. Recent data analysis from patients with PCa within the United States has shown an increased rate of metastatic disease particularly in men of age group 55–69 years [21]. The rate of metastasis incidence has significantly increased at 2.74% per year from 2012 for all recorded cases. Moreover, there has been a steady increase in the incidence of metastatic PCa among white men as opposed to other races. Furthermore, these rates are expected to increase at 0.38% per year accounting for almost 42% of

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metastatic PCa cases by 2025 [51], which is an alarming increase for the most prevalent male cancer.

Tumor metastasis is a multistep process that involves dissemination of cancer cells from the primary site, their survival in the circulatory system, extravasation to the metastatic sites and subsequent colonization [14, 38]. In addition to their own genetic susceptibilities, cancer cells disseminated from primary sites depend on several growth regulatory signals such as those from chemokines and cytokines in the metastatic niche in order to survive and proliferate at secondary locations [14]. Given the inherent heterogeneity in primary tumors, one can expect emergence of clones that are fit to survive the adversities encountered during the entire process of metastasis. These tumor cells evolve both genetically and epigenetically to surmount the barriers of survival outside their primary niche [38]. There is activation of genes that facilitate metastatic progression, including those that control the epithelial-to-mesenchymal (EMT) pathways, invasion-migratory pathways and allow proteolysis and degradation of the extracellular matrix (ECM). TWIST1, SNAI1, SNAI2 and ZEB1 are some of the important transcription factors that regulate this important step of EMT activation [62]. These essential initiators of metastasis are often regulated in most tumors including PCa by small specialized RNAs called microRNAs [36]. *ANGPTL4, MMP1, PTGS2, EREG* are some of the genes that allow invasion and survival in the circulation and *IL11, IL6, PTHRP* are genes that facilitate subsequent colonization [62]. Further, these cells cooperate with the microenvironment and its constituent cell types such as fibroblasts and immune cells so as to attain aggressive phenotypes that entail metastatic progression [38]. MicroRNAs (miRNAs) are small non-coding regulatory RNAs which are often deregulated in tumors. In this book chapter, we will provide an overview on PCa metastasis following which we will review the roles of different miRNAs and their contribution towards different steps in PCa metastasis.

5.2 Prostate Cancer Metastasis

Prostate tumors are clinically defined as either indolent or aggressive. Majority of these tumors are localized and treated according to their stage or Gleason score which is determined primarily by biopsy sampling. Most tumors that are identified as aggressive or advanced at the time of first diagnosis and are often accompanied by micrometastasis at secondary locations. Of the different sites in the body, the skeletal system has especially high propensity to develop metastatic lesions in patients with prostate cancer [14, 30, 60]. The presence of bone metastasis in PCa patients in addition to visceral metastasis is associated with a significantly lower overall survival of 14 months [30]. Thus, signifying the importance of metastatic locations in determining disease prognosis. There are different hypotheses to explain the genetics of metastatic tumors. Primary prostate tumors are heterogeneous and are often comprised of different clonal populations that may give rise to metastasis [70]. Liu *et al* studied the association of primary tumors with their corresponding

metastatic tumors and suggested a monoclonal origin for metastatic tumors. They analyzed the copy number alterations and genome wide nucleotide polymorphisms across tumors derived from different metastatic locations in the same patients and observed a genomically stable pattern among these different tumors [57]. However, a more recent study from Hong *et al.* suggests that metastatic tumors are different from primary tumors and that acquisition of favorable mutation such as those in tumor suppressor *TP53* contributes to metastatic tumor heterogeneity and confers metastatic potential to these cells. These mutations or variation in metastatic tumors may arise in order to provide better survival advantage against therapeutic interventions [41, 70]. Furthermore, they also suggest a cross-metastatic seeding pattern in patients where metastatic tumor subclones themselves seed new tumors in more secondary locations [41].

5.2.1 Factors Regulating PCa Metastasis

Several cell intrinsic and extrinsic factors play an important role in execution of a successful metastatic process. While factors intrinsic to a cell refer to the nature of cell type and its genetic composition, microenvironmental cues correspond to the extrinsic factors controlling metastasis. Tumors cells with extensive genomic instability are considered to be the potential candidates for metastatic initiation [62]. Prostate tumor cells are well known to harbor copy number alterations, chromosomal deletions, DNA rearrangements such as chromoplexy, chromothripsis, gene fusions and certain mutations [2, 3, 85]. Presence of *TMPRSS-ERG* gene fusions is a common event among prostate cancer cells. Fluorescence *in situ* hybridization in primary and corresponding metastatic tumors derived from patients undergoing radical prostectomies have shown association of *TMPRSS2-ERG* fusion among the tumors suggesting a common origin [35]. More recently, it has been shown that over-expression of *TMPRSS2 ERG* fusions in metastatic PCa cell line PC3M-Luc leads to increased bone metastasis in mice [25]. Epigenetic modifications also play an important role in imparting plasticity to the cell that facilitates initiation of metastatic events. EZH2, a histone lysine methyltransferase enzyme is over-expressed in prostate tumors from advanced stage prostate cancer patients and its expression directly correlates with metastatic progression of the disease [89]. Germline mutations in DNA repair genes such as *BRCA1*, *BRCA2*, *CHEK2*, *ATM*, *RAD51D* and *PALB2* is a significant prognostic factor for development of metastatic prostate cancer [66]. Stankiewicz *et al* recently identified *FBXL4* gene which is located on chromosome 6q to be deleted in metastatic bone tumors as well as the primary tumors suggesting its role as a possible tumor suppressor that is lost during metastatic progression [78]. Deletions/additions in parts of chromosomes is a commonly observed phenomenon in PCa tumors [70]. Chromosomal gain (1q, 3q, 7q, 8q, 17q and Xq) and loss (1q, 6q, 8p, 10q, 13q and 16q) are frequently observed chromosomal alterations in the prostate tumor genome [7]. Homeobox tumor suppressor gene *NKX3.1* that is involved in differentiation of the prostate gland is located at chromosome 8p

and its loss has been linked to disease progression [4, 6, 40]. Many tumor-suppressor miRNA genes are located on these chromosomes. Studies from our lab have shown that chromosome 8p is frequently lost during PCa progression, harbors miRNAs that have tumor-suppressor functions and plays an essential role in regressing EMT transition in tumor cell [8–10].

5.2.2 Microenvironment and PCa Metastasis

The extracellular microenvironment is another important determinant in the process of metastatic cancer progression. The extracellular matrix (ECM) is formed of diverse matrix proteins including laminin, fibronectin, vitronectin, collagen, osteopontin and others in which stromal cells such as adipocytes, fibroblasts, immune cells and many tissue-specific cells are embedded. Together with blood and lymphatic vessels, they form a niche necessary for sustenance and proliferation of tumor cells. The stromal compartment itself is a strong prognostic indicator of progressing disease. Gene expression analysis of the stromal and epithelial compartment of tumor tissue has revealed a distinct stroma signature that significantly varies with a patient's Gleason score, thereby serving as an independent indicator of high grade PCa [87].

Expression of many basement membrane and matrix proteins are altered during PCa progression [79]. In addition, PCa cells also demonstrate altered patterns of cell adhesion molecules (CAMs) including cadherins and integrins that allow interaction with the surrounding matrix and facilitate cancer progression. The expression of integrins varies with the metastatic stage that in turn, allows protection and easier passage of tumor cells from the external barriers in the primary site, blood vessels and metastatic locations [79, 84]. Signaling through these surface molecules mediated by their interaction with ECM proteins or endothelial cells control motility, growth and proliferation of the disseminated cancer cell [84]. Proteases such as matrix metalloproteases (MMPs), urokinase plasminogen activator (uPA), and cathepsins are upregulated in the invasive disease, thus allowing for degradation of the basement membrane and dissemination of cancer cells through the matrix [79].

Soluble growth promoting signaling molecules such as growth factors, chemokines and chemo-attractants are other important players in mediating successful colonization at metastatic sites. As mentioned earlier, most PCa patients often develop bone metastasis [30]. Stephan Paget's "seed and soil" hypothesis and James Ewing's circulatory connection theory help explain this preferential metastatic spread of prostate tumor cells to bone [14, 84]. Insulin-like growth factor 1 (IGF-1), transforming Growth Factor- β (TGF-β), uPA, bone morphogenetic proteins (BMPs) and parathyroid hormone-related protein (PTHrP) are some of the soluble factors that facilitate the growth and survival of tumor cells in the bone microenvironment [60, 84]. Runt-related transcription factor (Runx2) is found to be overexpressed in metastatic PCa cell lines and has been shown to mediate bone-specific metastatic behavior in tumor cells [1]. In addition to these biochemical mediators, physical

forces from the microenvironment also influence metastatic progression [45]. Pressure changes in the bone microenvironment have been shown to enhance the migratory behavior of PCa cell lines via induction of Chemokine (C-C motif) ligand 5 (CCL5) from the osteocytes [77].

5.2.3 Exosomes in Metastasis

Extracellular vesicles (EVs) are other important tumor cell messengers that facilitate cell-to-cell communication between primary tumor cells and distant metastatic locations. Exosomes are small EVs of size ranging from 30-100 nm that are secreted by several cell types [20]. These bilayer vesicles carry different biomolecules including: RNA, DNA, protein and lipids. A very recent study by Hoshino et al. have demonstrated that organ-specific metastatic patterns are governed by exosomes released by tumor cells. They injected tagged exosomes from breast and pancreatic cancer cells that specifically metastasize to lungs or bone to study patterns of their distribution *in vivo*. They observed organ-specific deposition of the exosomes that allows for colonization of the disseminated or injected tumor cells to the exact location of exosome deposition. This study suggests that exosomes functionally educate the metastatic locations beforehand to facilitate colonization by the tumor cells [42]. Further, these small vesicular particles are abundantly secreted in most biological fluids under normal and diseased conditions, thus profiling the exosomal particles offers a great opportunity to detect cancer and other pathological conditions at various stages of the disease. Specialized small RNAs such as microRNAs are often enriched in these EVs and are thought to mediate post-transcriptional gene controls in the receptor cells [88]. Thus, they not only function as biological mediators of metastatic progression but can also serve as markers for disease severity.

In conclusion, tumor metastasis is a dynamic process that is governed by several cell intrinsic and extrinsic factors and requires continuous changes in the tumor and surrounding cells for successful execution. Stable genetic changes in the tumors confer genetic fitness to initiate this process, however different steps in the process often require post-transcriptional regulation of several genes controlling the EMT pathways, proteolysis and secretion of factors that allow for cell survival and subsequent colonization. Small non-coding RNA molecules such as miRNAs are important mediators of such posttranscriptional gene controls. We will discuss in detail in the following sections how these specialized small RNAs influence prostate tumorigenesis with a focus on their roles in PCa metastasis.

5.3 MicroRNAs: Small Non–Coding Gene Regulators

MicroRNAs are 22–23 nt long, single stranded non-coding RNA molecules that play an important role in regulating gene transcription post-transcriptionally. They were first discovered in *Caenorhabditis elegans* in 1993 and since then many regulatory miRNAs have been identified. Several studies have demonstrated their role in tumor pathogenesis by regulation of cell growth, proliferation, apoptosis, cell cycle and cell differentiation [26]. Almost 60% of the human genome is regulated by these small RNAs [12]. They are synthesized as a long chain of polycistronic RNA that is cleaved by ribonucleases DROSHA and DICER into 22–23 nt long single stranded RNAs. They bind through complete or partial complementarity to the 3'-UTRs of target mRNAs and together with Argonaute protein form a RISC complex at the mRNA strand that catalyzes its degradation [26]. Their expression is often deregulated in cancer, where miRNAs that lose their expression in tumors are called as "tumor-suppressors" and those that are up-regulated are called "oncomirs". Studies from our and many other laboratories have provided insights into miRNAs and the important role they play in prostate tumorigenesis. In this chapter, we will be highlighting their contribution in the process of PCa metastasis.

5.3.1 MicroRNAs in Prostate Cancer Metastasis

Tumor cells undergo many gene expression changes to acquire metastatic ability. Gene expression analysis of metastatic tumors have revealed molecular signatures corresponding to cell cycle, transcription factors, signal transduction pathways that can be therapeutically targeted and can predict prognosis for disease recurrence [34, 52]. miRNAs also share this deregulatory behavior in metastatic tumors. Next generation sequencing of metastatic tumors have shown that a large number of small RNAs are differentially expressed and are believe to be important players in the metastatic process [71, 86, 90]. More recently, Xue *et al.* designed a computer algorithm to analyze different PCa metastasis datasets. Based on their observations they identified transcriptional factors AR, HOX6 and NKX2–2 that were altered in metastatic tumors and are believed to regulate the expression of various metastasis-related miRNAs. These TFs were then validated *in vitro* for their functional significance in controlling metastasis by miRNA regulation from prostate epithelial cell line RWPE1 [92]. Deregulation of miRNA expression is often accompanied by metastatic disease and miRNAs are essentially required for the process. We will be discussing roles of various miRNAs in different metastatic steps including: acquisition of EMT, regulation of factors responsible for tumor cell metastasis/colonization and regulation of the microenvironment to facilitate tumor progression.

5.3.1.1 miRNAs in Regulating Acquisition of EMT

Dissemination from the primary site is the first step in metastasis, which requires a tumor cell to undergo transition from an epithelial to a mesenchymal state. Epidermal Growth Factor Receptor (*EGFR)* is well known oncogene that is often overexpressed in many tumors and its expression is thought to promote bone metastasis in breast and prostate cancer. A very recent study by Day *et al.* has shown that circulating tumor cells in PCa patients with bone metastasis overexpress *EGFR* while tumor cells depend on *Her2* overexpression for their growth in the bone microenvironment [24]. Additionally, EGFR has been also shown to regulate the expression of miR-1 in PCa cell lines which in turn, controls EMT transcription factor TWIST1. Together, they form a mechanistic loop where EGFR expression increases with progression of disease and miR-1 expression decreases that leading to an increase in TWIST1, thus facilitating EMT progression [15]. It has been previously shown that miR-1 is implicated in metastatic disease and its expression is lost both in primary and metastatic tumors. It also functions as a tumor–suppressor that regulates genes related to the cell-cycle, apoptosis, DNA damage and inhibits the invasive phenotype of PCa cells lines [44]. miR-143 and miR145 are downregulated in bone metastatic tumors and their overexpression in PCa cell lines decreases invasion-migration and bone metastasis forming ability of cancer cell lines *in vi*vo [65]. miR-145, which is negatively correlated with HEF1 expression directly targets the 3'-UTR of *HEF1* mRNA and ablates its EMT conferring properties in PCa cell lines [37]. miR-29b when over-expressed in PC3 cancer cells reduces the invasiveness and lung and liver metastasis forming ability *in vivo*. Furthermore, it leads to up-regulation of E-Cadherin and down-regulation of EMT markers TWIST1, N-Cadherin and SNAI1 [69]. miR-182 and miR-203 target SNAI2 and induce epithelial phenotypes and self-sufficient growth ability in prostate cells EPT1 [68]. Human enhancer of filamentation-1 (HEF-1) is highly expressed in bone metastatic specimens from PCa patients and it regulates EMT and aggressiveness in PC3 cells. miR-409-3p/5p are members of the delta like 1 homolog-deiodinase, iodothyronine 3 (DLK1-DIO3) cluster which are known to be involved in prostate metastasis [47]. miR-409-3p and 5p have been shown to be overexpressed in PCa patient serum and are involved in mediating tumorigenicity, EMT and bone metastasis *in vivo* in PCa cell lines upon its overexpression [47]. miR-154 and miR-379 are other miRNAs found in the same DLK1-DIO3 cluster that are overexpressed in metastatic bone lesions from PCa patients. Inhibition of these miRNAs in bone metastatic PCa cell lines leads to acquisition of MET and reduced bone and soft tissue metastasis from these cell lines [39]. Furthermore, combined overexpression of all 4 miRNAs found in the DLK1-DIO3 cluster including miR-409-3p/5p, miR-154 and miR-379 was shown to promote EMT in PCa cell lines. They are believed to function in activating oncogenic pathways including Ras, hypoxia-inducible factor (HIF), WNT and TGFβ signaling by targeting tumor-suppressors cohesion sub-unit SA-2 (STAG2), SMAD7, Von Hippel-Lindau tumor-suppressor (VHL) and polyhomeotic-like protein 3 (PHC3) [39]. miR-195 located on chromosome 17p13.1 functions as a tumor-suppressor and is found to be downregulated in high grade prostate tumors. Ribosomal protein

S6 kinase (RPS6KB-1) was found to be a miR-195 target gene. RPS6KB-1 knockdown restored cell migration, invasion and increased apoptosis observed in PCa cells as a result of miR-195 overexpression. Alterations in the miR-195-RPS6KB-1 axis were shown to regulate expression of MMP-9, BAD, E-Cadherin and VEGF that are involved in PCa progression. This study established the role of miR-195 in preventing PCa metastasis [11]. More recently, it has been shown that increased expression of miR-301a is associated with PCa recurrence in patients undergoing radical prostatectomy. Ectopic overexpression of miR-301a in PCa cell lines PC3 and LNCaP led to increased cell growth, invasion and migration. miR-301a directly targets p63, a member of the p53 family, that in turn alters the expression of EMT proteins E-Cadherin and transcription factor ZEB1 [61]. Recent studies from our lab have demonstrated that genomic loss of chromosome 8p21 is associated with PCa progression. We have identified that miRNAs, miR-3622a and miR-3622b, which are located within this genomic region play an important role in regulating PCa progression [8, 9]. We found that miR-3622a is widely downregulated in PCa and that miR-3622a represses PCa EMT by directly targeting *ZEB1* and *SNAI2*. miR-3622a loss allows tumor cells to acquire a mesenchymal phenotype, promoting invasion and metastasis [8]. Ectopic overexpression of miR-3622b in PCa cell lines led to reduced cellular viability, proliferation, invasiveness, migration and increased apoptosis. miR-3622b overexpression *in vivo* induced regression of established prostate tumor xenografts and miR-3622b was found to directly target EGFR [9].

5.3.1.2 Regulatory Effect of miRNAs on PCa Metastasis–Associated Signaling Pathways and Other Factors

miR-1 has been shown be downregulated in PCa tumors, more so in the metastatic samples. It directly targets SRC which is known to be a promoter of PCa metastasis [59]. miR-30 is commonly downregulated in PCa tumors and increases with SRC inhibitors. Overexpression of miR-30 in VCaP cells leads to reduction in expression of EMT genes and downregulation of Ets- related genes (ERG). Thus, miR-30 plays an important role in modulating the SRC/EGF and ERG pathways in tumor cells [50]. In Ras-activated xenograft tumors, miR-34a negatively regulates the expression of WNT signaling protein transcription factor 7 (TCF7) and anti-apoptotic protein baculoviral inhibitor of apoptosis repeat containing 5 (BIRC5), both of which are required for successful PCa metastasis in Ras-driven tumors [17]. Levels of miR-194 are often elevated in serum as well as tissues of PCa patients and can serve as a marker for disease recurrence. Forced overexpression in PCa cell lines have shown a pro-metastatic and invasive role for miR-194 in PCa tumorigenesis and it has been shown to directly target ubiquitin ligase suppressor of cytokine signaling 2 (SOCS2) protein. SOCS2 further regulates ubiquitination of two important kinases JAK and FLT3 that in turn, deregulates STAT3-mediated expression of pro-metastatic genes [23]. Loss of miR-15,16 and a concomitant increase in miR-21 activates TGFβ signaling pathways and plays an important role in bone colonization of PCa cells [5]. Studies from our group have identified many miRNAs that exert an anti-metastatic

effect by targeting key metastatic genes. miR-203 is frequently lost in metastatic tumors and bone metastatic PCa cell lines. miR-203 over-expression in PCa cells alters the EMT markers, reduces invasion-migration and targets key metastatic genes including survivin, *ZEB2, SMAD4, DLX5* and *RUNX2* which is a known bone metastasis promoting transcription factor [73]. More recently, we identified miR-466 as another anti-metastasis miRNA that is downregulated in PCa tumors and its overexpression reduces PCa tumor and metastasis growth *in vivo*, targets *RUNX2* and alters the expression of *RUNX2* target genes including *MMP11, Angiopoietin (ANGPT1), ANGPT4*, *Osteopontin (OPN) and Osteocalcein (BGLAP)* [19].

Cancer stem cells (CSCs) are considered to be a precursor cell population for metastatic tumors and are defined by expression of cell surface markers such as CD44 [83]. These highly tumorigenic and metastatic cell populations have also been identified and characterized in PCa tumors [63, 64]. miR-34a is shown to be downregulated in CD44+ cell populations in PCa xenografts as well as purified CD44+ cells from PCa cell lines. Ectopic overexpression in PCa cell lines have shown that miR-34a directly represses CD44 expression and reduces the migratory and invasive phenotype of CD44+ cells, thus diminishing their metastatic potential [54]. Studies from our lab have demonstrated the role of miR-708 in reducing the tumorigenic potential of CD44+ PCa cells. It was shown to target CD44 and Ser/Thr kinase AKT2, thus altering tumor progression [72]. More recently, we identified that miR-383 located on chromosome 8p is lost during PCa progression and has an inhibitory effect on the CD44+ PCa cell population [10]. miR-128, miR-199-3p, miR-320 and miR141 are some of the other miRNAs that have been shown to regulate prostate metastasis by directly regulating the tumor-initiating stem populations in PCa [43, 46, 55, 56].

5.3.1.3 Microenvironmental Control of miRNAs in Regulation of PCa Metastasis

Metastasis is often marked by loss of BM protein that facilitates invasion of disseminated tumor cells. miR-205 plays an important role in deposition of the major BM protein laminin in prostate tissues. miR-205, along with TP63, regulates the deposition of BM protein and its expression is often lost with PCa tumor progression [31]. Expression of miR-25 is reduced in prostate cancer stem cells (PCSCs) when compared to differentiated luminal cells. Its overexpression has been shown to target expression of integrins αv and α6 in metastatic PCa cell lines and it leads to reduced migration and decreased metastasis *in vivo* [93]. miR-1207-3p is also lost during PCa progression. It has been shown to directly target fibronectin type III domain containing protein (FNDC1) that in turn, regulates fibronectin (FN1) and Androgen receptor (AR) in PCa cell lines. Loss of miR1207-3p is marked by increased expression of FNDC1/FN1/AR that is associated with PCa aggressiveness [22]. In order to understand the role of miRNAs with increasing Gleason grade, when tumors from Gleason grades 3, 4 and 5 were subjected to miRNA gene expression analysis, the results demonstrated miR-29c, miR-34a and mir-141 as

differentially expressed miRs that had reduced expression with increasing grade. miRNAs function as tumor-suppressors and their overexpression reduces tumor cell migration and downregulation of ECM, focal adhesion kinase and MAPK13 pathways [53]. Syndecan-1 is another ECM protein that positively regulates levels of miR-331-3p which in turn, contributes towards increased EMT and aggressiveness in prostate tumors [29].

Tumor-associated stroma also undergoes changes in response to progressing tumors. Mesenchymal stem cells (MSCs) have been shown to migrate to tumors and promote prostate tumorigenicity [49]. Co-culture of MSCs with PCa cell lines *in vitro* have been shown to induce more adipogenic differentiation in these cells that is mediated through IL6. Expression of IL6 in MSCs is further regulated by let-7 miRNA, which is downregulated in tumor cells co-cultured with MSCs, thus signifying an important regulatory role of miRNA let-7 in determining the reactivity of tumor stroma [80]. Pre-adipocytes have been shown to be associated with the prostate tumor microenvironment as opposed to normal prostate tissues. They enhance the invasiveness and metastasis of PCa cell lines via upregulation of miR-301a which targets AR expression in tumors and in turn, affects expression of metastasis associated genes *MMP9, SMAD3* and TGF-β3 in PCa cells [91]. Cancer-associated fibroblasts (CAFs) are reactive fibroblasts that are often found in the tumor microenvironment. miRNA analysis of CAFs derived from patients with PCa tumors revealed miR-133a as highly expressed miRNA in these cells. miR-133a released from CAFs functions as a soluble paracrine factor that activates adjacent normal fibroblast to attain a reactive phenotype [27]. In the bone microenvironment, osteoblasts are the main effector cells that allow metastatic colonization by tumor cells under the influence of various factors. It has been shown that osteoblasts secrete Wnt1-induced secreted protein 1 (WISP1) that is released in conditioned media and acts on PCa cell lines to increase their invasion/migration abilities as well increasing the expression of vascular cell adhesion molecule 1 (VCAM1). This effect is mediated by miR-126 downregulation driven by osteoblast-derived WISP1 which further regulates αvβ1/p38 and FAK pathways in PCa cell lines [81]. miR-409-3p/5p are other miRNA that are found elevated in CAFs in prostate tumors. They are released by EVs from CAFs and upon their uptake by PCa cells mediate tumor cell EMT and aggressiveness [48].

Prolyl 4-hydroxylase alpha polypeptide 1 (P4HA1) enzyme is involved in proper folding of pro-collagen chains. It has been shown to be overexpressed during aggressive PCa and is regulated directly by miR-124. miR-124 is downregulated in high grade PCa and is transcriptionally regulated by EZH2 and transcriptional co-repressor C-terminal binding protein 1 (CtBP1) both of which are increased in aggressive PCa tumors [13].

Comparisons of prostate epithelial cell line P69 with respect to its metastatic subline M12 have shown altered miRNA expression among the two cell lines [16]. miR-130b is down-regulated in the metastatic M12 cell line, PC3, DU145 as well as in prostate tumors. It functions as a tumor suppressor and reduces invasion-migration ability of tumor cells. MMP2 is a direct target of miR-130b and exerts its invasive effect on metastasis as a result of miR-130b down-regulation [16]. miR-296-3p is

over-expressed in M12, thereby down-regulating expression of intercellular cellular cell adhesion molecule 1 (ICAM1) which in turn, confers a protective effect to circulating tumor cells against natural killer (NK) cells [58].

miR-323 is upregulated in PCa cell lines and is shown to directly target adiponectin receptor 1 (AdipoR1) which in turn, negatively regulates vascular endothelial growth factor-A (VEGF-A)-mediated neovascularization. Thus, mir-323 mediated down-regulation of AdipoR1 facilitates formation of new blood vessels for the growing tumor [32]. Chemokine receptor CXCR4 is elevated in metastatic cell lines and is negatively regulated by miR-494-3p. Ectopic over-expression of miR-494-3p in PCa cell lines inhibits cell invasion and migration [75]. Circulating serum levels of miR-375 have been putatively linked to circulating tumor cells (CTCs) in metastatic CRPC patients. However, miR-375 have been shown to negatively regulate EMT and invasion in PCa cells and targets oncogene YAP1 which is often elevated in invasive PCa tumors. Furthermore, miR-375 is under negative regulation of ZEB1 which enables EMT in PCa cells, thus forming an axis of ZEB1, mir-375 and YAP1 that controls epithelial cell EMT and MET transitions [74].

5.4 Conclusions and Future Directions

Evidences from the literature suggest an essential role for miRNAs in the metastatic process (Fig. 5.1). In most PCa metastases, regulatory small RNAs are lost during tumor progression and function mostly as tumor suppressors. Thus, they inhibit

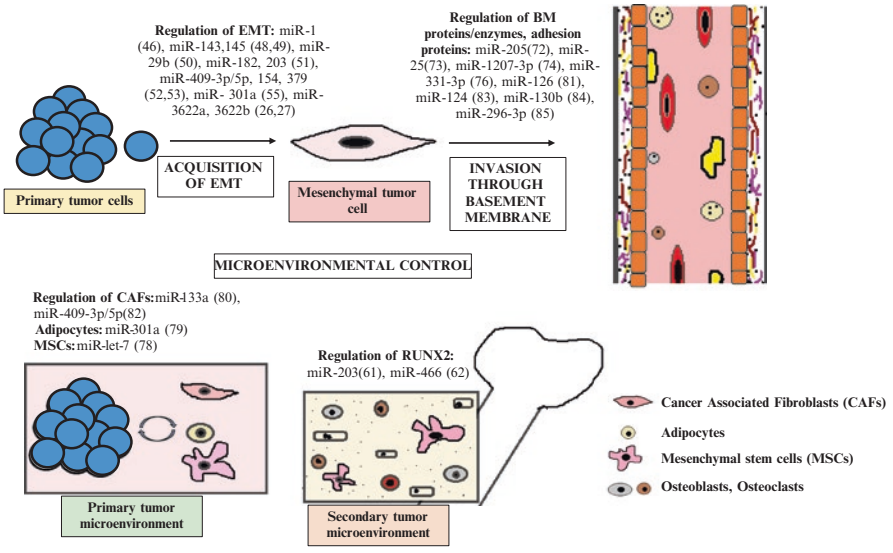

Fig. 5.1 Schematic representation of PCa metastasis and regulation of different steps by various miRNAs

metastatic initiation via EMT or regulate growth of primary tumor cells in the primary or metastatic microenvironment via control over important growth factors, chemokines, ECM and stromal components. In addition to their functional effects in mediating PCa metastasis, expression in tissues and circulatory are often important indicators of disease severity. Non-invasive sampling of cell free or EV-derived RNAs in the serum, plasma or urine offers a great opportunity for sensitive detection of metastatic disease [28]. Moreover, much research these days is focused on utilizing the therapeutic potential of miRNA in cancers. Different ways for delivering miRNA to their target cells i.e. via nanoparticles, liposomes, viral particle-mediated transfer or polyethylene glycol (PEG)-based particles, have been developed for systemic and local delivery of miRNAs [18, 66]. Atelocollagen particle-mediated delivery of miR-16 and chitosan nanoparticle-derived delivery of miR-34a to PCa xenografts *in vivo* have highlighted the promising effect of miRNA delivery in inhibiting advanced prostate cancer [33, 82]. Given the multifaceted roles of miRNAs, more research efforts are needed to improve PCa detection and the efficacy of disease therapeutics utilizing small regulatory miRNAs.

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Conflict of Interest None.

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