Minireview

MicroRNAs targeting prostate cancer stem cells

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Abstract

Prostate cancer is a frequently diagnosed cancer in males with high mortality in the world. As a heterogeneous tissue, the tumor mass contains a subpopulation that is called as cancer stem cells and displays stem-like properties such as self-renewal, epithelial–mesenchymal transition, metastasis, and drug resistance. Cancer stem cells have been identified in variant tumors and shown to be regulated by various molecules including microRNAs. MicroRNAs are a class of small non-coding RNAs, which can influence tumorigenesis via different mechanisms. In this review, we focus on the functions of microRNAs on regulating the stemness of prostate cancer stem cells with different mechanisms and propose the potential roles of microRNAs in prostate cancer therapy.

Keywords: miRNAs, PCSCs, EMT, metastasis, drug resistance

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Introduction

Prostate cancer (PCa) is the most commonly diagnosed cancer and the third leading cause of cancer-related deaths among men in developed countries.[1] According to American cancer statistics, approximately 233,000 new cases of PCa were diagnosed and approximately 29,480 PCa deaths occurred in the United States in 2014.[2] Up to now, the molecular mechanisms underlying PCa progressions are still unclear. However, recent cancer stem cell hypothesis has provided a novel sight for the diagnosis and treatment of PCa.[3]

According to this hypothesis, cancer stem cells (CSCs) are a subset of cancer cell subpopulations in the tumor mass and are considered to be responsible for tumor initiation, resistance to anti-cancer therapies, recurrence, and metastasis.[4] It was reported that CSCs exist in almost solid tumors including PCa.[5] In order to further understand and explore mechanisms for the regulation of stemness of CSCs, especially prostate cancer stem cells (PCSCs), several regulatory factors were identified and among them microRNA (miRNA) is one of the critical factors.[6] At present, miRNAs were found to regulate the stemness of PCSCs either directly by targeting stemness-related transcription factors and markers, or indirectly by targeting epithelial–mesenchymal transition (EMT), metastasis-related factors, and drug resistance-related factors. In addition, miRNAs were shown to be associated with regulation of several stemness-related pathways, such as TGF-β,[7–9] Wnt/beta-catenin,[10,11] and MAPK[12] pathway (Figure 1). The aim of this review is to summarize the involvement of specific miRNAs in regulating the stemness of PCSCs (Table 1) and prospect the potential therapeutic application of these miRNAs for PCa.

miRNAs directly regulate the stemness of PCSCs

CSCs are a dynamic and heterogeneous population,[13] and can be isolated by specific biomarkers, including cell surface markers and intracellular transcription factors. Up to date, several different cell surface markers have been employed to identify PCSCs from prostate tumors, including CD44,[14] CD133,[15] integrin α2β1,[16] ABCG2,[17] and Sca-1.[18,19] On the other hand, several well-known transcription factors that function in embryonic or pluripotency stem cells were also found to be involved in the maintenance of stemness in PCSCs.[20–23] Interestingly, these surface markers and transcription factors have been shown to be regulated by specific miRNAs, which affect expression and function of specific stemness-related surface markers as well as transcription factors.

miRNAs regulate the stemness of PCSCs by targeting PCSC-related surface markers

MiR-34a. By comparing the miRNA profiling between PCSCs and non-PCSCs, Liu et al.[24] have found that miR-34a is consistently under-expressed in six xenograft PCSC

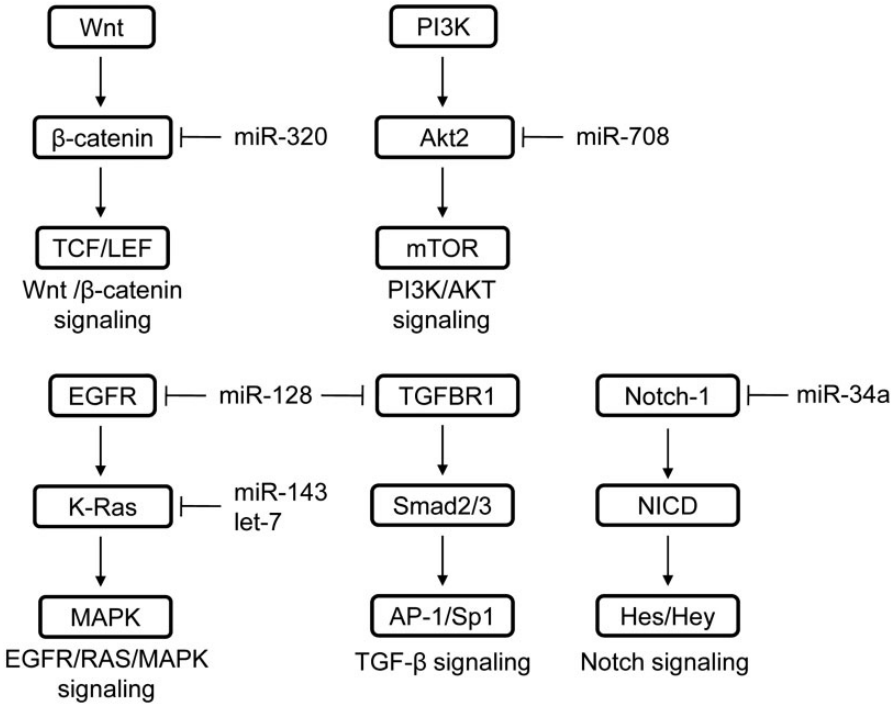

Figure 1 MiRNAs that have been reported to regulate the stemness-related pathways. Multiple signaling pathways, which are associated with the maintenance of stemness of PCSCs, have been identified to be regulated by miRNAs.

Table 1 MiRNAs and related targets for regulation of PCSCs' stemness. (A color version of this table is available in the online journal.)

	Target			
miRNA	**PCSC-related surface marker**	**PCSC-related transcription factor**	**EMT & metastasis related gene**	**Drug resistance-related gene**
miR-34a	CD44[24]	AR[25]	N/A	SIRT1, Bcl2[83]
miR-708	CD44[26]	N/A	N/A	N/A
let-7	N/A	c-Myc[12] EZH2[31]	N/A	N/A
miR-143	CD133, CD44[32]	OCT4, c-Myc, KLF4[32]	N/A	N/A
miR-145	CD133, CD44[32]	OCT4, c-Myc, KLF4[32]	HEF1[52] Zeb2[53]	N/A
miR-128	N/A	Bmi-1, Nanog[37]	N/A	N/A
miR-100	N/A	Argonaute 2[40]	N/A	N/A
miR-200b	N/A	N/A	Zeb1/2, Snail2[58] Slug[59]	N/A
miR-200c	N/A	N/A	Zeb1/2, Slug[60]	N/A
miR-205	N/A	N/A	Zeb1/2, Slug[60] PKCε[61], ΔNp63α[62]	N/A
miR-409-3p/-5p	N/A	N/A	STAG2, RSU1[67]	N/A
miR-21	N/A	N/A	BTG2[69], TGFBR2[71]	N/A
miR-30d miR-181a miR-199a-5p	N/A	N/A	N/A	GRP78[79]

N/A: data not reported (not appreciable)

populations (three CD44+, one CD133+, one $\alpha2\beta1^{high}$, and one SP) as well as in CD44+ PCSC subpopulations from primary tumors, suggesting that miR-34a may play a key role in negatively regulating PCSC features. Functional experiments further demonstrated that miR-34a overexpression inhibits holoclone formation, clonogenic capacity, and sphere establishment in Du145, LAPC4, and PPC-1 PCa cells. In addition, miR-34a was found to be down-regulated in CD44+ PCa and restoration of miR-34a in CD44+ PCa also inhibited PCSC characteristics.

To further explore the molecular mechanisms, they discovered that miR-34a inhibits PCSCs functions via suppressing CD44 expression and directly binding to the complimentary site in CD44 3′UTR. Collectively, these results suggest that miR-34a possesses tumor-inhibitory effects in PCa cells through negatively regulating stem cell properties in PCa cells. Besides CD44, miR-34a was also found to regulate self-renewal capacity of PCSCs via directly targeting AR and Notch-1 signaling, both of which are critically involved in growth and metastasis of PCa.[25]

MiR-708. Besides miR-34a, it was demonstrated that miR-708 is another PCSCs suppressive gene by targeting CD44 in PCa.[26] MiR-708 is down-regulated in CD44+ PCa population, indicating that miR-708 is associated with the stemness of PCSCs. Overexpression of miR-708 in CD44+ PCa represses sphere formation and clonogenic potential, while inhibition of miR-708 in CD44– cells increased growth and sphere formation ability. MiR-708 also directly regulates Akt2, a core member of PI3K/Akt signaling pathway, which plays a key role in tumor progression and maintenance of cancer stem-like cell features.[27]

miRNAs regulate PCSCs-related transcriptional factors

Besides cell surface markers, many intracellular stem cell-related transcription factors are also used to identify PCSCs, such as KLF4,[20] OCT4,[21] Sox2,[22] Nanog,[23] and Bmi-1.[28] miRNAs can regulate the stemness of CSCs also via targeting these key transcription factors and their downstream pathways.[6]

Let-7 family. Let-7 family had been demonstrated to possess strong CSC-suppressing functions in breast cancer,[29] lung cancer,[30] and PCa[12] through miRNA profiling assays. Kong et al.[31] reported that ectopic expression of let-7 suppresses stemness of PCSCs through inhibiting the expression of enhancer of Zeste homolog 2 which plays a key role in embryogenesis, normal stem cells, and CSCs. They also found that let-7 family expression is positively regulated by BR-DIM (metabolite 3,3′-diindolylmethan), which inhibits the growth of PCa cells. Moreover, it has been reported that let-7b overexpression inhibits clonal and sphere formation, via repressing c-Myc and K-Ras, both of which are oncogenic and self-renewal molecules.[12] These results indicated a complex regulatory network between let-7 and CSCs-associated genes, and an essential role of let-7 in regulating the stemness of PCSCs.

MiR-143 and miR-145. Overexpression of miR-143 and miR-145 in PC3 cells was found to inhibit the expression of PCSC markers and stemness factors, such as CD133, CD44, OCT4, c-Myc, and KLF4, and to suppress tumor sphere formation as well as tumorigenesis.[32] Moreover, the transcription of miR-145 is repressed by OCT4, which uncovers a double-negative feedback loop between OCT4 and miR-145.[33] This double-negative feedback loop was also found to modulate cell differentiation, suggesting an important role of miR-145 in repressing stemness of PCSCs.

MiR-128. MiR-128 has been reported to inhibit the growth of glioblastoma[34] and breast CSCs[35,36] by directly targeting Bmi-1 (B lymphoma Mo-ML V insertion region 1 homolog), a component of the PRC2 polycomb repressor complex and a critical regulator of stem cell self-renewal and malignant transformation.[28] Jin et al.[37] demonstrated that overexpression of miR-128 has a similar effect on suppressing proliferation, invasion, clonogenic, and sphere-forming capacities in PCa. They further found that endogenous miR-128 levels in PCa are reversely correlated with their clonogenic and tumorigenic potential. These findings suggest that miR-128 shows inhibitory effects in PCa initiation. By mechanistic study, miR-128 regulates a cohort of oncogenic and stem cell-related genes in PCa cells, including Bmi-1, Nanog, TGFBR1, and EGFR, all of which are implicated in maintenance of CSCs' stemness.[37] In addition, several PCSC populations, including CD133+ and CD44+ show a reduced miR-128 expression level. Forced miRNA-128 expression in CD44+ PCSCs strongly suppresses PCSC properties, while down-regulation of miRNA-128 in CD44– PCa enhances the stemness. These data indicate that miR-128 can weaken the stemness of cancer stem-like cells.

MiR-320. MiR-320 has been reported to be down-regulated in multiple cancers, such as breast cancer,[38] colon cancer,[39] as well as PCa.[10] In PCa cell line PC3 and DU145, overexpression of miR-320 directly inhibits β-catenin and significantly represses the expression of Wnt/β-catenin pathway regulatory factors (c-Myc, LFF1, CD44, Sox9, OCT4, cyclin D1) and stem cell markers (CD133, CD117, CXCR4, ABCG2), so as to suppress the stemness of PCSCs.[10]

MiR-100. It was found that miR-100 impairs stemness properties of PCa cells through directly targeting Argonaute 2, an oncogene that directly regulates expression of stemness factors, such as Oct4, Sox2, Nanog, KLF4, and c-Myc, following its binding to their regulatory regions.[40] MiR-100 negatively regulates colony formation and spheroid formation of PCSCs and its expression is dramatically down-regulated in PCa especially in the bone metastasis patients.[40,41] On the other hand, it was also reported that a higher miR-100 expression level is positively correlated with biochemical recurrence after radical prostatectomy.[42] Therefore, these studies indicate a possible context-dependent role shift of miR-100 between a tumor suppressor and an oncogene.

Thus, examples above demonstrate that tumor suppressor miRNAs can impair PCSCs' stemness by directly targeting stemness-related transcription factors as well as stemness-associated markers and in turn inhibit tumor progression.

miRNAs regulate PCSCs indirectly through targeting EMT- and metastasis-associated factors

Epithelial–mesenchymal transition (EMT) is a multistep change in which epithelial characteristics are lost and

mesenchymal phenotypes are acquired.[43] EMT endows cancer cells with malignant properties of metastasis, invasiveness as well as stemness.[44–46] On the other hand, CSCs also have mesenchymal-like features.[47] On the molecular mechanism, EMT and CSC development may share common signaling pathways, such as Wnt, Notch, and hedgehog (Hh) pathways.[48] The EMT process is regulated by multiple transcription factors including N-cadherin, Snail1/2, and Zeb1/2, most of which are implicated in CSCs features and are regulated by miRNAs, suggesting that miRNAs can modulate the stemness of CSCs via regulating these EMT-associated factors. In PCa, there are multiple miRNAs reported to regulate EMT, such as miR-145, miR-200 family, miR-205, and so on.

MiR-145

MiR-145 has been reported to regulate EMT and CSCs in breast cancer,[49] lung cancer,[50] and renal cancer.[51] In PCa, Guo et al. demonstrated that miR-145 suppresses EMT and invasion partially through repressing HEF1.[52] Ren et al.[53] also found that miR-145 directly suppresses Zeb2 expression and reversely Zeb2 inhibits transcription of miR-145. Hence, there is a negative feedback loop between Zeb2 and miR-145. Functional experiments showed that this feedback loop inhibits EMT and stem cell properties in PCa. Additionally, it has also been reported that p53 inhibits EMT and the stemness of PC3 and DU145 PCa cells by directly increasing the expression of miR-145 at the transcriptional level.[54] All these data indicate that miR-145 plays an essential role in regulating the stemness of PCSCs and EMT.

MiR-200 family

All the four miR-200 family members (miR-200a/b/c and miR-141) have been reported to be associated with EMT.[55] Among these, miR-200b is a critical regulator for EMT, CSC maintenance, and cancer chemosensitivity.[56,57] In PDGF-D (platelet-derived growth factor-D) induced EMT phenotype in PC3 cells, miR-200b is down-regulated and restoration of miR-200b leads to a reversal of the EMT phenotype and enhanced expression of epithelial markers following down-regulation of Zeb1/2 and Snail2.[58] Liu et al. further demonstrated that miR-200b along with miR-1 directly targets Slug and in turn inhibits tumor proliferation and delays tumorigenesis.[59] Furthermore, they also found that Slug inhibits miR-200b expression by directly binding to the promoter of pri-miR-200b, showing that there is a mutually inhibitory feedback loop between miR-200b and Slug. Besides miR-200b, overexpression of another member miR-200c as well as miR-205 is also found to enhance E-cad expression and to reduce expression of Zeb1/2 and Slug, two key components closely associated with both mesenchymal phenotypes and stemness of PCSCs.[60]

MiR-205

Gandellini et al. have reported that miR-205 reverses EMT progression by negatively regulating expression of multiple genes, such as Zeb2 and PKCε, and in turn inhibits cancer stem-like properties in PCa.[61] Ectopic overexpression of miR-205 inhibits prostasphere formation and decreases the proportion of $CD44^{high}/CD24^{low}$ and CD133+ cancer stem-like cells in PCa cells. Moreover, they also found that HIF-1a (hypoxia-inducible factor-1a), a malignant transformation factor, directly represses miR-205 expression at a transcriptional level.[61] In addition, miR-205 can also repress invasion through a miR-205-ΔNp63α autoregulatory network, which is essential for the maintenance of the basement membrane in the prostate epithelium.[62]

MiR-409-3p and -5p

Besides the tumor-suppressor miRNAs described above, there are several important oncogenic miRNAs that enhance EMT and the stemness of PCSCs, including miR-409-3p and −5p.[63] These two miRNAs are members of the DLK1-DIO3 (delta-like 1 homolog–deiodinase, iodothyronine 3) gene cluster which are associated with pluripotency levels in embryonic stem cells[64] and cancer development.[65,66] MiR-409-3p has been reported to be up-regulated in the serum of patients with high risks compared to those with low risks.[63] Josson et al. further demonstrated that miR-409-3p and −5p are notably up-regulated in two aggressive, bone metastatic PCa models and play an important role in facilitating tumor growth, EMT, and bone metastasis through inhibiting multiple tumor suppressors[67] such as STAG2 (stromal antigen 2) and RSU1 (ras suppressor protein 1). MiR-409-3p and -5p were also found to be involved in prostatic tumorigenesis via tumor–stromal interactions. Up-regulation of miR-409 in normal prostate fibroblasts confers a cancer-associated stroma-like phenotype and releases miR-409 into the tumor microenvironment to promote tumor induction and EMT.[68]

MiR-21

A well-known oncogenic miRNA, miR-21, has also been found to contribute to EMT by directly targeting BTG2 (basal protein B-cell translocation gene 2), which is implicated in PCa transformation and progression.[69] Bao et al. reported that miR-21 is induced by hypoxia and HIF and involved in acquisition of EMT, maintenance of CSCs functions, and therapeutic resistance.[70] They also demonstrated that miR-21 down-regulation represses prostasphere formations, expression of CSC markers CD44, and EpCAM as well as CSCs-related factors VEGF.[70] In addition, AR was reported to activate the transcription of miR-21 and in turn inhibit TGF-β receptor II (TGFBR2) expression in PCa cells, so to escape the growth inhibition from the TGF-β/Smad 3 pathway.[71] These results indicate that miR-21 plays an important role in enhancing PCSCs' stemness by enhancing EMT.

Thus, besides repressing the expression of stemness factors directly, tumor suppressive miRNAs can also impair PCSCs' stemness indirectly by reversing EMT and inhibiting metastasis. On the contrary, oncogenic miRNAs can also enhance PCSCs' stemness indirectly by repressing the expression of these EMT-inhibiting factors.

miRNAs regulate PCSCs indirectly through targeting drug resistance-associated factors

As mentioned above, one feature of CSCs is therapeutic resistance.[72,73] In PCa, after androgen deprivation and chemoradiotherapy, most patients eventually progress to castration resistance prostate cancers and metastasis.[74] PCSCs have been reported to be tightly associated with drug resistance and to express related genes at a high level.[75,76] Several signaling pathways regulating the self-renewal behavior of CSCs, including PI3K/Akt and Ras pathway, are identified to be associated with chemotherapy.[77,78] Underlying mechanisms of CSCs-related therapy resistance are associated with DNA damage response, apoptosis resistance, autophage, etc.[72] Thus, multiple miRNAs related to drug resistance, including miR-143 and miR-34a, are reported to regulate the stemness of PCSCs indirectly.

MiR-143

Xu et al. found that PC3 and DU145 transfected with miR-143 inhibits cell growth and enhances the sensitivity to docetaxel through suppressing K-Ras, a key component in EGFR/RAS/MAPK pathway, which is associated with PCSCs' stemness.[78]

MiR-30d, miR-181a, and miR-199a-5p

Su et al. demonstrated that miR-30d, miR-181a, and miR-199a-5p cooperatively increases the sensitivity of several different human cancer cell lines including C42B (a PCa cell line) to an HDAC inhibitor TSA through suppressing a key signaling regulator GRP78,[79] which is significantly associated with stemness maintenance, drug resistance, and apoptosis resistance in different types of cancers.[80–82]

MiR-34a

In addition to the direct regulatory function of miR-34a on repression of PCSCs' stemness, Kojima et al. reported that miR-34a attenuates paclitaxel-resistance of castration resistance prostate cancers through directly targeting SIRT1 (silent mating type information regulation 2 homolog 1) and Bcl2, both of which play a crucial role in promoting tumorigenesis and developing drug resistance.[83] Moreover, Corcoran et al. also demonstrated that miR-34a is essential in reducing chemoresistance to docetaxel in PCa patients.[84]

The above studies demonstrate that besides repressing the expression of stemness factors directly, tumor suppressive miRNAs can also impair PCSCs' stemness indirectly by reducing chemoresistance and increasing their drug sensitivity.

Conclusion

Mounting evidences indicate that tumor suppressive miRNAs can repress the stemness of PCSCs both directly (via inhibiting the expression of stemness-related transcription factors and stemness-associated markers) and indirectly (via reversing EMT and restoring the drug sensitivity). In the future, identification of more miRNAs that specifically block the PCSCs' stemness or stemness-associated pathways (Figure 1) directly or indirectly and elucidation of their acting mechanisms will still be helpful. We predict that specific stemness-related miRNAs will become not only very useful markers for the diagnosis and prognosis but also potential therapeutic molecules for PCa.

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