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Role of microRNA in prostate cancer stem/progenitor cells regulation

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Abstract. – Most of the human tumors contain a population of cells with stem cell properties, called cancer stem cells (CSCs), which are believed to be responsible for tumor establishment, metastasis, and resistance to clinical therapy. It's crucial to understand the regulatory mechanisms unique to CSCs, in order to design CSC-specific therapeutics. Recent discoveries of microRNA (miRNA) have provided a new avenue for understanding the regulatory mechanisms of cancer. The present review article will discuss important milestones associated with mircroRNA regulation during prostate carcinogenesis.

Key Words:
Micro RNA, Prostate cancer, Cancer stem cells.

Introduction

Prostate cancer is one of the most common cancers among men and is the second leading cause of death following lung cancer[1]. Despite the extensive studies on prostate cancer, there are still many uncertainties/questions about the origin of the prostate cancer, molecular mechanisms driving the cancer cells, tumor recurrence mechanisms and metastasis. The major cause of this spread is the failure of existing clinical therapies including surgical and chemical treatments such as androgen deprivation treatment (ADT). There is emerging evidence that a small population of cells with stem cell properties called cancer stem cells (CSCs) driving tumor initiation, progression, metastasis and response to the clinical treatments[2,3]. The CSC hypothesis has provided significant implications for better understanding of the biology of cancer cells and helping in great extent in the development of new cures for cancer.

MicroRNAs (miRNA) are evolutionally conserved, small non-coding RNAs that are about 19-22 nucleotides (nt) long[4]. MiRNAs modulate gene expression by repressing their targets' translation or inducing mRNA degradation through binding to the complementary sequence in target messenger RNA. Although tiny, miRNAs have been shown to play important roles in regulating developmental, physiological and oncogenic processes. So far, there are 1921 potential mature miRNAs that have been reported in human (miRbase, v18, www.mirbase.org), and they are thought to be involved in the regulation of more than 60% genes coding for the human protein.

miRNA and Stem Cells

The discovery of the first miRNA was in association with stem cell (SC) development in *Caenorhabditis elegans*. This miRNA was required for the transition from the L1 to L2 stage[5]. Let-7-associated family members (miR-48, miR-84 and miR-241) were later shown to be involved in the transition from L2 to L3, further implicating miRNAs in the maintenance and progression of SCs. Let-7 itself is involved in the transition from L4 to adult phenotypes[6,7]. In the absence of lin-4 and members of the let-7 family, the SC lineage fails to differentiate and continues a proliferative cycle. The fact that lin-4 and let-7 are conserved over a number of species suggested that their developmental regulatory functions mi-

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ght also be conserved[8]. There are several additional examples of miRNAs being key regulators of cell proliferation and death[9], cell differentiation[10], skeletal and cardiac muscle development[11] and brain and neural development[12]. Also, ongoing research is building solid evidence for the role of miRNAs in regulating SC division and other SC properties[13,14].

miRNA in Cancer

The first study of miRNA in cancer discovered that miR-15a/16-1 is often lost in chronic lymphocytic leukemia (CLL)[15]. The advances in profiling techniques such as high-throughput screening and miRNA microarray in the past few years have facilitated investigator to identify aberrant expression of miRNAs in lung cancer, breast cancer, glioblastomas, pancreatic tumors and prostate cancers[16]. Differential miRNA expression profiles were also observed between primary tumors and metastatic tumors. These differential expression profiles of miRNA are informative for the diagnosis as well as prognosis of cancer[17]. The mechanisms involved in the differential expression of miRNA included the genetic location of the miRNA at cancer-related regions[18], the epigenetic regulation of miRNA expression[19], or the abnormalities in genes and proteins involved in miRNA maturation processes. MiRNA could be also regulated by an important oncogenes or tumor suppressor genes with transcription activities[20] such as p53, Myc and lin-28.

miRNA as an Oncogene or Tumor Suppressor Gene

The aberrant expressions of miRNAs in cancer suggested that these miRNAs might be involved in a pathological process of cancer development. Indeed, more and more studies showed that miRNAs might function as either oncogene or tumor suppressor gene. For example, miR-21, which is upregulated in cancers of the breast, lung, prostate, colon and glioblastomas, has been shown to be self-sufficient in promoting cancer cell proliferation and could evade apoptosis by targeting several tumor suppressors like PTEN and PDCD4[21]. Moreover, transgenic over-expression of miR-21 resulted in B cell lymphoma[22]. Many other miRNAs that display oncogenic functions included miR-17-92 cluster, which are transactivated by oncogene c-Myc. These mirRNAS have been reported to promote tumor growth in a lymphoma mouse model[23]. On the other hand, many miRNAs that are under-expressed in cancers might exert tumor suppressor functions. For example, loss of miR-15 and miR-16 is often found in CLL patients. It has been shown that miR-15/16 cluster is often deleted in various cancers including CLL, prostate cancer, and pancreatic cancer. Moreover, this loss of miR-15/16 is negatively correlated with the level of antiapoptotic protein BCL2 and is therefore associated with evasion of apoptosis[24]. Other important tumor suppressive miRNAs included the let-7 family, which is widely underexpressed miRNAs in various tumors, and can suppress tumor development by targeting multiple oncogenic factors involved in tumor initiation and progression such as RAS, MYC and HMGA2[25].

miRNA in Cancer Therapeutics

It is a well-known fact now that miRNAs are differentially expressed in cancer compared to normal tissue and are involved in various aspects of cancer development. So, investigators are in the process of development of miRNA-based anti-cancer diagnostics and therapeutics[26]. Primarily, miRNA expression profiles could be utilized as informative biomarkers in cancer diagnosis and prognosis. In a study in the recent past, miRNA scored better in comparison with traditional mRNA diagnosis in studying classification as well as the correlation of cancer with tissue origin. Also, miRNA levels in serum and other body fluids have been observed to be associated well with the metastatic status of cancer and are being developed as powerful biomarkers for cancer diagnosis[27].

Researchers are working also on new techniques to either replace tumor suppressive miRNAs or target oncogenic miRNAs so as to negatively regulate tumor development. The advantage of using miRNAs as therapeutic targets is understandable. Each miRNA has the ability to target multiple factors in an oncogenic pathway, therefore, might exert a better synergistic effect as compared to the traditional therapeutic methods, which generally target a single molecule. The miRNA mimic oligonucleotides (oligos), which are synthetic double-stranded RNA oligos that mimic the mature endogenous miRNAs[28]. On the other hand, anti-sense oligos against miRNAs (antagomiRs) have been developed to block the oncogenic activities of the over-expressed miRNAs. For example, systemic delivery of antagomir miR-10b, which has been shown to promote metastasis in breast cancer, has successfully suppressed the metastasis of breast cancer cells to the lung[29].

miRNA in Development Regulation

Lin-4 and let-7 are the first two miRNAs to be identified involved in the development process. Interestingly, both of them were discovered during Caenorhabditis elegans (C. elegans) development. Lin-4 controlled the larval stage transition from L1 to L2, by repressing lin-14, a factor required for proper developmental timing[30]. Since then, evidence from several systems has implicated that miRNA might play an important role in regulating embryonic stem cell (ESC) and embryonic development. The global functions of miRNA have been evaluated by the consequences of Dicer and DGCR8 mutants in human and mouse ESCs. Specifically, complete blockage of miRNA maturation by deletion of Dicer in mouse caused embryonic lethality[31]. Both dicer deficient, as well as DGCR8-deficient mouse ESCs, exhibited a defect in differentiation and G1 cell-cycle arrest[32]. In addition to the overall involvement of the miRNA pathway in ESCs, other researchers have also revealed the specific miRNA expressions and functions in ESCs. For example, a set of 32 miRNAs differentially expressed in human ESCs was reported[33]. In addition, miR-290-295 cluster, miR-296, miR-302, miR-17-92 cluster and miR-15b-16 cluster have been reported to be over-expressed in ESCs. However, reports also confirmed that these miRNA got decreased during differentiation and are absent in adult tissue, whereas miR-21 and 22 are found abundantly expressed in differentiated and adult tissue[34]. Of note, several regulatory circuits between miRNAs and the pluripotent genes required for maintaining the stemness have been reported, revealing an amazing network of stem cell regulation. For example, the core transcription factors of stem cells pluripotency, OCT-4, NANOG, SOX2, TCF3, occupied the promoter regions of a set of miRNAs that are preferentially expressed in mouse ESCs such as miR-290-295 cluster and transcriptionally activated their expressions[35]. On the other hand, some of these pluripotent genes are regulated by miRNAs at the post-transcriptional level as well[36].

miRNA in Cancer Stem Cells Regulation

Increasing evidence suggested that miRNAs might also be involved in regulating CSC properties. First of all, miRNA expression signature specific for CSC populations has been confirmed in several cancers. In breast cancer, Yu et al[37] reported that let-7, as well as some of other miRNAs including miR-16, miR-107, miR-128 and miR-20b, were significantly reduced in breast CSC (BCSC) enriched by consecutively passaging breast cancer cell line SKBR3 in mice treated with chemotherapy. Clarke's group used the cell surface marker CD44 and CD24 expression to enrich BCSC, and identified a set of 37 miRNAs to be a differentially expression in CD44+/CD24-/lo BCSC population, in which three clusters, miR-200c-141, miR-200b-200a-429, and miR-183-96-182 were significantly downregulated[38]. MiRNA deregulation has also been reported in glioblastoma and other brain CSCs[39]. In hepatic CSC identified by EpCAM+AFP+ profile, researchers also discovered a unique miRNA signature in which miR-181 family and several miR-17-92 cluster members were up-regulated in the CSC population[40]. Furthermore, miRNAs have been shown to regulate various CSCs properties, including self-renewal and differentiation, tumorigenesis, metastasis and chemoresistance. For instance, under-expression of let-7 in BCSCs seems to be important for maintaining the stem cell properties. Thus, overexpression of let-7 with let-7-lentivirus inhibited proliferation, mammosphere formation, tumor formation and metastasis in NOD/SCID mice. In contrast, antagonizing let-7 enhanced the in vitro self-renewal of non-CSC. Interestingly, over-expression of miR-30, another miRNA that was also markedly reduced in BCSCs, not only inhibited their ability of self-renewal, but also inhibited anoikis resistance and increased apoptosis by directly targeting UBC9 (ubiquitin-conjugating enzyme 9) and ITGB3 (integrin β3)[41]. Importantly, a complete inhibition of self-renewal and mammosphere formation in BCSC was observed when introducing both let-7 and miR-30 at the same time. This synergistic inhibitory effect of let-7 and miR-30 on self-renewal indicated that multiple miRNAs distinctively and concertedly regulated CSC properties.

Several other miRNAs like miR-200c, miR-128 have been reported to be under-expressed in BCSCs, normal mammary stem cells and in glioma stem cells[42]. miR-34, a p53 downstream target, has also been shown to negatively regulate the stem cell properties of pancreatic and gastric CSCs; thus, over-expression of miR-34 inhibited sphere formation in vitro and tumor formation in vivo via modulating downstream targets viz. Bcl-2, NOTCH, and HMGA2[43]. miRNAs such as miR-205 and miR-200 might have been observed to be involved in regulation of metastasis, by affecting epithelial-mesenchymal transition (EMT)[44]. Mo-

reover, the overexpression of miR-200 negatively regulated the expression of EMT-activator ZEB1 and ZEB2. These findings established a negative feedback loop between miR-200 family and ZEB1/ZEB2 that regulated important biological processes in cancer metastasis[45].

Conclusions

It is evident from above literature that different miRNAs are involved in the regulation of different aspects of tumor development. Also, they showed distinct CSC properties, and work coordinately in order to control the tumor progression. However, there is a need for more comprehensive studies to elucidate more detailed mechanistic information on the role of miRNAs in prostate cancer progression as well as metastasis.

Conflicts of interest

The authors declare no conflicts of interest.

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