MicroRNA Regulation of Cancer Stem Cells

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Abstract

Cancer stem cells (CSC), or cancer cells with stem cell properties, have been reported in many human tumors and are thought to be responsible for tumor initiation, therapy resistance, progression, relapse, and metastasis. Despite their potential clinical importance, how CSCs are regulated at the molecular level is not well understood. MicroRNAs (miRNA), small noncoding RNAs that play critical roles in normal stem cell functions during development, have emerged as important regulators of CSCs as well. In this review, we summarize the recent major findings of miRNA regulation of various CSCs and discuss our recent findings that miR-34a suppresses prostate CSCs and metastasis by directly repressing CD44. This recent progress has important implications for understanding how CSCs are intrinsically regulated by networks of miRNAs and for developing novel mechanism-based miRNA therapeutics that specifically target CSCs.

Introduction

Research in the past decade suggests the presence of cancer stem cells (CSC) that can both regenerate themselves and differentiate into a spectrum of maturing daughter cells, which create the cellular heterogeneity of cancer. CSCs were first discovered in acute myeloid leukemia and, since 2003, have also been reported in most solid tumors (1). Emerging evidence indicates that CSCs may be involved in tumor maintenance, therapy resistance, tumor progression, and distant metastasis. Despite their potential clinical significance, how intrinsic CSC properties are regulated at the molecular level is poorly understood. Recent discoveries of microRNAs (miRNA) have provided a new avenue in understanding the regulatory mechanisms in CSCs.

miRNAs are 21- to 25-nucleotide (nt)–long, noncoding RNAs that induce the target mRNA degradation or repress mRNA translation by imperfect binding to their 3′-untranslated region (2). The miRNA gene is first transcribed by RNA polymerase II into primary transcript (pri-miRNA) in the nucleus, where the hairpin stem-loop structure is processed into precursor miRNA (pre-miRNA) by a microprocessing complex, including Drosha and DGCR8. The ~70-nt-long pre-miRNA is then exported into cytoplasm, where it undergoes a second processing by Dicer, in which one strand of the hairpin is incorporated into the ribonucleoprotein complex called microRNA-induced silencing complex (2). A single miRNA may target dozens of mRNAs, and one mRNA can be regulated by multiple miRNAs. Although small, miRNAs play a powerful role in biological processes including development, proliferation, and apoptosis. Early studies have linked miRNAs to controlling the self-renewal and differentiation of embryonic stem cells (ESC), and later, aberrant expression and/or functions of miRNAs are implicated in tumorigenesis (3). More recent studies suggest that miRNAs may also regulate CSC properties.

miRNA Regulation of Development and Embryonic Stem Cells

The first 2 miRNAs, lin-4 and let-7, were both discovered during Caenorhabditis elegans development. Since then, miRNAs have emerged as important regulators of embryonic development and stem cell functions in mammals. The overall roles of miRNAs in both mouse and human ESCs have been evaluated by analyzing the phenotypes of Dicer and DGCR8 mutants. Deletion of Dicer in mouse causes embryonic lethality (4), and Dicer-deficient mouse ESCs exhibit defects in differentiation and G1 cell-cycle arrest (5). Similarly, DGCR8-deficient mouse ESCs show problems in cell-cycle progression and differentiation, evidenced by failing to silence self-renewal genes, such as OCT4, REX1, NANOG, and SOX2, as well as delayed expression of differentiation markers (6). Other studies have also revealed specific expression and functions of individual miRNAs in ESCs (7).

A regulatory circuitry between miRNAs and "pluripotency" genes required for maintaining ESC stemness has been identified. On one hand, the master regulators of stem cell pluripotency, including OCT-4, NANOG, SOX2, and TCF3, all

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miRNA Regulation of Cancer and Cancer Stem Cells

Interestingly, the miRNA expression patterns in tumor cells
often bear resemblance to those in ESCs. Let-7, for instance, is
excluded in ESCs and often lost in cancers, including breast, lung, and ovarian cancers. Such cancer-specific miRNA
expression signature(s) may become very informative for
diagnostic and prognostic purposes. Functional studies of
the dysregulated miRNAs indicate that they regulate mole-
cular pathways in cancer via targeting different oncogenes
and/or tumor suppressors. More recent evidence suggests
that miRNAs may also be involved in tumor development
by critically regulating CSCs. Here, we discuss the major
findings of some recent studies highlighting the roles of
certain "CSC-specific" miRNAs in several representative
cancer types. From these discussions, we present an emerging
theme that several miRNAs may distinctively and concertedly (coordinate) regulate the key biological properties of CSCs.

Differential expression of miRNAs in cancer stem cells

Yu and colleagues were the first to examine the miRNA
expression in breast CSCs (BCSC; ref. 11). The authors
enriched BCSCs by consecutively passing breast cancer
cell SKBR3 in mice treated with chemotherapeutics. The tumors
were shown to contain a high percentage of CD44+/CD24⁻/lo
cells and high ability to form mammospheres in vitro and
tumors in vivo. Importantly, the BCSC-enriched cells
expressed much lower levels of let-7 as well as a number
of other miRNAs, including miR-16, miR-107, miR-128, and
miR-20b, than the parental cells and the in vitro differentiated progeny (11). Later, Shimono and colleagues identified
37 miRNAs to be differentially expressed in CD44+/CD24⁻/lo
BCSCs, in which 3 clusters, miR-200c-141, miR-200b-200a-429, and miR-183-96-182, were significantly downregulated
(12). Notably, these miRNAs were markedly reduced in normal mammary stem and/or progenitor cells as well. In
glioblastoma multiforme (GBM), some miRNAs, including
miR-451, miR-486, miR-425, miR-16, miR-107, and miR-185,
were decreased in the CD133⁻ population (13). In hepatocel-
lar carcinoma (HCC), EpCAM<sup>+</sup>AFP⁺CSCs expressed a
unique miRNA signature with upregulation of miR-181
family members and several miR-17-92 cluster members
(14). Through unbiased miRNA expression profiling, our
group recently showed that prostate cancer stem and/or
progenitor cell populations enriched with surface markers
CD44, CD133, or α2β1 prominently and commonly under-
express miR-34a and let-7b (15).

Breast cancer stem cells

BCSCs were the first CSCs to be reported and are among the
best characterized of all CSCs in solid tumors. BCSCs are most
commonly enriched using the CD44<sup>+</sup>CD24⁻/lo marker
profile (12) or Aldefluor assays (16). Because of the early discovery
and better understanding of BCSCs, miRNA studies in these
cells are also more advanced than in other CSCs. On the basis
of profiling results that let-7 was significantly reduced in
BCSCs (11). Yu and colleagues further unraveled that let-7 regulated the stem cell properties, that is, self-renewal
and differentiation. Lentiviral-mediated overexpression of
let-7a inhibited cell proliferation, mammosphere formation, tumor
formation, and metastasis in nonobese diabetic (NOD)/severe
combined immunodeficient mice (SCID) mice and reduced the
proportion of undifferentiated cells in vitro. In contrast,
antagonizing let-7 by antisense oligonucleotides enhanced
in vitro propagation of non-CSCs. H-RAS and HMGA2 were
identified as the direct downstream targets that partially
mediated the let-7 effects (11).

Interestingly, a recent study from the same group suggested
that miRNAs besides let-7 might also play a role in regulating
BCSCs because overexpression of let-7 alone was not sufficient
to completely block the tumor formation and progression (17).
Subsequently, miR-30 was found to be one of the miRNAs
markedly reduced in BCSCs and to negatively modulate the
stemness of BCSCs. Overexpression of miR-30 in BCSCs not
only diminished their self-renewal ability but also reduced
anoikis resistance and increased apoptosis by directly target-
ing ubiquitin-conjugating enzyme 9 (UBC9) and integrin β3
(β3). Conversely, knocking down endogenous miR-30 with
antagomirs enhanced self-renewal, tumor regeneration, and
metastasis in differentiated breast cancer cells. Impressively, a
more complete inhibition of self-renewal and mammospheres
in BCSCs was observed when both let-7 and miR-30 were
introduced at the same time, compared with transfecting
either miRNA alone (17). The synergistic BCSC-inhibitory
effects of let-7 and miR-30 on BCSC self-renewal suggest that
multiple miRNAs may distinctively and concertedly regulate
CSC properties (Fig. 1A).

miRNA expression profiling in purified CD44<sup>+</sup>CD24⁻/lo
BCSCs identified 37 miRNAs to be differentially expressed in
these cells with miR-200 family significantly downregulated
in both BCSCs and normal mammary stem and/or
progenitor cells (12). Functional studies showed that overexpression of
miR-200c reduced the clonogenic and tumor-initiation activ-
ities in BCSCs and suppressed formation of mammary ducts
by normal mammary stem cells. The stem cell factor BMI-1
was directly modulated by miR-200c. This work (12), thus,
provides a molecular link between normal breast stem cells
and BCSCs.

Recently, aldehyde dehydrogenase (ALDH) has emerged
as a functional marker for both normal and malignant stem
and/or progenitor cell populations in various tissues,
miR-205 levels in metastatic breast cancer cell lines and clinical samples (19).

CSCs are morphologically and phenotypically plastic and possess high migratory and invasive capacities. Several groups have observed that miR-205 and miR-200 family members regulate epithelial–mesenchymal transition (EMT), a process thought to be critical in the metastatic cascade. For example, miR-200 miRNAs and miR-205 are significantly downregulated in cancer cells undergoing EMT and in metastatic breast cancer specimens (20, 21). Overexpression of miR-200 miRNAs prevents TGFβ-induced EMT by negatively regulating the expression of EMT activator ZEB1 (also known as TCF8) and ZEB2 (also known as ZFXH1B and SMAD interacting protein 1 or SIP1). Interestingly, ZEB1 and ZEB2 can also transcriptionally repress the expression of miR-200 miRNAs by binding to their promoter regions, leading to strong activation of EMT. These findings (20, 21) establish a double-negative feedback loop between ZEB1/ZEB2 and miR-200 family miRNAs that, together, regulate an important biological process in tumor development and cancer metastasis.

The studies on miRNAs and BCSCs suggest an emerging theme that may also be applicable to understanding how miRNAs regulate other CSCs. BCSCs possess several fundamental biological properties, including self-renewal, quiescence associated with slow cell-cycle kinetics or differentiation associated with cell-cycle exit, prosurvival and antistress mechanisms (e.g., resistance to anoikis), and high capacities to undergo EMT and to invade, all of which likely contribute to their resistance to anticancer therapies and enhanced tumor-initiating and metastatic potential (Fig. 1A). Distinct miRNAs, via their respective downstream targets, distinctively and concertedly regulate these critical CSC properties. Thus, let-7 mainly restricts cell-cycle progression by targeting RAS, HMGA2, and EZF2; miR-30 may preferentially be involved in modulating the survival and stress responses; miR-200 miRNAs negatively regulate the self-renewal by targeting molecules such as BMI-1; and miR-200 (and miR-205) may regulate EMT, migration, and invasiveness in BCSCs (Fig. 1A).

Glioblastoma multiforme and other brain cancer stem cells

Specific miRNA dysregulation in GBM and other brain CSCs has recently been reported in several studies. By comparing miRNA expression in CD133− versus CD133+ GBM cells, one group reported underexpression of tumor-suppressor miR-451 in the CD133− population (13). miR-451 is well known to repress Myc expression. Another miRNA expression profiling in human GBM specimens revealed a significant reduction of miR-128 compared with adjacent normal brain tissue (22). Subsequently, miR-128 was shown to inhibit glioma stem cell proliferation in vitro and glioma xenograft growth in vivo. Overexpression of miR-128 significantly blocked glioma CSC self-renewal by directly targeting BMI-1 (22). Finally, miR-34a was found to be downregulated in human glioblastomas (23). Transfection of miR-34a into bulk GBM cells or GBM CSCs caused cell-cycle arrest or apoptosis and also inhibited xenograft growth, mediated by downregulation of multiple

including human (16) and mouse (18) mammary gland. In human mammary epithelial cells, for example, ALDH+ cells were shown to possess high-proliferative and broad-lineage differentiation potential and were able to regenerate mammary ductal structures in vivo. Likewise, breast cancer cells with high ALDH activity were capable of self-renewal and generating tumors in mouse models (16). miRNA expression profiling revealed that miR-205 and miR-22 were most abundant, whereas let-7 family members and miR-93 were depleted in ALDH+, CD133+, and Sca-1+ mouse mammary epithelial cells (18). Interestingly, although miR-205 was most abundant in ALDH+ normal mouse mammary progenitor cells, its expression in breast cancer cells remains heterogeneous, varying in different subtypes of breast cancer and at different stages of tumor progression. One group reported high levels of miR-205 in ER+ PR+ Her2+ breast cancers, whereas others reported both high miR-205 expression in triple-negative tumors and low miR-205 expression in triple-negative breast cancer cell lines and clinical samples (19).
oncogenic targets, including c-MET, Notch-1/2, and CDK6 (23). These studies in GBM (13, 22, 23) support the concept that several major miRNAs may distinctively and concertedly act together to restrict the key GBM CSC properties (Fig. 1B). miR-199-5p was downregulated in medulloblastoma, and overexpression of miR-199-5p inhibited proliferation and anchorage-independent growth of medulloblastoma cells by targeting HES-1 (24), a transcription factor of the Notch signaling pathway. Significantly, overexpression of miR-199-5p decreased the CD133+ subpopulation of cells and inhibited tumor development of medulloblastomas cells.

Prostate cancer stem cells

Our group was the first to profile miRNA expression in prostate cancer stem and/or progenitor cells (15). Prostate CSCs (PCSC) with high tumor-initiating and metastatic potential are enriched in the side population (25), CD44+ (26), and CD44+ CD28+ (27) subpopulations. Prostate cancer cells with CD133+ CD44+ CD28+ phenotype also show enhanced clonogenic potential in vitro (28). Through an unbiased miRNA expression profiling in 5 PCSC and/or progenitor cell populations purified from prostate cancer xenografts, including 3 CD44+ populations from the LAPC9, LAPC4, and Du145 tumors, CD133+ cells from LAPC4 tumors, and CD28+ cells from Du145 tumors, we identified miR-34a, together with let-7b, to be commonly underexpressed in all marker-positive cell populations (15). The underexpression of miR-34a was subsequently corroborated in CD44+ prostate cancer cells purified from ~20 patient prostate tumors. Overexpression of miR-34a in bulk prostate cancer cells or purified CD44+ cells by transfecting with mature oligonucleotide mimics or infecting with lentiviral vectors encoding pre-miR-34a exerted pronounced inhibitory effects on tumor growth and metastasis in vivo. In contrast, neutralizing endogenous miR-34a using antagonims in bulk or CD44+ prostate cancer cells promoted tumor regeneration and metastasis. Strikingly, delivery of miR-34a oligos systemically through tail vein inhibited metastasis to the lung and other organs and prolonged the survival of animals bearing orthotopic human prostate cancer, indicating the therapeutic potential of this miRNA. Mechanistically, miR-34a suppressed PCSC properties as it inhibited prostasphere establishment, migration and invasiveness of CD44+ prostate cancer cells, and serial prostasphere passaging and serial tumor transplantation. Of significance, we showed that CD44 itself represented a direct and relevant downstream target of miR-34a. Hence, the CD44 protein levels decreased in cells overexpressing miR-34a, and knocking down of CD44 functionally phenocopied the miR-34a effects in inhibiting tumor development and metastasis. Our findings (15) shed new light on the mechanisms of miRNA regulation of PCSCs.

Other cancer stem cells

Interestingly, miR-34, a transcriptional target of p53, not only inhibits the GBM CSCs (23) and PCSCs (15) but also restrains the biological properties of pancreatic and gastric CSCs (29, 30). Restoration of miR-34 expression in these latter CSCs inhibits sphere formation in vitro and tumor regeneration in vivo (29, 30). HCC CSCs identified by EpCAM+ AFP+ marker profile overexpressed the miR-181 family and several miR-17-92 cluster members (14). Inhibition of miR-181 led to a reduction in the number of EpCAM+ HCC cells and in tumor-initiating ability, whereas overexpression of miR-181 increased the EpCAM+ cells. The biological effects of miR-181 might be mediated via targeting caudal type homeobox transcription factor 2 (CDX2), GATA6, and nemo-like kinase (NLK), a Wnt/β-catenin pathway inhibitor (14).

Therapeutic Implications and Perspectives

Dysregulation of miRNAs has been intimately implicated in tumor development, and miRNAs may regulate tumorigenesis via modulating CSC properties. Thus, let-7 miRNAs control the cell-cycle and differentiation properties of BCSCs, miR-200c modulates the self-renewal of BCSCs by targeting Bmi-1, and miR-34a restricts the migratory and invasive properties of PCSCs by directly repressing CD44. The new findings discussed above better our understanding of CSC regulation and provide novel insight on developing new strategies to target therapy-resistant cancer cells. Given that CSCs seem to be involved in multiple steps of tumorigenesis, including tumor initiation, tumor maintenance, metastasis, and therapy resistance, and that miRNAs exert a broad regulatory role on tumor development, miRNA-based therapeutics that specifically target CSCs may add novel firepower to the anticancer arsenal, as exemplified by our recent demonstrations of the impressive therapeutic efficacies of systemically delivered miR-34a on preestablished human prostate cancers. As distinct miRNAs seem to distinctively and concertedly regulate key and interconnected biological properties of CSCs (Fig. 1), complete eradication of CSCs and residual tumors may entail manipulations or targeting of multiple miRNAs. In addition to developing miRNAs as anti-CSC therapeutics, miRNA expression profiling in CSCs or specific subtypes of cancer and at various clinical stages may have diagnostic and prognostic values.

Disclosure of Potential Conflict of Interest

No potential conflicts of interest were disclosed.

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