Published in final edited form as:

Wiley Interdiscip Rev Membr Transp Signal. 2012 January 1; 1(1): 83–95. doi:10.1002/wdev.5.

The miRNA Regulation of Stem Cells

Xiao Albert Huang and Haifan Lin

Yale Stem Cell Center & Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06511

Abstract

The miRNA pathway, as a fundamental mechanism of gene regulation, plays a key role in controlling the establishment, self-renewal, and differentiation of stem cells. Such regulation is manifested as fine-tuning the temporal- and tissue-specificity of gene expression. This fine-tuning function is achieved by (1) miRNAs form positive and negative feedback loops with transcription factors and epigenetic factors to exert concerted control of given biological processes; and/or (2) different miRNAs converge to control one or more mRNA targets in a signaling pathway. These regulatory mechanisms are found in embryonic stem cells, iPS cells, and adult tissue stem cells. The distinct expression profiles of miRNAs and their regulatory roles in various types of stem cells render these RNAs potentially effective tools for clinical diagnosis and therapy.

Introduction

How gene regulation defines the fate, self-renewal, and differentiation of stem cells represents a central question in stem cell biology. Towards this question, much of the current effort has been devoted to niche-signaling, epigenetic, and transcriptional regulation of gene activities in stem cells. In contrast, gene regulation at post-transcriptional levels, such as translational and post-translational regulation, remains largely unexplored. This situation, however, is being improved by the recent studies that reveal the key regulatory role of miRNAs in controlling stem cell function and animal development through modulating gene regulation network. The microRNA (miRNA), first discovered in *C. elegans*, is a family of small non-coding RNAs existing in diverse organisms that bind to the 3' untranslated region (3' UTR) of mRNAs via incomplete sequence complementarity to regulate their translation and stability^{1–3}. In the human genome, miRNA genes constitute ~4% of genes and regulate the expression of more than a third of the protein-coding genes in a target specific manner at the post-transcriptional level⁴. In this review, we summarize the latest knowledge of the regulatory function of miRNAs in the self-renewal and differentiation of embryonic stem cells and tissue stem cells, starting with a brief review of miRNA biogenesis and regulatory mechanism as background.

The biogenesis and regulatory mechanism of miRNAs

miRNAs are produced in distinct steps in nuclear and cytoplasmic compartments. In the canonical miRNA biogenesis pathway (Fig. 1, left panel), primary miRNA transcripts (pri-miRNAs) are generated by RNA polymerase II and then cleaved into ~70-nucleotide precursor-miRNAs (pre-miRNAs) in the nucleus by the microprocessor complex, comprised of a RNase III enzyme called Drosha and a dsRNA binding protein DiGeorge syndrome Critical Region Gene 8 (DGCR8)^{5, 6}. Pre-miRNAs are subsequently trafficked by Exportin 5 to the cytoplasm, where they are further cleaved by another RNase III enzyme called Dicer at a site 22nt away from the Drosha cleavage site into mature miRNA duplexes^{5, 7}. Dicer is in a complex that contains the dsRNA binding protein (Transactivating Region Binding Protein) TRBP, PACT (Protein Activator of PRK), and Loquious (Loqs). This complex further separates the two RNA strands of the miRNA/miRNA* duplex, incorporates one

RNA strand (miRNA) into Ago and releases the other strand (miRNA*), which is typically subject to degradation^{8–10}. The miRNA-containing Ago is then assembled into miRNA-induced Silencing Complex (miRISC), which recognizes the target mRNA via incomplete sequence complementarity between the miRNA and mRNA targets. Nucleotides 2–8 of miRNAs are critical for the target recognition through perfect complementarity with the target, and are thus called the “seed motif”^{11, 12}. A single miRNA can recognize multiple targets, regulating up to several hundred different species of mRNAs. In addition, multiple miRNAs can act simultaneously on one mRNA target. Such combinatorial modes of action allow several hundreds of miRNAs to modulate the expression of thousands of mRNA species at the posttranslational levels. Such regulation is conceivably achieved via decreased translational efficiency and/or reduced mRNA stability¹³. Recently, Bartel and colleagues found that decreased mRNA levels constitutes most (~80%) of the reduced protein production¹⁴.

In addition to the canonical pathway, there are microprocessor-independent and Dicer-independent pathways (Fig. 1, middle panel). In the microprocessor-independent pathway (Fig. 1, middle panel), the cropping function by Drosha is replaced by three different mechanisms: (1) by spliceosome for mirtron pathway; (2) by Dicer for snoRNA-, tRNA-, and shRNA-derived precursor; and (3) by tRNase Z for tRNA-derived pathway^{15–20}. Recently, Dicer-independent miRNAs were discovered in zebrafish and mammals^{21, 22}. In this pathway (Fig. 1, right panel), the RNA cleavage activity of Ago2 mediates the maturation of pre-miR-451. miR-451 is unique in that its 5'-end was defined by Drosha cut, yet its 3'-end is flexible and extends over the loop region of the hairpin with a length range of 20–30 nt. Moreover, 1–5 non-templated uridine residues were also found at the 3'-end of longer reads, implying that the Ago2 cleavage products undergo uridylation and subsequent trimming by a nuclease. These alternative pathways illustrate the ability of cells to exploit a wide variety of mechanisms to generate miRNAs and to execute gene regulation.

The role of miRNAs in pluripotent stem cells

Embryonic stem cells (ESCs) are derived from the inner cell mass (ICM) of mammalian embryos at the blastocyst-stage. They possess two unique properties: the ability to differentiate into any fetal or adult cell type (pluripotency) and the capacity to replicate for many generations (self-renewal). Such properties are sustained by delicate regulatory mechanisms including extracellular signaling, epigenetic programming, and epigenetic regulation. The chromatin state in ESC is largely open, rendering it accessible for transcriptional activation, while specific epigenetic regulators control the chromatin state locally to block the activation of differentiation-specific genes. In this chromatin context, ESC core transcriptional factors (Oct4, Sox2, Nanog etc.) together with a variety of signaling pathways govern ESC pluripotency.^{23, 24} In addition, the molecular program underlying the ESC pluripotency and self-renewal has increasingly been linked to miRNAs, which will be reviewed in this section.

miRNA expression in ESCs

miRNA expression profile is distinct in ESC, with most of the ESC-enriched miRNAs sharing a 5'-proximal AAGUGC sequence signature^{19, 25–28}. In humans, the miR-371 family (homologous to the miR-290 family) and the miR-302 family represent the majority of ESC-specific miRNAs. Additionally, these miRNAs tend to be cotranscribed as polycistronic transcripts, and share a common upstream transcriptional regulation by a set of core stem cell transcription factors²⁹. The levels of these miRNAs decrease as the ESCs differentiate, which indicates the reducing function in differentiated cells³⁰.

Intrigued by the distinct expression pattern, researchers have investigated the roles of miRNAs in regulating ESC self-renewal and differentiation. These investigations revealed the following three mechanisms of miRNAs in establishing and maintaining ESC stemness (Fig. 2), while directing ESC differentiation into different lineages.

miRNAs regulate the expression of core transcriptional factors of ESCs

In order for ESCs to differentiate properly, miRNAs suppress the expression of ESC core transcription factors (Fig. 2, left branch). For example, miR-145 represses c-Myc, Sox2, Oct4 and Klf4^{31, 32}. In mouse ESCs (mESCs), Oct4, Sox2 and Nanog are regulated by miR-134, miR-296 and miR-470, whereas Sox2 and Klf4 are cooperatively modulated by miR-200c, miR-203 and miR-183^{33–35}. One common theme in miRNAs and the ESC pluripotency transcription machinery is double-negative feedback. On one hand, the aforementioned miRNAs repress the expression of pluripotency transcription factors at the posttranscriptional level. On the other hand, pluripotency transcription factors in return silence the promoter regions of those miRNAs through co-occupation of Polycomb group proteins and the resultant H3K27me3²⁹.

miRNAs regulate the ESC cell cycle

ESCs exhibit a significantly shorter cell cycle. For example, cell cycle for mESCs is 12 hours instead of approximately 24 hours for somatic cells. The key underlying this short cell cycle is a shortened G1 phase, where G1-to-S transition is primarily unchecked, via a constitutively active cyclin E-Cdk2 complex³⁶. The global depletion of miRNAs in Dgcr8 and Dicer1 mutant mESCs show proliferation defects^{37–39}. Especially, DGCR8-deficient mESCs show accumulated cells arrested at G1 phase, indicating the function of miRNAs in mediating the constitutive activation of cyclin E-Cdk2 complex, thus promoting G1-to-S transition. This activation of cyclin E-Cdk2 by miRNAs was intensively studied, which revealed that miR-290 and miR-302 suppress cyclin E-Cdk2 upstream inhibitors (including Cdkn1a, Rbl2, and Lats2) in mESCs, therefore enabling the unrestricted G1-to-S transition and short cell cycle (Fig. 2, middle branch)^{36, 40}.

Such function in regulating cell cycle is also conserved in hESCs, where G1-to-S transition checkpoint genes Cdkn1a and Cdkn1c are suppressed by miR-372 and miR-92a, respectively^{41, 42}. miR-372 belongs to the miR-371 family, which is homologous to mouse the miR-290 family, indicating such suppression of cell cycle checkpoint is highly conserved. Moreover, G2-to-M transition is regulated by miRNAs as well. miR-195 represses WEE1, an inhibitory kinase in G2-to-M transition that blocks the G2 cyclin B-Cdk complex⁴².

One interesting mode of action is convergence of multiple miRNAs on one pathway. It is noteworthy that miR-290 and miR-302 possess the same target recognition motif³⁶. Such a shared motif allows multiple miRNAs to target multiple cyclin E-Cdk2 upstream inhibitors, thus promoting mitosis progression beyond G1-to-S transition check point.

miRNAs control epigenetic programming in ESCs

ESCs harbor a relatively open chromatin, possibly as a unique property underlying their pluripotency and self-renewal. During differentiation, distinctive regions on the chromatin undergo de novo DNA methylation and mediate proper differentiation. In Dicer-null ESCs with global miRNA depletion, the levels of DNA methyltransferases are reduced, which compromises de novo DNA methylation. This implicates the miRNA function in epigenetic regulation (Fig. 2, right branch)^{40, 43}. Notably, Oct4 is incompletely and reversibly silenced due to DNA methylation defects. Such compromised silencing of Oct4 may well explain the failure of differentiation in Dicer-null ESCs. Transfection of miR-290 family was shown to

partially rescue this DNA methylation phenotype by restoring the levels of Dnmt3a and Dnmt3b, but not that of Dnmt1. Such restoration is mediated by miR-290 family miRNAs suppressing their direct targets, retinoblastoma-like 2 protein (Rb12), a transcriptional repressor of Dnmts. Specifically, upon transfection of miR-290 cluster, Oct4 promoter methylation was restored^{40, 43}. One less explored question is whether miRNAs also shape the histone code to regulate ESC self-renewal and differentiation.

miRNAs control the generation of iPS cells

Five years ago, Yamanaka's group discovered that fibroblasts could be reprogrammed into induced pluripotent stem cells (iPSCs) by expressing Sox2, Oct4, Klf4 and c-Myc⁴⁴. The combination of Sox2, Oct4, Lin28, and Nanog also achieved the iPSC reprogramming⁴⁵. As we are gaining more and more insights into the mechanisms underlying somatic reprogramming, the roles of miRNAs in regulating this process have begun to be explored. miRNAs regulate somatic reprogramming at two different levels, cell proliferation and epigenetic programming. At the cell proliferation level, ES cell cycle-specific miRNAs miR-291-3p, miR-294 and miR-295 can replace c-Myc to facilitate Sox2-, Oct4- and Klf4-induced somatic reprogramming with a similar efficiency⁴⁶. Given the fact that these miRNAs are under the control of c-myc and other reprogramming transcription factors and that these miRNAs are not able to further increase somatic reprogramming efficiency in the presence of c-myc, it is likely that these miRNAs are downstream effectors of c-myc-mediated cell proliferation pathway^{13, 29}.

In addition to replacing c-Myc, miRNAs can promote reprogramming through epigenetic regulation. It was already known that the decreased Dnmt activity leads to elevated efficiency of iPS generation^{47, 48}. miR-302, abundantly expressed in ES cells, was recently shown to regulate Dnmt and enhance iPS generation⁴⁹. In hair follicle cells, overexpression of miR-302 to levels comparable to those of hESCs leads to suppression of AOF2, a stabilizer for Dnmt1. Therefore, induced expression of miR-302 promotes global DNA demethylation and in turn fosters iPS generation. Consistent with this, miR-302b and miR-372 were recently also shown to promote reprogramming of human fibroblast to iPS, through targeting multiple targets and regulating cell cycle, epithelial-mesenchymal transition, epigenetic regulation and vesicular transport⁵⁰. In addition, as previously discussed, the miR-290 family suppresses the repressor of Dnmt, therefore promoting Dnmt activity. Therefore, it would be of interest to examine whether miR-290 family antagonists could enhance the somatic reprogramming efficiency.

The role of miRNAs in tissue stem cells

Tissue stem cells reside in diverse types of adult tissues and are self-renewing progenitor cells responsible for the establishment and/or maintenance of their resident tissues. Current studies on miRNAs have revealed their roles in ectodermal and mesodermal tissues. In this section, we will first use germline stem cell system to illustrate the miRNA regulation of tissue stem cell in *Drosophila* and *C. elegans*, since the germline represents most of the studies on miRNAs in stem cells in lower model systems. We will then highlight one mammalian ectodermal tissue (neural system) and three mammalian mesodermal tissues (blood, muscle, and bone) in which the role of miRNAs is best known. It is only a matter of time before the role of miRNAs in endodermal tissues will be revealed.

miRNAs regulate germline development

Gametogenesis is a highly active process driven by germline stem cells in the gonad, with the exception of oogenesis in mammals. At present, little is known about the role of miRNAs in the *C. elegans* and mammalian germline. However, the *Drosophila* ovary

provides a powerful platform to dissect the role of miRNA in regulating germline stem cells. During *Drosophila* oogenesis, germline stem cell (GSC) resides at the anterior tip of the ovary in direct contact with their niche cells called cap cells, and divides asymmetrically to produce a daughter GSC and a differentiating cell called the cystoblast. The daughter GSC remains anchored to cap cells; whereas the cystoblast undergoes further oogenic differentiation⁵¹. miRNA was found to play a role in regulating GSC division and maintenance when Loquacious, Dicer-1 and Ago-1 were demonstrated to be important in *Drosophila* oogenesis^{52–55}. More specifically, *bantam* miRNA was shown to be essential for GSC maintenance, where *bantam* represses primordial germ cell differentiation and regulates GSCs as an extrinsic factor^{56–58}. In addition, miR-7 and miR-278 were found to regulate the cell cycle of GSCs. miR-278 depletion causes GSCs to divide slower whereas miR-7 depletion results in abnormal cell cycle progression. Such two microRNAs targets the 3' UTR of *dacapo* mRNA, which encodes a cyclin-dependent kinase inhibitor, that governs the G1/S transition^{59–61}. These results illustrate a common theme where multiple miRNAs converge to regulate the same pathway to fine tune the developmental process (Fig. 4B).

miRNAs regulate neurogenesis

Neurogenesis, starting from neural stem cells and neural progenitor cells, yields new neurons and supporting cells during both embryonic development and adult neural system maintenance (Fig. 3). In the nervous system, recent progress has identified several miRNAs important for neural development in multiple model organisms. In zebrafish, depletion of maternal and zygotic Dicer causes severe morphogenesis defect including incomplete neural tube closure, indicating that miRNAs regulate brain morphogenesis⁶². In *Drosophila*, miR-9a inhibits excess sensory organ precursors production by targeting Sens^{63, 64}, whereas miR-124a promotes dendritic branching of dendritic arborization sensory neurons by regulating unknown targets⁶⁵.

The study of miRNAs in mammalian neurogenesis is currently focused on miRNAs that show abundant or exclusive expression in the brain, or rapidly increased expression upon differentiation of ESCs to neural stem cells. The latter include miR-9 and miR-124. The respective nucleotide sequences of these two miRNAs are highly conserved⁶⁶.

A miRNA can regulate different mRNA targets at different stages of neurogenesis. For example, miR-9 stimulates the division and limits migration of ESC-derived neural progenitors by regulating its target Stathmin in hESCs⁶⁷. During the later differentiation of multipotent neural stem cells, miR-9 promotes the neuronal differentiation and suppresses neuronal stem cell self-renewal by down-regulating the expression level of its target, TLX, a highly conserved nuclear receptor (Fig. 3)⁶⁸. Conversely, miR-9 is also subject to the regulation of TLX, as the promoter region of miR-9 is occupied by TLX and the corepressor HDAC5. Such a feedback regulatory loop is exploited to ensure the delicate regulation of key players in developmental processes. Such feedback loop raises new questions about the sequence of actions for miR-9 and transcription factor TLX. Since both miR-9 and TLX coexists during the neuronal differentiation, it would be interesting if one could examine the temporal order of miR-9 and TLX expression to determine whether miR-9 regulate TLX first or vice versa. In addition to promoting neuronal differentiation, miR-9 also inhibits the astrocytic fate⁶⁹. In addition, miR-9 and miR-124 act in a concerted manner to control neurogenesis in ESCs^{67, 69}. Both miRNAs contribute to the decreased STAT3 phosphorylation level, thereby inhibiting the astrocytic fate during neural differentiation.

Recently, miR-9* and miR-124 were found to guide the directly conversion of human fibroblasts to neurons through SWI/SNF-like BAF chromatin-remodelling complexes. This indicates that miRNA could control cell lineage through epigenetic regulation⁷⁰.

From the above review, three common principles underlying miRNA-mediated regulation have emerged (Fig. 4): (1) miRNAs act as an fine-tuning developmental switch in a stage- and tissue-specific manner (Fig. 4A); (2) different miRNAs converge to control the same signaling pathways by regulating one or more component simultaneously (Fig. 4B); and (3) miRNAs and key developmental regulators form feedback loops to ensure delicate control of fundamental biological processes (Fig. 4C). These modes of miRNA regulation repeatedly appear as common themes in other tissues, as further reviewed below. However, these are not parallel or exclusive modes in action. Often, the fine-tuning function of miRNAs is achieved via either their targeting of signaling pathways and/or forming feedback loops with other types of developmental regulators such as epigenetic factors or transcriptional factors.

miRNAs regulate hematopoiesis

Hematopoiesis, initiated by multipotent hematopoietic stem cell (HSC) and giving rise to all types of blood cells, is a multi-stage and highly regulated proliferation and differentiation process. Since little is known about the function of miRNA in hematopoiesis in *Drosophila*, this section is dedicated to mammalian hematopoiesis. During hematopoiesis in mammals, transcriptional factors have differential expression patterns in hematopoietic stem cells and different progenitors, where they control gene expression, which in turn regulate lineage specification and differentiation of those stem cells and progenitors. More and more recent studies show that miRNAs also exhibit lineage specific expression profile and control stem cell self-renewal and differentiation, yet through a different mechanism--posttranscriptional regulation. The aforementioned features of miRNA-mediated regulation make them prime candidates as key regulators of hematopoiesis.

A common feature of miRNA regulation in hematopoiesis is that miRNAs display distinct stage- and lineage-specific expression profiles and functions (Fig. 3)⁷¹. Based on bioinformatic, miRNA profiling, and reverse genetic analyses, the role of miRNAs in regulating hematopoiesis has been increasingly revealed. For example, miR-181 and miR-128 are highly enriched in early hematopoietic stem cells (HSCs), and maintain the stem cell identity by limiting the differentiation of HSCs into all hematopoietic lineages. On the other hand, miR-223 promotes the differentiation of HSCs⁷². Therefore, miRNAs with opposite effect act together on the same HSC differentiation pathway to exert tight regulation. As another example of such antagonistic regulation, miR-155, miR-24a, and miR-17 inhibit multipotent progenitor differentiation into myeloid lineage; whereas miR-146 and miR-181 inhibit and promote the lymphoid lineage differentiation, respectively. Such regulation also occurs during the terminal differentiation. miR-181a, highly enriched in the CD4⁺CD8⁺ double positive cells, promotes the T-cell positive selection⁷³, whereas miR-150 acts as a block to limit pro-B to pre-B transition⁷⁴.

The second common feature of miRNA regulation of hematopoiesis is the feedback loop between miRNAs and transcription factors, whereby miRNAs modulate the expression level of transcription factors or the inhibitors and stabilizers of transcription factors. Conversely, transcription factors bind to miRNA genes and regulate their transcription. For example, during granulopoiesis, the interplay among miR-223, transcription factors NFI-A, and C/EBP α reveals the fine and concerted control of granulocytic differentiation⁷⁵. NFI-A binds to the promoter region of pre-miR-223 and represses miR-223 expression, while upon retinoic acid (RA)-induced differentiation, C/EBP α outcompetes NFI-A for the same promoter region and positively regulates miR-223 expression. The replacement of NFI-A by C/EBP α and the resulting granulocytic differentiation is also strengthened by the feedback loop regulation of miR-223, which suppresses NFI-A expression at the posttranscriptional level.

The third common feature is that one miRNA could regulate several different lineages. For instance, miR-155 controls both T cell and B cell differentiation. On one hand, miR-155 regulates B cell polyclonal expansion, as evidenced by the pre-B cell over proliferation in E μ -miR-155⁷⁶. On the other hand, miR-155 is important for T cell differentiation into Treg and Th1, as miR-155 knockout mice show reduced Treg cells, immunodeficiency and compromised inflammation^{77, 78}. This one-to-many relationship between miRNAs and biological processes is accompanied by the combinatorial regulation of miRNAs to ensure accurate and tight control over stem cell proliferation and differentiation.

miRNAs control bone and muscle development

Bone formation (osteogenesis) consists of a series of differentiation events starting from multipotent mesenchymal stem cell and resulting in the development of the bone. The commitment of mesenchymal stem cell to the bone lineage is mediated by transcription factors *Dlx5*, *Runx2*, and growth factor BMP. Recent studies showed that the interaction between miRNAs and these key regulators are also very important. For example, miR-125b inhibits BMP4-mediated mesenchymal stem cell differentiation to osteoblasts in mice⁷⁹, while miR-141 and miR-200a suppress pre-osteoblast differentiation through targeting transcription factor *Dlx5*⁸⁰. As opposed to these miRNAs, miR-2861 shows a stimulatory effect on BMP2-induced osteoblast differentiation, in part by maintaining the expression level of *Runx2*⁸¹. Interestingly, this role is mediated through miR-2861 targeting an epigenetic factor, HDAC5, which accelerates the degradation of *Runx2*.

miRNAs also play a role in chondrogenesis, which is initiated by the differentiation of mesenchymal stem cells into chondrogenic progenitors. A BMP-2 early responsive target miRNA, miR-199a*, inhibits early stage of chondrogenesis and the production of cartilage oligomeric matrix protein and collagen, primarily through inhibiting *Smad1* posttranscriptionally⁸².

Like the bone, skeletal and cardiac muscles are derived from mesoderm progenitors. Skeletal muscle development is subject to the stimulatory miRNAs (miR-1, -26a, -206 and -214) and inhibitory miRNAs (miR-133, -221 and -222). Notably, miR-1 and miR-26a execute such regulation through targeting epigenetic factors, *Hdac4* and *Ezh2*, respectively^{83–85}. During the development of cardiac muscle development, positive and negative feedback regulatory loops co-exist. In the positive feedback loop, transcription factor MEF2 activates the expression of miR-1, which then down-regulates the epigenetic repression of HDAC towards MEF2, which further elevates the expression of MEF2. This positive feedback loop could ensure a rapid and lasting control of cardiac muscle development. On the other hand, a negative feedback loop could allow precise and fine regulation, where miR-133 suppresses the transcription factor SRF that activates the expression of miR-133. Therefore, the recurring theme of feedback loop mechanism enables precise control of key biological processes. Unlike cardiac and skeletal muscles, smooth muscle is produced from the ectoderm-derived neural crest stem cell. Recently, miR-145 was shown to be necessary for neural crest stem cell differentiation into smooth muscle cells, through targeting transcription factors *Klf4*, *Myocardin*, and *Elk1*⁸⁶.

The therapeutic roles of miRNAs

Given the complex and fine-tuned nature of miRNA regulation of gene expression, its deregulation may lead to a variety of diseases including cancer. Indeed, miRNA expression profiles are often skewed in tumor cells^{87, 88}. Moreover, certain miRNAs display oncogenic or tumor suppressive properties⁸⁹. Therefore, miRNAs show promising potential as tools for cancer diagnosis, prognosis and treatment. Mounting evidence indicates that miRNA expression profile can be used to distinguish between normal tissues and tumor tissues, and

among different cancer subtypes^{90–92}. More specifically, in a breast cancer study, nine miRNAs provide the discriminating power to classify basal vs. luminal cancer subtypes⁹³. In addition to diagnosis, miRNAs also have prognostic value. For example, miR-21 has the prediction power for breast cancer aggression, whereas high miR-155 and low let-7a levels are associated with poor survival^{94, 95}. Based on this, it would be of interest to examine whether these miRNAs also could predict the patient's response to treatments. Moreover, miRNAs have also been used in treating cancer. Viral delivery of let-7 and miR-26a to mice suffering from lung cancer and liver cancer exhibited decreased tumor growth, through targeting oncogenes or cell cycle regulators, respectively^{96–98}. As seen in the success of these studies, miRNAs showed unique therapeutic advantages in cancer, probably because of the low risk of off-target effect and the broader impact on their target genes. However, a viral miRNA delivery system also has its limitations. First, the viral delivery system imposes the risk of adverse effect including immune response, viral integration into genome, and cytotoxicity. Second, such delivery has minimal, if any, cellular targeting specificity, thus affecting both cancer cell and normal cells. One possible approach to address these drawbacks is to develop tumor cell-targeting liposome, which employs Transferrin (Tf) to target Tf-Receptor enriched tumor cells⁹⁹. With rapid new development in miRNA-based therapy, we are entering an age when the fundamental research on miRNAs will soon be applied to clinical practice.

Acknowledgments

We are grateful to Robert Ross, Jianquan Wang, and anonymous reviewers for critical reading of this review. The research in the Lin lab is supported by the NIH (DP1OD006825, R01 HD33760, and R01HD42012), the G. Harold and Leila Y. Mathers Foundation Award, and the Ellison Medical Foundation Senior Scholar Award, and the Connecticut Stem cell Research Funds (08-SCD-Yale-004).

References

1. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell*. 1993; 75:843–854. [PubMed: 8252621]
2. Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degan B, Müller P, et al. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature*. 2000; 408:86–89. [PubMed: 11081512]
3. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettlinger JC, Ruvkun G, Horvitz HR, Ruvkun G. The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature*. 2000; 403:901–906. [PubMed: 10706289]
4. Mallanna SK, Rizzino A. Emerging roles of microRNAs in the control of embryonic stem cells and the generation of induced pluripotent stem cells. *Dev Biol*. 2010; 344:16–25. [PubMed: 20478297]
5. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Rådmark O, Kim S, et al. The nuclear RNase III Drosha initiates microRNA processing. *Nature*. 2003; 425:415–419. [PubMed: 14508493]
6. Zeng Y, Cullen BR. Sequence requirements for micro RNA processing and function in human cells. *RNA*. 2003; 9:112–123. [PubMed: 12554881]
7. Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes and Development*. 2003; 17:3011–3016. [PubMed: 14681208]
8. Chendrimada TP, Gregory RI, Kumaraswamy E, Norman J, Cooch N, Nishikura K, Shiekhattar R. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature*. 2005; 436:740–744. [PubMed: 15973356]
9. Maniataki E, Mourelatos Z. A human, ATP-independent, RISC assembly machine fueled by pre-miRNA. *Genes and Development*. 2005; 19:2979–2990. [PubMed: 16357216]
10. Haase AD, Jaskiewicz L, Zhang H, Lainé S, Sack R, Gatignol A, Filipowicz W. TRBP, a regulator of cellular PKR and HIV-1 virus expression, interacts with Dicer and functions in RNA silencing. *EMBO Reports*. 2005; 6:961–967. [PubMed: 16142218]

11. Brennecke J, Stark A, Russell RB, Cohen SM. Principles of microRNA-target recognition. *PLoS Biology*. 2005; 3:0404–0418.
12. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*. 2005; 120:15–20. [PubMed: 15652477]
13. Martinez NJ, Gregory RI. MicroRNA gene regulatory pathways in the establishment and maintenance of ESC identity. *Cell Stem Cell*. 2010; 7:31–35. [PubMed: 20621047]
14. Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature*. 2010; 466:835–840. [PubMed: 20703300]
15. Okamura K, Hagen JW, Duan H, Tyler DM, Lai EC. The Mirtron Pathway Generates microRNA-Class Regulatory RNAs in *Drosophila*. *Cell*. 2007; 130:89–100. [PubMed: 17599402]
16. Ruby JG, Jan CH, Bartel DP. Intronic microRNA precursors that bypass Drosha processing. *Nature*. 2007; 448:83–86. [PubMed: 17589500]
17. Ender C, Krek A, Friedländer MR, Beitzinger M, Weinmann L, Chen W, Pfeffer S, Rajewsky N, Meister G. A Human snoRNA with MicroRNA-Like Functions. *Molecular Cell*. 2008; 32:519–528. [PubMed: 19026782]
18. Saraiya AA, Wang CC. snoRNA, a novel precursor of microRNA in *Giardia lamblia*. *PLoS Pathogens*. 2008;4.
19. Babiarz JE, Ruby JG, Wang Y, Bartel DP, Blelloch R. Mouse ES cells express endogenous shRNAs, siRNAs, and other Microprocessor-independent, Dicer-dependent small RNAs. *Genes Dev*. 2008; 22:2773–2785. [PubMed: 18923076]
20. Bogerd HP, Karnowski HW, Cai X, Shin J, Pohlers M, Cullen BR. A Mammalian Herpesvirus Uses Noncanonical Expression and Processing Mechanisms to Generate Viral MicroRNAs. *Molecular Cell*. 2010; 37:135–142. [PubMed: 20129062]
21. Cheloufi S, Dos Santos CO, Chong MMW, Hannon GJ. A dicer-independent miRNA biogenesis pathway that requires Ago catalysis. *Nature*. 2010; 465:584–589. [PubMed: 20424607]
22. Cifuentes D, Xue H, Taylor DW, Patnode H, Mishima Y, Cheloufi S, Ma E, Mane S, Hannon GJ, Lawson ND, et al. A novel miRNA processing pathway independent of dicer requires argonaute2 catalytic activity. *Science*. 2010; 328:1694–1698. [PubMed: 20448148]
23. Silva J, Smith A. Capturing Pluripotency. *Cell*. 2008; 132:532–536. [PubMed: 18295569]
24. Gaspar-Maia A, Alajem A, Meshorer E, Ramalho-Santos M. Open chromatin in pluripotency and reprogramming. *Nature Reviews Molecular Cell Biology*. 2011; 12:36–47.
25. Houbavii HB, Murray MF, Sharp PA. Embryonic stem cell-specific microRNAs. *Developmental Cell*. 2003; 5:351–358. [PubMed: 12919684]
26. Wilson KD, Venkatasubrahmanyam S, Jia F, Sun N, Butte AJ, Wu JC. MicroRNA profiling of human-induced pluripotent stem cells. *Stem Cells and Development*. 2009; 18:749–757. [PubMed: 19284351]
27. Landgraf P. A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell*. 2007; 129:1401–1414. [PubMed: 17604727]
28. Suh MR. Human embryonic stem cells express a unique set of microRNAs. *Dev Biol*. 2004; 270:488–498. [PubMed: 15183728]
29. Marson A, Levine SS, Cole MF, Frampton GM, Brambrink T, Johnstone S, Guenther MG, Johnston WK, Wernig M, Newman J, et al. Connecting microRNA Genes to the Core Transcriptional Regulatory Circuitry of Embryonic Stem Cells. *Cell*. 2008; 134:521–533. [PubMed: 18692474]
30. Gangaraju VK, Lin H. MicroRNAs: Key regulators of stem cells. *Nature Reviews Molecular Cell Biology*. 2009; 10:116–125.
31. Sachdeva M, Zhu S, Wu F, Wu H, Walia V, Kumar S, Elble R, Watabe K, Mo YY. p53 represses c-Myc through induction of the tumor suppressor miR-145. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:3207–3212. [PubMed: 19202062]
32. Xu N, Papagiannakopoulos T, Pan G, Thomson JA, Kosik KS. MicroRNA-145 Regulates OCT4, SOX2, and KLF4 and Represses Pluripotency in Human Embryonic Stem Cells. *Cell*. 2009; 137:647–658. [PubMed: 19409607]

33. Tay Y, Zhang J, Thomson AM, Lim B, Rigoutsos I. MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. *Nature*. 2008; 455:1124–1128. [PubMed: 18806776]
34. Tay YMS, Tam WL, Ang YS, Gaughwin PM, Yang H, Wang W, Liu R, George J, Ng HH, Perera RJ, et al. MicroRNA-134 modulates the differentiation of mouse embryonic stem cells, where it causes post-transcriptional attenuation of Nanog and LRH1. *Stem Cells*. 2008; 26:17–29. [PubMed: 17916804]
35. Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, Waldvogel B, Vannier C, Darling D, zur Hausen A, et al. The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. *Nature Cell Biology*. 2009; 11:1487–1495.
36. Wang Y, Baskerville S, Shenoy A, Babiarz JE, Baehner L, Bluelloch R. Embryonic stem cell-specific microRNAs regulate the G1-S transition and promote rapid proliferation. *Nature Genetics*. 2008; 40:1478–1483. [PubMed: 18978791]
37. Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, Livingston DM, Rajewsky K. Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. *Genes and Development*. 2005; 19:489–501. [PubMed: 15713842]
38. Murchison EP, Partridge JF, Tam OH, Cheloufi S, Hannon GJ. Characterization of Dicer-deficient murine embryonic stem cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:12135–12140. [PubMed: 16099834]
39. Wang Y, Medvid R, Melton C, Jaenisch R, Bluelloch R. DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. *Nature Genetics*. 2007; 39:380–385. [PubMed: 17259983]
40. Sinkkonen L, Hugenschmidt T, Berninger P, Gaidatzis D, Mohn F, Artus-Revel CG, Zavolan M, Svoboda P, Filipowicz W. MicroRNAs control de novo DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells. *Nature Structural and Molecular Biology*. 2008; 15:259–267.
41. Sengupta S, Nie J, Wagner RJ, Yang C, Stewart R, Thomson JA. MicroRNA 92b controls the G1/S checkpoint gene p57 in human embryonic stem cells. *Stem Cells*. 2009; 27:1524–1528. [PubMed: 19544458]
42. Qi J, Yu JY, Shcherbata HR, Mathieu J, Wang AJ, Seal S, Zhou W, Stadler BM, Bourgin D, Wang L, et al. microRNAs regulate human embryonic stem cell division. *Cell Cycle*. 2009; 8:3729–3741. [PubMed: 19823043]
43. Benetti R, Gonzalo S, Jaco I, Muñoz P, Gonzalez S, Schoeftner S, Murchison E, Andl T, Chen T, Klatt P, et al. A mammalian microRNA cluster controls DNA methylation and telomere recombination via Rbl2-dependent regulation of DNA methyltransferases. *Nature Structural and Molecular Biology*. 2008; 15:268–279.
44. Takahashi K, Yamanaka S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell*. 2006; 126:663–676. [PubMed: 16904174]
45. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007; 318:1917–1920. [PubMed: 18029452]
46. Judson RL, Babiarz JE, Venere M, Bluelloch R. Embryonic stem cell-specific microRNAs promote induced pluripotency. *Nature Biotechnology*. 2009; 27:459–461.
47. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, Melton DA. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nature Biotechnology*. 2008; 26:795–797.
48. Shi Y, Despons C, Do JT, Hahm HS, Schöler HR, Ding S. Induction of Pluripotent Stem Cells from Mouse Embryonic Fibroblasts by Oct4 and Klf4 with Small-Molecule Compounds. *Cell Stem Cell*. 2008; 3:568–574. [PubMed: 18983970]
49. Lin SL, Chang DC, Lin CH, Ying SY, Leu D, Wu DT. Regulation of somatic cell reprogramming through inducible mir-302 expression. *Nucleic Acids Res*. 2010
50. Subramanyam D, Lamouille S, Judson RL, Liu JY, Bucay N, Derynck R, Bluelloch R. Multiple targets of miR-302 and miR-372 promote reprogramming of human fibroblasts to induced pluripotent stem cells. *Nat Biotech*. 2011; 29:443–448.

51. Dansereau DA, Lasko P. The development of germline stem cells in drosophila. 2008; 450:3–26. Available at: <http://www.scopus.com/inward/record.url?eid=2-s2.0-44049108146&partnerID=40&md5=befb6ab4e64da1b1de8ba24ae478130>.
52. Forstemann K, Tomari Y, Du T, Vagin VV, Denli AM, Bratu DP, Klattenhoff C, Theurkauf WE, Zamore PD. Normal microRNA maturation and germ-line stem cell maintenance requires Loquacious, a double-stranded RNA-binding domain protein. *PLoS Biol.* 2005; 3:e236. [PubMed: 15918770]
53. Hatfield SD, Shcherbata HR, Fischer KA, Nakahara K, Carthew RW, Ruohola-Baker H. Stem cell division is regulated by the microRNA pathway. *Nature.* 2005; 435:974–978. [PubMed: 15944714]
54. Jin Z, Xie T. Dcr-1 Maintains Drosophila Ovarian Stem Cells. *Current Biology.* 2007; 17:539–544. [PubMed: 17306537]
55. Yang L, Chen D, Duan R, Xia L, Wang J, Qurashi A, Jin P. Argonaute 1 regulates the fate of germline stem cells in Drosophila. *Development.* 2007; 134:4265–4272. [PubMed: 17993467]
56. Shcherbata HR, Ward EJ, Fischer KA, Yu JY, Reynolds SH, Chen CH, Xu P, Hay BA, Ruohola-Baker H. Stage-specific differences in the requirements for germline stem cell maintenance in the Drosophila ovary. *Cell Stem Cell.* 2007; 1:698–709. [PubMed: 18213359]
57. Neumuller RA, Betschinger J, Fischer A, Bushati N, Poernbacher I, Mechtler K, Cohen SM, Knoblich JA. Mei-P26 regulates microRNAs and cell growth in the Drosophila ovarian stem cell lineage. *Nature.* 2008; 454:241–245. [PubMed: 18528333]
58. Yang Y, Xu S, Xia L, Wang J, Wen S, Jin P, Chen D. The bantam microRNA is associated with drosophila fragile X mental retardation protein and regulates the fate of germline stem cells. *PLoS Genet.* 2009; 5:e1000444. [PubMed: 19343200]
59. de Nooij JC, Letendre MA, Hariharan IK. A cyclin-dependent kinase inhibitor, Dacapo, is necessary for timely exit from the cell cycle during Drosophila embryogenesis. *Cell.* 1996; 87:1237–1247. [PubMed: 8980230]
60. Lane ME, Sauer K, Wallace K, Jan YN, Lehner CF, Vaessin H. Dacapo, a cyclin-dependent kinase inhibitor, stops cell proliferation during Drosophila development. *Cell.* 1996; 87:1225–1235. [PubMed: 8980229]
61. Yu JY, Reynolds SH, Hatfield SD, Shcherbata HR, Fischer KA, Ward EJ, Long D, Ding Y, Ruohola-Baker H. Dicer-1-dependent Dacapo suppression acts downstream of Insulin receptor in regulating cell division of Drosophila germline stem cells. *Development.* 2009; 136:1497–1507. [PubMed: 19336466]
62. Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM, Baskerville S, Hammond SM, Bartel DP, Schier AF. MicroRNAs regulate brain morphogenesis in zebrafish. *Science.* 2005; 308:833–838. [PubMed: 15774722]
63. Li Y, Wang F, Lee JA, Gao FB. MicroRNA-9a ensures the precise specification of sensory organ precursors in Drosophila. *Genes Dev.* 2006; 20:2793–2805. [PubMed: 17015424]
64. Bejarano F, Smibert P, Lai EC. miR-9a prevents apoptosis during wing development by repressing Drosophila LIM-only. *Developmental Biology.* 2010; 338:63–73. [PubMed: 19944676]
65. Xu XL, Li Y, Wang F, Gao FB. The steady-state level of the nervous-system-specific microRNA-124a is regulated by dFMR1 in Drosophila. *Journal of Neuroscience.* 2008; 28:11883–11889. [PubMed: 19005053]
66. Gao FB. Context-dependent functions of specific microRNAs in neuronal development. *Neural Dev.* 2010; 5:25. [PubMed: 20920300]
67. Delaloy C, Liu L, Lee JA, Su H, Shen F, Yang GY, Young WL, Ivey KN, Gao FB. MicroRNA-9 Coordinates Proliferation and Migration of Human Embryonic Stem Cell-Derived Neural Progenitors. *Cell Stem Cell.* 2010; 6:323–335. [PubMed: 20362537]
68. Zhao C, Sun G, Li S, Shi Y. A feedback regulatory loop involving microRNA-9 and nuclear receptor TLX in neural stem cell fate determination. *Nature Structural and Molecular Biology.* 2009; 16:365–371.
69. Krichevsky AM, Sonntag KC, Isacson O, Kosik KS. Specific MicroRNAs modulate embryonic stem cell-derived neurogenesis. *Stem Cells.* 2006; 24:857–864. [PubMed: 16357340]

70. Yoo AS, Sun AX, Li L, Shcheglovitov A, Portmann T, Li Y, Lee-Messer C, Dolmetsch RE, Tsien RW, Crabtree GR. MicroRNA-mediated conversion of human fibroblasts to neurons. *Nature*. 2011 advance online publication.
71. Garzon R, Croce CM. MicroRNAs in normal and malignant hematopoiesis. *Curr Opin Hematol*. 2008; 15:352–358. [PubMed: 18536574]
72. Georgantas RW 3rd, Hildreth R, Morisot S, Alder J, Liu CG, Heimfeld S, Calin GA, Croce CM, Civin CI. CD34+ hematopoietic stem-progenitor cell microRNA expression and function: a circuit diagram of differentiation control. *Proc Natl Acad Sci U S A*. 2007; 104:2750–2755. [PubMed: 17293455]
73. Neilson JR, Zheng GXY, Burge CB, Sharp PA. Dynamic regulation of miRNA expression in ordered stages of cellular development. *Genes and Development*. 2007; 21:578–589. [PubMed: 17344418]
74. Xiao C, Calado DP, Galler G, Thai TH, Patterson HC, Wang J, Rajewsky N, Bender TP, Rajewsky K. MiR-150 controls B cell differentiation by targeting the transcription factor c-Myb. *Cell*. 2007; 131:146–159. [PubMed: 17923094]
75. Fazi F, Rosa A, Fatica A, Gelmetti V, De Marchis ML, Nervi C, Bozzoni I. A minicircuitry comprised of microRNA-223 and transcription factors NFI-A and C/EBPalpha regulates human granulopoiesis. *Cell*. 2005; 123:819–831. [PubMed: 16325577]
76. Costinean S, Zanesi N, Pekarsky Y, Tili E, Volinia S, Heerema N, Croce CM. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. *Proc Natl Acad Sci U S A*. 2006; 103:7024–7029. [PubMed: 16641092]
77. Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, van Dongen S, Grocock RJ, Das PP, Miska EA, et al. Requirement of bic/microRNA-155 for normal immune function. *Science*. 2007; 316:608–611. [PubMed: 17463290]
78. Thai TH, Calado DP, Casola S, Ansel KM, Xiao C, Xue Y, Murphy A, Frendewey D, Valenzuela D, Kutok JL, et al. Regulation of the germinal center response by microRNA-155. *Science*. 2007; 316:604–608. [PubMed: 17463289]
79. Mizuno Y, Yagi K, Tokuzawa Y, Kanesaki-Yatsuka Y, Suda T, Katagiri T, Fukuda T, Maruyama M, Okuda A, Amemiya T, et al. miR-125b inhibits osteoblastic differentiation by down-regulation of cell proliferation. *Biochemical and Biophysical Research Communications*. 2008; 368:267–272. [PubMed: 18230348]
80. Itoh T, Nozawa Y, Akao Y. MicroRNA-141 and -200a are involved in bone morphogenetic protein-2-induced mouse pre-osteoblast differentiation by targeting distal-less homeobox 5. *J Biol Chem*. 2009; 284:19272–19279. [PubMed: 19454767]
81. Li H, Xie H, Liu W, Hu R, Huang B, Tan YF, Xu K, Sheng ZF, Zhou HD, Wu XP, et al. A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans. *J Clin Invest*. 2009; 119:3666–3677. [PubMed: 19920351]
82. Lin EA, Kong L, Bai XH, Luan Y, Liu CJ. miR-199a, a bone morphogenic protein 2-responsive MicroRNA, regulates chondrogenesis via direct targeting to Smad1. *J Biol Chem*. 2009; 284:11326–11335. [PubMed: 19251704]
83. Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, Conlon FL, Wang DZ. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nature Genetics*. 2006; 38:228–233. [PubMed: 16380711]
84. Anderson C, Catoe H, Werner R. MIR-206 regulates connexin43 expression during skeletal muscle development. *Nucleic Acids Research*. 2006; 34:5863–5871. [PubMed: 17062625]
85. Caretti G, Di Padova M, Micales B, Lyons GE, Sartorelli V. The Polycomb Ezh2 methyltransferase regulates muscle gene expression and skeletal muscle differentiation. *Genes and Development*. 2004; 18:2627–2638. [PubMed: 15520282]
86. Cordes KR, Sheehy NT, White MP, Berry EC, Morton SU, Muth AN, Lee TH, Miano JM, Ivey KN, Srivastava D. MiR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature*. 2009; 460:705–710. [PubMed: 19578358]
87. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, et al. MicroRNA expression profiles classify human cancers. *Nature*. 2005; 435:834–838. [PubMed: 15944708]

88. Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nature Reviews Cancer*. 2006; 6:857–866.
89. Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. *Dev Biol*. 2007; 302:1–12. [PubMed: 16989803]
90. Mattie MD, Benz CC, Bowers J, Sensinger K, Wong L, Scott GK, Fedele V, Ginzinger D, Getts R, Haqq C. Optimized high-throughput microRNA expression profiling provides novel biomarker assessment of clinical prostate and breast cancer biopsies. *Molecular Cancer*. 2006;5. [PubMed: 16457726]
91. Raponi M, Dossey L, Jatke T, Wu X, Chen G, Fan H, Beer DG. MicroRNA classifiers for predicting prognosis of squamous cell lung cancer. *Cancer Research*. 2009; 69:5776–5783. [PubMed: 19584273]
92. Yang H, Kong W, He L, Zhao JJ, O'Donnell JD, Wang J, Wenham RM, Coppola D, Kruk PA, Nicosia SV, et al. MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. *Cancer Research*. 2008; 68:425–433. [PubMed: 18199536]
93. Blenkinson C, Goldstein LD, Thorne NP, Spiteri I, Chin SF, Dunning MJ, Barbosa-Morais NL, Teschendorff AE, Green AR, Ellis IO, et al. MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype. *Genome Biology*. 2007;8.
94. Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell*. 2006; 9:189–198. [PubMed: 16530703]
95. Yan LX, Huang XF, Shao Q, Huang MY, Deng L, Wu QL, Zeng YX, Shao JY. MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. *RNA*. 2008; 14:2348–2360. [PubMed: 18812439]
96. Kota J, Chivukula RR, O'Donnell KA, Wentzel EA, Montgomery CL, Hwang HW, Chang TC, Vivekanandan P, Torbenson M, Clark KR, et al. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell*. 2009; 137:1005–1017. [PubMed: 19524505]
97. Esquela-Kerscher A, Trang P, Wiggins JF, Patrawala L, Cheng A, Ford L, Weidhaas JB, Brown D, Bader AG, Slack FJ. The let-7 microRNA reduces tumor growth in mouse models of lung cancer. *Cell Cycle*. 2008; 7:759–764. [PubMed: 18344688]
98. Kumar MS, Erkeland SJ, Pester RE, Chen CY, Ebert MS, Sharp PA, Jacks T. Suppression of non-small cell lung tumor development by the let-7 microRNA family. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:3903–3908. [PubMed: 18308936]
99. DeSano JT, Xu L. MicroRNA regulation of cancer stem cells and therapeutic implications. *AAPS J*. 2009; 11:682–692. [PubMed: 19842044]

Figure 1. Three different pathways of miRNA biogenesis: the canonical pathway (left panel), the microprocessor –independent pathway (middle panel), and the Dicer-independent pathways (right panel). For details, see text.

Figure 2.
Three different mechanisms through which miRNAs regulate the proliferation, self-renewal, and differentiation of embryonic stem cells. For details, see text.

Figure 3.

The function of miRNAs in regulating the proliferation, self-renewal, and differentiation of adult tissue stem cells. Individual miRNAs are indicated by numbers next to the process regulated by them. Red numbers indicate miRNAs that promote proliferation and self-renewal; whereas green numbers indicate miRNAs that promote differentiation. For details, see text.

Figure 4. Three different modes of miRNA regulation in stem cell proliferation, self-renewal, and differentiation. For details, see text.

Table 1

List of microRNAs involved in stem cell regulation

Germ layer	Process	miRNA	Regulatory Mechanism
ESC	ESC maintenance and differentiation	miR-145	Represses c-Myc, Sox2, Oct4 and Klf4 ^{31, 32}
		miR-134, miR-296 and miR-470	Regulate Oct4, Sox2 and Nanog ^{33–35}
		miR-200c, miR-203 and miR-183	Regulate Sox2 and Klf4 ^{33–35}
		miR-195	Represses WEE1, an inhibitory kinase in G2-to-M transition that blocks the G2 cyclin B-Cdk complex ⁴² .
		miR-290 and miR-302	Suppress cyclin E-Cdk2 upstream inhibitors in mESCs, therefore enabling the unrestricted G1-to-S transition and short cell cycle ^{36, 40} .
		miR-290 family miRNAs	Suppresses Rbl2, a transcriptional repressor of Dnmts ^{40, 43} .
	iPS Generation	miR-302	Regulates Dnmt and enhance iPS generation ⁴⁹
		miR-302b and miR-372	Promote reprogramming of human fibroblast to iPS ⁵⁰
Germline	Germline Development	bantam miRNA	Represses primordial germ cell differentiation and regulates germline stem cell as an extrinsic factor ^{56–58}
		miR-7 and miR-278	Target dacapo, a cyclin-dependent kinase inhibitor and control G1/S transition ^{59–61}
Ectoderm	Neurogenesis	miR-9a	Inhibits excess sensory organ precursors production by targeting Sens in Drosophila ^{63, 64}
		miR-124a	Promotes dendritic branching through unknown targets in Drosophila ⁶⁵
		miR-9	Promotes the neuronal differentiation and suppresses neuronal stem cell self-renewal by repressing TLX and is subjective to TLX transcriptional regulation ⁶⁸ .
		miR-9	Inhibits the astrocytic fate ⁶⁹
		miR-9* and miR-124	Promotes human fibroblasts directly conversion to neuron, through BAF ⁷⁰
Mesoderm	Hematopoiesis	miR-155, miR-24a, and miR-17	Inhibit multipotent progenitor differentiation into myeloid lineage ⁷³
		miR-150	Inhibits pro-B to pre-B transition ⁷⁴
		miR-223	Targets transcription factors NFI-A, and C/EBP α and controls granulocytic differentiation ⁷⁵
		miR-155	Controls T cell and B cell differentiation and regulates B cell polyclonal expansion ⁷⁶
	Osteogenesis	miR-125b	Inhibits BMP4-mediated mesenchymal stem cell differentiation to osteoblasts in mice ⁷⁹
		miR-141 and miR-200a	Suppress pre-osteoblast differentiation through targeting transcription factor Dlx5 ⁸⁰
		miR-2861	Stimulates BMP2-induced osteoblast differentiation by maintaining the expression level of Runx2 ⁸¹
	Chondrogenesis	miR-199a*	Inhibits early stage of chondrogenesis and production of cartilage oligomeric matrix protein and collagen, by inhibiting Smad1 posttranscriptionally ⁸²
	Skeletal muscle development	miR-1 and miR-26a	Stimulates skeletal muscle through targeting epigenetic factors, Hdac4 and Ezh2, respectively ^{83–85}

Germ layer	Process	miRNA	Regulatory Mechanism
	Smooth muscle cells development	miR-145	Promotes neural crest stem cell differentiation into smooth muscle cells through targeting transcription factors Klf4, Myocardin, and Elk1 ⁸⁶