miRNAs in ESC differentiation

Emanuele Berardi,1* Matthias Pues,1* Lieven Thorrez,1 and Maurilio Sampaolesi1,2
1Laboratory of Translational Cardiomyology, Department of Development and Regeneration, Katholieke Universiteit Leuven, Leuven, Belgium; and 2Human Anatomy Institute, Department of Public Health, Neuroscience, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy

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Am J Physiol Heart Circ Physiol 303: H931–H939, 2012. First published August 10, 2012; doi:10.1152/ajpheart.00338.2012.—MicroRNAs (miRNAs) are small sequences of noncoding RNAs that regulate gene expression by two basic processes: direct degradation of mRNA and translation inhibition. miRNAs are key molecules in gene regulation for embryonic stem cells, since they are able to repress target pluripotent mRNA genes, including Oct4, Sox2, and Nanog. miRNAs are unlike other small noncoding RNAs in their biogenesis, since they derive from precursors that fold back to form a distinctive hairpin structure, whereas other classes of small RNAs are formed from longer hairpins or bimolecular RNA duplexes (siRNAs) or precursors without double-stranded character (piRNAs). An increasing amount of evidence suggests that miRNAs may have a critical role in the maintenance of the pluripotent cell state and in the regulation of early mammalian development. This review gives an overview of the current state of the art of miRNA expression and regulation in embryonic stem cell differentiation. Current insights on controlling stem cell fate toward mesodermal, endodermal and ectodermal differentiation, and cell reprogramming are also highlighted.

miRNA; embryonic stem cells; early differentiation

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Introduction

Embryonic stem cells (ESCs) can be isolated from the inner cell mass of the blastocyst (54, 61), and they can be expanded in vitro for several passages without losing their pluripotency. In fact, they can be differentiated into all germ layer cells at late passages upon appropriate culture conditions. Embryonic stem-like cells, termed induced pluripotent stem cells (iPSCs), can be obtained by the expression of pluripotency genes including Oct4 (official gene symbol Pou5f1), Sox2, Klf4, Nanog, Lin28, and c-Myc. ESCs and iPSCs, referred to as pluripotent stem cells (PSCs), are a promising source for drug screening and cell therapy in degenerative diseases (56). However, developmental biologists and stem cell researchers concern the concerns regarding our poor knowledge of molecular mechanisms regulating the ESC switch between pluripotency and differentiation. The molecular programs responsible for different PSC fates are mediated by internal regulatory elements, such as transcription factors, but these programs are influenced by external elements such as physical interactions with the stem cell niche.

Most of these transcription factors are regulated at the posttranscriptional level by the presence of microRNAs (miRNAs) in the ESCs, often grouped as family or cluster. miRNA clusters are located on polycistronic transcripts and transcribed as a single primary precursor, whereas miRNA families consist in a collection of miRNAs sharing high-sequence homology. miRNAs are around 22 nucleotides in length and are present in the genome as independent transcription units or as clusters. Occasionally, miRNAs are located in the exonic/intronic sequences and regulated by the host gene transcription. miRtrons are referred to as miRNAs hosted in the intronic sequences of protein-coding genes. They are cotranscribed with the host gene or regulated by their own specific promoters, similar to those of protein-coding genes. However, the majority of characterized miRNAs are intergenic and often oriented antisense to neighboring genes. Therefore, they are suspected to act as independent units. In fact, the miRNA promoter consists of a core promoter containing binding sites for RNA polymerase and transcription factors, a proximal promoter containing regulatory binding sites for specific transcription factors and cytosine-phosphate-guanosine motif (CpG) islands for methylation and a distal domain for secondary regulatory elements.

Typically, miRNAs are able to bind and repress miRNAs through complementary base pairing, and miRNA-miRNA duplexes are generated with at least six consecutive nucleotides that undergo base pairing to establish a miRNA-miRNA duplex. The miRNA seed sequence, localized between nucleotides 2–8, is responsible to specifically bind the complementary sites of target miRNAs mostly located in the 3′ untranslated regions (3′-UTRs). miRNAs are transcribed by RNA polymerase II into primary miRNAs (pri-miRNAs) that are
several kilobases long and characterized by hairpin structures. RNA polymerase III is also able to generate pri-miRNAs although limited to the miRNA cluster of the human chromosome 19 among repetitive Alu sequences. The double-stranded specific endonuclease RNaseIII Drosha processes the pri-miRNAs into a 60–70-nt stem loop precursor (pre-miRNA) to be exported into the cytoplasm by nuclear transport receptor exportin-5 (Fig. 1).

The final 22-nt miRNA/miRNA* duplex is finally generated in the cytoplasm by the action of ds-RNaseIII Dicer. The guide strand at the 5’ end pairing is recruited by Argonaute proteins (AGOs) and incorporated into the RNA-induced silencing complex (RISC). The less-stable strand (miRNA* or passenger) is then degraded. After integration in the RISC complex, miRNAs are able to target specific mRNAs, although the entire process is still largely unknown (2, 4) (Fig. 1).

The core RISC components interact with several proteins responsible for RNA remodeling and the generation of foci known as processing bodies (P-bodies) or glycine-tryptophan bodies (GW-bodies) accountable for mRNA decapping, deacylation, and degradation (66). Recently, some authors reported that circulating HDL particles (59), exosomes (58), and liposomes (22) transport endogenous miRNAs to recipient cells for functional mRNA targeting in a non-cell autonomous manner. It has recently been reported that miRNAs can be regulated by long noncoding RNAs (19).

In the last decade several reports identified miRNAs as key players in ESC differentiation toward ectodermal, mesodermal, and endodermal cell types (15). In this review, we highlight the emerging role of miRNAs in ESC differentiation and describe the molecular mechanisms controlling miRNA expression and function during those particularly complex processes.

**miRNAs Regulating Early Differentiation**

When stem cells give rise to their progenies during differentiation, the transition at the cellular level closely resembles the developmental progression at the organismal level that is the progression from the inner cell mass to the three germ layers and eventually to fully functional tissues and organs. Embryonic development requires a precise spatial-temporal control of cell fate decision factors. Two main events are necessary to promote the switch from a pluripotent to a differentiating state: reduction of proliferation rate and cell lineage commitment. Cell-cycle control plays a key role during these events, and the contribution of miRNAs has been reported. Self-renewal capacity and pluripotency in ESCs are maintained by a shortening of their cell cycle that leads to a rapid cell division (23, 39, 50). At this stage, cell cycle is characterized by a truncated G1 phase, elevated expression of G1-associated cyclins, active cyclin-dependent kinases (CDKs), and low levels of inhibitory cell cycle proteins such as p21, p27, and p57 (5).

**Fig. 1. MicroRNA (miRNA) biogenesis.** miRNAs are transcribed as long transcripts [primary RNAs (pri-miRNAs)] by RNA polymerase (Pol) II or III, processed into hairpin RNAs [precursor RNAs (pre-miRNAs)] by Drosha and then exported to the cytoplasm by exportin. The distal domain contains secondary regulatory elements; the proximal promoter is responsible for specific transcription factor bindings as well as the core promoter in which the RNA polymerase binding sites are also located. Pre-miRNAs are further cleaved into a 22-nt miRNA/miRNA* duplex by Dicer. The weakest strand is loaded into the RNA-induced silencing complex (RISC) and functions as a guide for mRNA target recognition and final degradation.
As pluripotent cells adopt particular fates, lineage-specific genes are transcripationally activated. In addition, it is equally critical to suppress the expression of genes that would otherwise drive differentiation toward alternative fates or inhibit cell type-specific processes, so called disallowed genes (55). While this occurs at the transcriptional level, miRNAs also contribute to this process by clearing miRNAs expressed in a previous developmental phase.

Let-7. Studies on lin-4 and let-7 miRNAs provided the first evidence that miRNAs have essential roles in development. Let-7, the “antistemness” miRNA, promotes the exit from cell cycle by repressing CDK6, CDC25A, and CCND2 that control G1/S and G2/M cycle progression (33). Inhibition of let-7 production by Lin28 also has an important role in the maintenance of ESCs. In turn, upon activation, let-7 targets the 3’-UTR of some stemness factors, including c-Myc, Sall4, and Lin28, destabilizing the self-renewing capacity and promoting the differentiation of ESCs (35) (Fig. 3). Besides let-7, several other miRNAs control the expression of pluripotency markers during cell differentiation.

Others. Human ESC (hESC)-enriched miRNAs, including the miR-17 and -520 families, and the miR-371 cluster are rapidly downregulated in response to differentiation (49).

Differentiation of ESCs to embryoid bodies in mouse is correlated with G1 phase elongation, up to 6–8 h longer than as observed in adult cells (63). During differentiation of hESCs, P53 drives active expression of P21, which supports elongation of G1 phase and counteracts cell division. When activated, p53 targets miR-34a and miR-145, which inhibit the expression of several stem cell factors and antagonize pluripotency (Fig. 3). miR-145, in particular, is expressed at low levels in self-renewing hESCs but is substantially upregulated during differentiation (49). miR-145, in particular, is expressed at low levels in self-renewing hESCs but is substantially upregulated during differentiation (49). miR-145, in particular, is expressed at low levels in self-renewing hESCs but is substantially upregulated during differentiation (49).

miRNAs in Ectodermal Differentiation

Until the eight-cell stage of mouse development, blastomeres are totipotent and retain the ability to contribute to all embryonic and extraembryonic cell lineages (24, 65). At the late eight-cell stage, blastomeres increase cell-cell interactions to form a compacted morula. Subsequent asymmetric cell divisions produce two distinct cell populations: an outside population in contact with the environment and an inside population completely surrounded by the outside cells (25). As development proceeds to the blastocyst stage, the outside cell population becomes more epithelial-like and gives rise to the trophectoderm. The inside cell population forms the inner cell mass, which subsequently segregates into two populations: the epiblast (which gives rise to all the tissues of the embryo) and the primitive endoderm (65).

During trophectoderm development (between the morula and blastocyst stages of embryonic development), specification of trophectoderm in mouse ESCs is sustained by miR-297, miR-96, miR-21, miR-29c, let-7, miR-214, miR-125a, miR-424, and miR-376a. Those miRNAs are responsible of ecto-

<p>| Table 1. Summary of miRNA expression in germ layers derived from activated embryonic stem cell |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Germ Layers</th>
<th>Species</th>
<th>miRNAs</th>
<th>Target Genes</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Ectoderm</td>
<td>Human</td>
<td>miR-125</td>
<td>SMAD4</td>
<td>(7)</td>
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<tr>
<td></td>
<td></td>
<td>miR-30b, miR-30c</td>
<td>Embryonic ectoderm development protein (EED)</td>
<td>(48)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>miR-297, miR-96, miR-21, miR-29c, let-7, miR-214, miR-125a, miR-424 and miR-376a</td>
<td>All Ras and Mapk (p38 pathways)</td>
<td>(60)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>miR-200a,b,c, miR-141, miR-429</td>
<td>Snail</td>
<td>(16)</td>
</tr>
<tr>
<td>Endoderm</td>
<td>Human</td>
<td>miR-145</td>
<td>OCT4, SOX2, KLF4</td>
<td>(64)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>miR-10a</td>
<td>HOXA1</td>
<td>(57)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>miR-24</td>
<td>NOTCH1-MAPK14</td>
<td>(57)</td>
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<tr>
<td></td>
<td></td>
<td>miR-275*/</td>
<td>TIMM8A, MTPN, JAK2, CIQBP, UUSP1, ADISPOR1</td>
<td>(21, 57)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>miR-122*, miR-192*</td>
<td>CAT1, SIP1</td>
<td>(57, 21)</td>
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<td></td>
<td>Mouse</td>
<td>miR-93</td>
<td>Stat3</td>
<td>(13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>miR-338-5p, miR-340-3p</td>
<td>Members of Hdac and related pathways</td>
<td>(14)</td>
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<td></td>
<td></td>
<td>miR-302, miR-427</td>
<td>Lefty1, Lefty2 (Tgfβ/Nodal Smaud2/3)</td>
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<tr>
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<td>miR-145</td>
<td>OCT4, SOX2, KLF4</td>
<td>(64)</td>
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<td></td>
<td>miR-290</td>
<td>Dkk1 (affecting Wnt pathway)</td>
<td>(68)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>miR-200a,b,c, miR-141, miR-429</td>
<td>Snail</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>miR-93, miR-17-5p</td>
<td>Stat3</td>
<td>(13)</td>
</tr>
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miRNA, microRNA (miR); Hdac, histone deacetylase. *Liver or /pancreatic islet-specific miRNAs.
dermal progression by targeting key proteins of Ras and MAPK/p38 pathways (Fig. 2) (60).

In hESCs, the early ectodermal differentiation is controlled by miR-30b and miR-30c, which regulate the embryonic ectoderm development protein (EED) involved in (patho)physiological neural tube formation (48).

Functional analysis of several miRNAs involved in neural commitment showed an important contribution by two isoforms of miR-125 by regulation of SMAD4 (7).

**miRNAs in Endodermal Differentiation**

During early embryo development, cells migrate inward along the archenteron to form the inner layer of the gastrula, which then develops into the endoderm. A potential role for miR-338–5p and miR-340–3p has been reported. These miRNAs promote definitive endodermal differentiation in murine ESCs by direct involvement of histone deacetylase (Hdac) activity (14).

In 2008, Tzur and colleagues (57) compared miRNA expression in two different ESC lines (HES1 and HES2) during endodermal differentiation triggered by sodium butyrate, an hdac inhibitor. Comparative analysis showed a key role for miR-10a and miR-24. miRNA expression profiling also revealed the involvement of several liver-enriched miRNAs, including miR-122 and miR-192.

Further studies identified miR-375, implicated in pancreatic development, as an important player involved in commitment toward definitive endoderm by direct targeting of TIMM8A mRNA in differentiating hESCs (21).

**miRNAs in Mesodermal Differentiation**

During gastrulation, inward migrating cells contribute to the mesoderm, an additional layer between the endoderm and the ectoderm.

**miR-302 cluster.** A potential role for miR-302 cluster during gastrulation is suggested by its involvement in the specification of definitive endoderm in murine ESCs (14).

**miR-200 family.** Five members of the miR-200 family (miR-200c, miR-141, miR-200b, miR-200a, and miR-429) have been proposed to have a role in epithelial-mesenchymal transition (EMT), occurring at several stages of development, including gastrulation in mouse (Fig. 2 and Table 1). Members of the miR-200 family are counteracted by Snail to promote EMT and early mesoderm commitment at day 2 of ESC differentiation (16). Also a general cytoprotective effect of miR-210, mediated by reducing mitochondrial reactive oxygen species production in cardiomyocytes, has been described (38).

**miR-17 family.** miR-17 family members (miR-17–5p, miR-20a, miR-93, and miR-106a) are expressed during embryonic development and regulate cell differentiation by targeting 3’-UTR of specific mRNAs, such as signal transducer and activator of transcription 3 (13) (Fig. 2 and Table 1). Further
evidence in humans confirms the involvement of miRNAs in early mesoderm formation.

Others. Lee and colleagues (28) found an active role of miR-124 during gastrulation by in vitro analysis of human embryoid body differentiation. miR-124, which targets two key regulators of dynamic rearrangement of cytoskeleton and cell migrations (SLUG and IQGAP1), is highly expressed in hESC and gradually degraded during cell migration occurring in mesoderm development. In addition, it has been reported that miR-145 promotes mesodermal and ectodermal differentiation by inhibition of self-renewal and pluripotency factors, such as OSK (64) (Table 1). miR-302 promotes the mesendoderm lineage at the expense of neuroectoderm formation (44).

miRNAs Regulating Pluripotency

PSCs preserve their identity by promoting self-renewal and preventing differentiation. miRNAs have been highlighted as an integral part of the gene network that regulates self-renewal, pluripotency, and cell-type specification in ESCs. Indeed, Dicer1-null ESCs do not fully downregulate pluripotency markers and show alterations in the differentiation program (37). Also, when a second major miRNA-processing gene, DGCR8, is ablated in ESCs, similar defects in differentiation are observed (62).

Several miRNAs are expressed preferentially in ESCs and help maintain the ESC phenotype by rapid cell proliferation and cell-cycle progression. ESCs are defined by a specific miRNA signature, and the majority of these miRNAs are transcribed from two clusters in the human genome: the miR-371 and the miR-302 clusters.

miR-290/371 cluster. The miR-371 cluster is found within a 1,050-bp region on chromosome 19 and encodes miR-371, miR-372, miR-373, and miR-373* (52). In mouse, the ortholog of this cluster is the miR-290–295 cluster, encoding miR-290, miR-291a, miR-291b, miR-292, miR-293, miR-294, and miR-295. In ESCs, the overexpression of the miR-290 cluster withholds them from early differentiation and ensures their high proliferation rate (30). This effect is mediated by preventing an accumulation in G1 phase. The miR-290 cluster assists G1-S progression and also regulates the G2-M transition. The cell cycle regulators Weel1 and Fbxl5 have been identified in vitro as targets of miR-290–295 miRNAs (30). ESCs lack the G1 checkpoint in their cell cycle (8) and show very short G1 phase. A short G1 and a long S phase assist stem cells in maintaining their pluripotency. The G1/S transition in cells is regulated by two cyclin/Cdk complexes: cyclin D/Cdk4,6 and cyclin E/Cdk2. In ESCs, the cyclin D/Cdk4,6 complex is not present, whereas the cyclin E/Cdk2 complex is constitutively active. Mouse ESC-specific members of the miR-290 family promote G1/S transition by suppression of several key inhibitors of the cyclin E-Cdk2 pathway including Cdkn1a, Rbl2, and Lat2 (Fig. 3) (61). Members of the miR-290 cluster also inhibit ESC differentiation by targeting Dkk1, a Wnt pathway inhibitor, and increase pluripotency, thus preventing mesodermal formation (68). Many of the defects in Dicer-deficient cells can be reversed by transfection with miR-290 family miRNAs (47). Furthermore, some miRNAs are also regulators of epigenetic effects. De novo DNA methylation in differentiating mouse ESCs is controlled by the miR-290 cluster by targeting repressors of DNA methyl transferases (Fig. 3) (6, 47).

miR-302 cluster. The miR-302 gene, located within a 700-bp region on chromosome 4, encodes a cluster of eight miRNAs (miR-302b*-b-c*-c*-a*-a-d-367) that are specifically expressed in hESCs and embryonal carcinoma cells (52). In hESCs and iPSCs, miR-302 is the most predominant miRNA species (52). In ESCs, key transcriptional regulators such as Oct4, Sox2, Nanog, and Tcf3 specifically occupy the promoters of both active and silent miRNA genes to activate and repress their expression, respectively (34). Oct4 and Sox2 are required for the transcriptional regulation of miR-302 (Fig. 3) (9). The expression of miR-302a also promotes G1/S transition, mediated by repression of cyclin D1 (9). MiR-302b and miR-372 repress multiple target genes, with downregulation of individual targets only partially recapitulating the total miRNA effects. These targets regulate various cellular processes, including cell cycle, EMT, epigenetic regulation, and vesicular transport (Fig. 3) (51). It was suggested that the relative expression of miR-302b might serve as diagnostic indicator in defining the developmental state of embryonic cells and other stem cell lines, such as iPSCs (49). In mouse, miR-302b is expressed at higher levels in murine epiblast stem cells (mEpiSCs) compared with an earlier developmental state, mouse ESCs. Similarly, in hESCs and iPSCs, the miR-302 family is mildly downregulated when the cells regress to an earlier developmental state.

miR-520 cluster. Some species-specific miRNAs involved in hESC propagation, such as the miR-520 cluster, have been reported to be critical for cell proliferation and chromatin remodeling in hESCs (42, 49). The miR-520 cluster is a large miRNA cluster, located on chromosome 19, and contains 21 miRNAs (miR-518b, miR-518c, miR-519b, miR-519c, miR-520a, miR-520c, miR-520e, miR-520g, miR-524*, miR-515–5p, miR-517a, miR-517b, miR-517c, miR-519e, miR-520b,
miR-502d, miR-520f, miR-520h, miR-521, miR-525–3p, and miR-526b*). The miR-520 cluster members share a consensus 7-mer seed sequence with the miR-302 members and have many common targets (42).

miR-17 cluster. The miR-17 family consists of 14 mature miRNAs located on chromosomes 13, X, and 7 in humans (13). miR-17 family members, miR-17–5p, miR-20a, miR-93, and miR-106a, have been identified as miRNAs that are specifically expressed in undifferentiated or differentiating ESCs (13). The miR-17 cluster is amplified in lymphoma and solid tumors (40).

miRNAs for Reprogramming Differentiated Cells to PSCs

Several methods to reprogram somatic cells to iPSCs already exist, but they are hampered by various drawbacks. These include low efficiencies, high costs, genetic insertions, and the requirement of forced expression of one or more PSC transcription factors (17). Recent insights in miRNAs involved in pluripotency, however, have led to new and improved reprogramming methods.

miRNAs improving retroviral reprogramming. MIR-302/367 CLUSTER. Later, they also showed that addition of synthetic mimics of the miRNAs from the miR-302/367 cluster enhances retroviral reprogramming of human fibroblasts by OSK and OSKM (51). This cluster (consisting of miR-302a/b/c/d and miR-367) silences AOF2 (a lysine-specific histone demethylase), HDAC2 (histone deacetylase 2), and MECP1/2 (methyl CpG-binding proteins) activities. Together with a subsequent degradation of DNMT1 [DNA (cytosine-5)-methyltransferase 1], this ultimately results in total genomic DNA demethylation and H3K4 modification. These epigenetic reprogramming events induce expression of OCT3/4, SOX2, and NANOG, necessary for somatic cell reprogramming (29). miR-302/367 also functions through mesenchymal-epithelial transition (MET) by targeting TGF-β receptor and RHOC, a part of the RAC subfamily of the RHO family of GTPases (51).

OTHERS. MEFs obtained from miR-34a knockout mice are more susceptible to reprogramming, resulting in a 4.5-fold increase in reprogramming efficiency, and iPSC-like colonies appeared after 5 instead of 7 days (10). miR-34 is a major downstream transcriptional target of the tumor suppressor p53, which is known for repressing reprogramming (18). miR-34 does not repress cell proliferation but acts (at least in part) by direct suppression of the pluripotency genes Nanog, Sox2, and N-Myc. Another group of miRNAs, namely miR-93 and miR-106b, was shown to play a role in reprogramming (29). Both of them have a very similar seed sequence, and miRNA mimics improved the reprogramming efficiency of MEFs by retroviral transfection with OSK or OSKM four to six times. miR-93 and miR-106b directly target TGF-β receptor II. This receptor is partially responsible for the MET, a hallmark during the initial stage of reprogramming.

A library screen of murine miRNAs revealed a new family (miR-130/301/721) of miRNAs that is able to improve early phase reprogramming of MEFs (41). These miRNAs, all with the same seed sequence, target Meox2, a transcription factor interfering with Tgf-β and thereby promote the MET. MET is also induced by bone morphogenic protein (Bmp), which functions by inducing miR-205 and the miR-200 family of miRNAs. Indeed, the addition of Bmp7 or mimics of miR-200b and miR-200c (2 of its downstream miRNAs) was shown to synergize with the OSKM transcription factors to accelerate the progression through the initiation phase of reprogramming (45).

Previous discussed papers provide ample evidence of the great potential of miRNAs in somatic cell reprogramming. Nonetheless, above methods merely ameliorate the existing method of retroviral transduction of OSK and/or OSKM transcription factors. This leads to higher efficiencies but does not circumvent the other disadvantages.

Reprogramming using only miRNAs. MIR-302/367 CLUSTER. In 2008, Lin and colleagues (31, 32) discovered the possibility of reprogramming human skin cancer cells (and later human hair follicle cells) to iPSCs, by mere transfection of miRNAs from the miR-302/367 cluster, without the need of transcription factors.

Anokye-Danso and colleagues (1) demonstrated a similar result. Both mouse and human fibroblasts were solely reprogrammed by forced expression of the miR-302/367 cluster. The efficiency amounted up to nearly 10%, almost two orders of magnitude greater than with lentiviral transfection of OSKM and at much higher speed.

OTHERS. The approach used by Miyoshi et al. (36) completely circumvents vector-based gene transfer by direct transfection of mature double-stranded miRNAs in mouse and human cells. They used a combination of miR-200c, the miR-302 and miR-369 families. Despite the lower reprogramming efficiency, this methodology entails great benefits for clinical and translational applications, especially for large-scale reprogramming. In fact, the production of miRNA mimics is fairly easy and at relative low cost.

The amount of miRNAs known to be involved in iPSC reprogramming is already significant; however, it seems that only a tip of the iceberg has been unveiled with new discoveries being published in fast succession. Protocols involving miRNAs, completely steering clear of viral reprogramming and transcription factors, will make it possible to generate iPSCs on a much larger scale. The lack of genomic insertions makes it possible to consider these cells for use in a clinical setting.

Open Questions and Future Directions

Although miRNAs are definitely relevant for the transcriptomic changes occurring in ESC differentiation, the exact contribution of each of the individual miRNAs is still questionable. The understanding of such regulation could also be
informative for ESC handling and directing to a specific cell fate.

Since the miR-290 cluster in mouse ESCs is relatively well known, it could be interesting to see whether the homologous human clusters, miR-371/2/3 and miR-302, could have similar target miRNAs and effects.

In addition, this information could be useful to verify potential roles for those miRNAs in regulating the cell cycle of adult multipotent stem cells.

The regulation of miRNA expression during ESC differentiation and in the terminally differentiated cell types are also open questions (20).

Furthermore, manipulating the expression of pluripotent-related miRNAs, as miR-290 and miR-302 clusters known to be active on TCF3, NANOG, SOX2, and OCT4 promoters pave potential clinical applications. In fact, overexpression of these miRNAs could be useful for the expansion of adult stem cells while their inhibition could be relevant for controlling tumor progression. Finally, since our group recently identified a miRNA family involved in muscle lineage switch of adult stem cells (11, 12), it could be interesting to verify whether this phenomenon can be reproduced in pluripotent cell types.

Conclusion

PSCs hold tremendous promise in regenerative medicine applications since they univocally are capable to differentiate in any cell type and can be easily expanded in large amounts. ESC self-renewal and differentiation depend on soluble signals, which activate pathways responsible for changes in gene transcription signature and cell differentiation state. In addition, cell-cell contact is also a critical parameter, since at low cell densities cell growth rates diminish, whereas at high cell densities spontaneous differentiation occurs. In this staggering scenario, miRNAs seem to have a prominent role in complex molecular mechanisms responsible for ESC self-renewal and differentiation.

Discovering novel pluripotency genes and stem cell pathways regulated by miRNAs will be the future directions in this scientific area. It is likely that some of those genes and pathways will be relevant to overcome hurdles facing the use of ESCs, such as the lack of reliable methods to differentiate ESCs to desired cell types in large amount.

Molecular and cellular mechanisms promoting miRNA processing, localization, and turnover are not well understood, and more basic research and preclinical investigation are required to move a step forward for future clinical application of miRNAs in PSC technology.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

E.B., L.T., and M.S. prepared figures; E.B., M.P., L.T., and M.S. drafted manuscript; E.B., M.P., L.T., and M.S. approved final version of manuscript; L.T. and M.S. edited and revised manuscript.

REFERENCES

MicroRNAs and stem cells: control of cell cycle phase distribution in mouse embryonic stem cells. The miR-290–295 cluster promotes pluripotency maintenance by regulating cell cycle pathways and exerts cytoprotective effects.

Doki Y, Mori M.


