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Dissecting microRNA-mediated regulation of stemness, reprogramming, and pluripotency

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Abstract

Increasing evidence indicates that microRNAs (miRNAs), endogenous short non-coding RNAs 19–24 nucleotides in length, play key regulatory roles in various biological events at the post-transcriptional level. Embryonic stem cells (ESCs) represent a valuable tool for disease modeling, drug discovery, developmental studies, and potential cell-based therapies in regenerative medicine due to their unlimited self-renewal and pluripotency. Therefore, remarkable progress has been made in recent decades toward understanding the expression and functions of specific miRNAs in the establishment and maintenance of pluripotency. Here, we summarize the recent knowledge regarding the regulatory roles of miRNAs in self-renewal of pluripotent ESCs and during cellular reprogramming, as well as the potential role of miRNAs in two distinct pluripotent states (naïve and primed).

Keywords: miRNAs, Embryonic stem cells, Pluripotency, Reprogramming, Self-renewal

Background

MicroRNAs (miRNAs) are endogenous short non-coding RNAs 19–24 nucleotides in length that regulate gene expression at the post-transcriptional level [1]. The first miRNA was identified in *C. elegans* by Lee and colleagues [2], who demonstrated that the lin-4 miRNA downregulated the expression of the LIN-14 protein via an antisense RNA-RNA interaction [2]. Since the term miRNA was coined in 2001 [3], numerous miRNAs have been identified in various organisms from plants to mammals. Further, miRNAs are evolutionarily conserved and are thus recognized as one of the essential regulators in the control of many different processes including development, homeostasis, and metabolism [4]. In addition, aberrant miRNA expression is involved in several diseases including cancer and chronic obstructive pulmonary disease [5, 6]. Because each miRNA targets a large number of mRNAs and multiple miRNAs can bind to one specific mRNA, the potential impact of miRNAs on the expression of a large number of proteins and on transcriptome regulation is increasingly being investigated to determine the crucial role of miRNAs in various biological events.

Recent findings have revealed that molecular mechanisms underlying the maintenance of embryonic stem cell (ESC) pluripotency and cellular reprogramming have been linked to miRNAs [1, 7]. ESCs are pluripotent cell lines derived from the inner cell mass of blastocysts [8, 9] and are characterized by two major properties that define them: an unlimited self-renewal capacity in vitro and pluripotency. Also, ESCs are able to form all three germ layers and give rise to all cell types in the tissues of the body [8, 9]. Due to these important properties, ESCs represent a valuable tool for disease modeling, drug discovery, developmental studies, and potential cell-based therapies in regenerative medicine [10, 11]. A complex set of intrinsic and extrinsic factors regulate the balance between self-renewal and lineage commitment in ESCs [12]. However, several aspects regarding the proliferation and differentiation of ESCs at the post-transcriptional level remain unknown. Numerous studies have described the

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expression of unique clusters of miRNAs in ESCs including miRNAs in the human and mouse miR-302 clusters, the mouse miR-290 cluster, and the human miR-371 cluster [7, 13]. It has been definitively predicted that miRNAs may be a valuable means to regulate the proliferation and the differentiation of ESCs. Here, we have reviewed the recent discovery of miRNAs in ESCs and ESC-like stem cells and their role in the regulation of self-renewal and during cellular reprogramming.

Biogenesis and biological action of microRNAs (miRNAs)

MiRNAss regulate gene expression at the post-transcriptional level by binding to the 3′-untranslated regions (3′ UTRs) or the open reading frames of target genes, resulting in the degradation of target mRNA or the inhibition of mRNA translation [1, 4]. MiRNAs represent ~4 % of the genes in the human genome and regulate the expression of more than one third of the protein-coding genes at the post-transcriptional level [14]. Gene expression is controlled by mRNA sequestration, translation repression, or miRNA-mediated mRNA decay [15]. Approximately half of miRNA genes are located in intergenic regions and can be controlled from their own promoters or as polycistronic clusters from a shared promoter, whereas the remaining miRNAs are embedded within protein-coding genes and are co-transcribed with their host genes or from miRNA-specific promoters [1, 4, 16]. Mature miRNAs are generated by multiple sequential endonucleolytic cleavage steps. The microprocessing complex consists of the RNAse III-like enzyme Drosha and its cofactor DiGeorge syndrome critical region gene 8 (DGCR8) [17–22]. Pre-miRNAs are further cleaved by Dicer, an RNAse III enzyme, which gives rise to a double-stranded RNA 22–24 nucleotide comprised of the mature miRNA (guide strand) and the miRNA passenger strand [23, 24]. Subsequently, the double-stranded mature RNA with a less thermodynamically stable 5′-end (the guide strand) is recruited by Argonaute proteins (AGOs) and is loaded into the RNA-induced silencing complex (RISC) to act as an miRNA [25, 26]. The RISC acts as an effector that facilitates miRNA-dependent silencing via binding of miRNAs to the 3′ UTR of the target mRNA transcript based on complementarity between the miRNA and the miRNA target. Nucleotides 2–8 (from the 5′ end) of the mature miRNA ("seed region") are crucial for target identification, as perfect complementarity guides the miRNA-induced degradation of the target mRNA through AGO2 endonuclease activity [27, 28]. Partial pairing results in repression of the target mRNA translation at the initiation step, or in sequestration of the target mRNAs into cytoplasmic processing bodies, which happens by engaging poly(A) nucleases to degrade mRNA through deadenylation pathways [29].

Embryonic stem cell (ESC)-specific microRNAs (miRNAs)

The molecular basis of miRNAs for mouse ESCs (mESCs) was initially demonstrated in mESCs lacking Dicer and Dgcr8 [30–32]. Although Dicer- or Dgcr8-deficient mESCs are viable, Dicer or Dgcr8 loss compromises the biogenesis of miRNA and causes severe defects in the proliferation and differentiation of mESCs. Furthermore, Dicer- and Dgcr8-deficient mESCs fail to generate detectable teratomas and chimeric mice when subcutaneously injected into nude mice and into blastocysts [30, 33]. These findings demonstrated the importance of miRNA synthesis in mESC pluripotency and during early embryonic development [34].

In parallel with studies on the role of essential factors for miRNA biogenesis in ESCs, the identification of ESC-specific miRNAs has been explored in mESCs and human ESCs (hESCs) to understand the post-transcriptional regulation of genes related to the self-renewal and pluripotency of ESCs [34]. Several techniques including cloning, qPCR, microarray, and deep sequencing have been employed to examine the expression of miRNAs in undifferentiated ESCs and their differentiated counterparts. Interestingly, only a few ESC-enriched miRNAs are transcribed and are unique to ESCs, whereas other miRNAs are widely expressed but decrease dramatically during differentiation. Thus, ESCs are comprised of a unique set of ESC-specific miRNAs [7, 35–37].

A large portion of these ESC-specific miRNAs constitute two clusters: the miR-290 cluster in mice and their human homologs in the miR-371-373 family and the miR-302-367 cluster in both mice and humans [7]. Later, Suh et al. identified several novel miRNAs from an undifferentiated human ESC cDNA library belonging to the miR-302 and miR-371 clusters [13]. In comparison with Houbaviy's data set, there are three common miRNAs (miR-296, miR-301, and miR-302) between the mESCs and hESCs data sets. Taken together, these findings strongly suggest that combinatorial regulation of ESC-specific miRNAs and their target networks plays a critical role in the maintenance of ESC pluripotency and in the regulation of early embryonic development.

Role of embryonic stem cell (ESC)-specific microRNAs (miRNAs)

Substantial evidence has shown that the miRNAs clusters miR-302, miR-209, and miR-371 represent key regulators of pluripotent stem cells [34, 38]. All of these miRNAs clusters play critical roles in cellular processes, such as maintaining pluripotency, proliferation, and differentiation, which are the characteristics of stemness. Recent findings suggest that ESC-specific transcription factors regulate ESC-specific miRNAs and, in turn, these