Cell Stem Cell
Previews

MicroRNA Regulation of Stem Cell Fate

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DOI 10.1016/j.stem.2008.02.008

MicroRNAs modulate target gene expression and are essential for normal development, but how does this pathway impact cell fate decisions? In this issue of *Cell Stem Cell*, Ivey et al. (2008) find that muscle-specific microRNAs repress nonmuscle genes to direct embryonic stem cell differentiation to mesoderm and muscle.

Pluripotent embryonic stem (ES) cells are capable of differentiating to all possible cell types. This unique property promises future medical applications, but to fulfill this potential it will be necessary to control ES cell differentiation. Much progress has been made toward understanding the role of signaling pathways and transcription factors for these cell fate choices, yet our current understanding remains incomplete. Until now, the role of individual microRNAs (miRNAs) remained largely unaddressed. miRNAs comprise a large family of regulatory RNAs with critical roles in diverse developmental and physiological pathways (Kloosterman and Plasterk, 2006). Sequential cleavage of long primary transcripts by the Microprocessor and Dicer complexes yields mature ~22 nucleotide miRNAs that direct messenger RNA cleavage or translational repression (Eulalio et al., 2008). Mouse ES cells lacking miRNAs are unable to downregulate pluripotency markers after induction of differentiation and retain the ability to produce ES cell colonies (Kloosterman and Plasterk, 2006). Therefore, an important question is how individual miRNAs contribute to cell differentiation. In this issue, Ivey and colleagues demonstrate a new role for miRNAs in regulating ES cell differentiation. They find that miR-1 and miR-133 direct mesoderm formation and regulate differentiation to cardiac muscle by suppressing gene expression of alternative lineages (Ivey et al., 2008).

Ivey et al. began by identifying miRNAs that are enriched in cardiac progenitors derived from in vitro-differentiated mouse ES cells. Using fluorescence-activated cell sorting (FACS) they isolated purified cell populations expressing a β-myosin-driven green fluorescent protein (GFP) and performed miRNA expression profiling using microarrays. Comparison of GFP^+ and GFP^- cells collected from differentiated embryoid bodies (EBs) revealed nine miRNAs enriched in cardiac progenitors and undetectable in undifferentiated ES cells. Among this list were miR-1 and miR-133, muscle-specific miRNAs that are important for heart development and physiology (Chen et al., 2006; Zhao et al., 2007). Next, to investigate the function of miR-1 and miR-133 in ES cell differentiation the authors used lentiviruses to introduce these miRNAs and derived stable ES cell lines expressing the individual miRNAs. Interestingly, expression of either of these miRNAs alone was insufficient to drive ES cell differentiation. However, during embryoid body formation, expression of either miR-1 or miR-133 led to dramatically increased levels of early mesoderm markers. Importantly, this increase in mesoderm gene expression was due to an increased number of expressing cells as well as increased levels per cell, suggesting that both miRNAs promote mesoderm formation. However, whereas miR-1 also promoted further differentiation to cardiac and skeletal muscle, expression of miR-133 had an inhibitory effect on myogenesis. Therefore, these in vitro ES cell differentiation assays largely recapitulate several aspects of miR-1 and miR-133 function in vivo (Chen et al., 2006; Zhao et al., 2007). One major conundrum is that miR-1 and miR-133 have opposing roles in cardiac development, yet they are processed from the same primary transcript. Therefore, unless unidentified mechanisms exist to selectively regulate the biogenesis of these miRNAs, they should be coexpressed in individual cells. It will therefore be important to identify the mechanisms that can explain this apparent discrepancy.

How does expression of these miRNAs enhance mesoderm formation? One possibility is that they could increase the proportion of cells expressing mesodermal mRNAs by suppressing differentiation into alternative lineages. To test this hypothesis, the authors compared gene expression profiles of wild-type and miR-1- and miR-133-expressing cells by microarray analyses. Expression of markers associated with endoderm specification and differentiation was significantly lower in the miRNA-expressing cells. Interestingly, markers associated with neuroectoderm specification were upregulated with a corresponding decrease in markers of differentiated neurons. Ivey et. al further examined the repression of neuroectoderm differentiation in a more in vivo setting using teratoma formation assays. Indeed, compared to control teratomas, those derived from miR-1- and miR-133-expressing ES cells had more neuronal progenitor cells, but were dramatically depleted of differentiated neuronal cells. The physiological relevance of promoting neuronal, but not endoderm, progenitor cells by these muscle-specific miRNAs is not clear, but it certainly warrants further investigation. Nevertheless, it is clear that both miRNAs suppress cell differentiation into nonmuscle lineages. Furthermore, both miRNAs seemed to have comparable functions in human ES cells.

Because Notch signaling can promote neural differentiation and inhibit muscle differentiation in ES cells (which is opposite to the effects of miR-1 expression), together with their previous identification of the Notch ligand *delta* as the major effector of miR-1 function in flies, Ivey

et al. asked whether mouse *delta* homologs are also under the same control. They showed that among three mouse homologs of *delta*, only Dll-1 is regulated by miR-1. Importantly, ablation of Dll-1 by shRNA mimicked the effects of ectopic miR-1 expression in ES cells, such that EBs formed from Dll-1-depleted ES cells had a greater propensity toward cardiomyocyte differentiation.

How do miRNAs control ES cell self-renewal and differentiation? First, though a few ES cell-specific miRNAs have been identified, their function awaits elucidation (Houbaviy et al., 2003). However, miRNA-deficient ES cells exhibit delayed cell-cycle progression, suggesting that certain miRNA(s) may be important for ES cell proliferation (Kloosterman and Plasterk, 2006). Second, miRNAs can initiate differentiation. For example, ectopic let-7 expression causes cancer stem and progenitor cells to differentiate, while let-7 depletion enhances self-renewal of those cells (Ibarra et al., 2007; Yu et al., 2007). Because let-7 levels increase upon ES cell differentiation, it is likely that let-7 also promotes ES cell differentiation. Finally, Ivey et al. demonstrated that tissue-specific miRNAs direct differentiation toward corresponding lineages by suppressing alternative cell fates (Figure 1). This concept may be widely applicable, because it was previously reported that ectopic expression of muscle (miR-1)- and brain (miR-124)-specific miRNAs in HeLa cells caused global shifts in gene expression toward patterns observed in the appropriate tissues (Lim et al., 2005). A comprehensive profiling of miRNA expressions in different tissues has recently been completed (Landgraf et al., 2007). It will be of great interest to determine whether other tissue-specific miRNAs function in a similar fashion to direct differentiation into corresponding lineages. Perhaps, utilizing the right combination of miRNAs to reinforce cell fate decisions will facilitate the generation of homogeneous cell populations of desired lineages from ES cells, and may be therapeutically exploited.

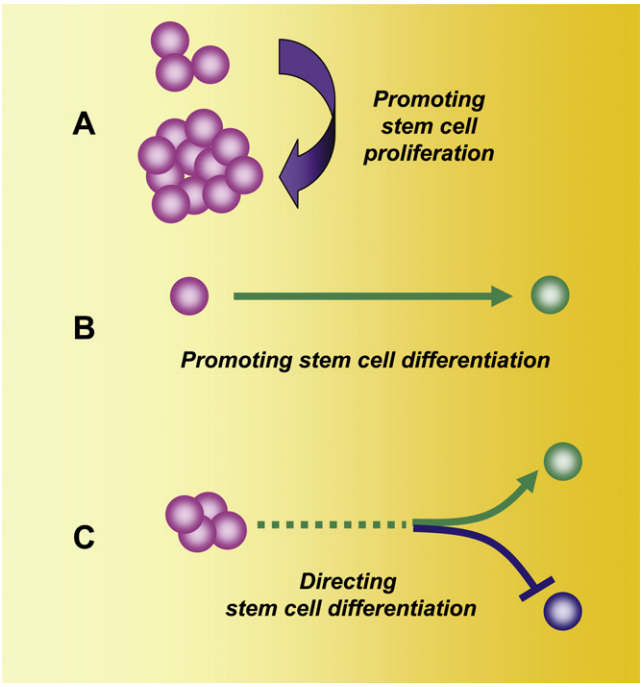

Figure 1. Potential Roles of Different miRNAs in Embryonic Stem Cell Self-Renewal and Differentiation

(A) Though not essential for ES cell viability, miRNAs are important for normal cell-cycle progression and stem cell proliferation. The particular miRNA or miRNAs important for this regulation have yet to be defined.

(B) The developmentally regulated let-7 family miRNAs promote stem cell differentiation and may act by repressing expression of the self-renewal machinery.

(C) miR-1 and miR-133 do not initiate ES cell differentiation but rather direct cell fate choices through the repression of alternative cell lineage gene expression.

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