CHAPTER 8

MicroRNA REGULATION OF EMBRYONIC STEM CELL SELF-RENEWAL AND DIFFERENTIATION

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Abstract: Stem cell differentiation requires a complex coordination of events to transition from a self-renewing to a differentiated cell fate. Stem cells can be pluripotent (capable of giving rise to all embryonic lineages), multipotent (possessing the potential to give rise to multiple lineages) and unipotent (capable of given rise to a single cell lineage). Regardless of their potency all stem cells must silence their self-renewal program during differentiation. The self-renewal program can be defined as the integration of external and internal stimuli that enables a cell to proliferate while maintaining its potency. Two hallmarks of the self-renewal program are a self-reinforcing transcriptional network and a specialized cell-cycle profile. In this chapter we discuss the impact of various microRNAs (miRNAs) to either reinforce or inhibit the self-renewal program of stem cells and how this added regulatory layer provides robustness to cell-fate decisions. We will focus on embryonic stem cells (ESCs) describing miRNA function in self-renewal, differentiation and de-differentiation. We will compare and contrast miRNA functions in ESCs with miRNA function in lineage specific somatic stem cells and in cancer.

INTRODUCTION: THE SELF-RENEWAL PROGRAM

The stem cell self-renewal program in both embryonic and somatic stem cell populations functions to maintain potency during successive rounds of replication. The degree of potency and proliferative rate vary greatly among stem cell populations in accordance with the evolutionary pressures and biological functions of these populations.

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ESCs are derived from the inner cell mass of the developing blastocyst and resemble cells of the developing epiblast. The epiblast gives rise to the embryonic endoderm, mesoderm and ectoderm, as well as the germ lineage and hence is pluripotent.[1] Epiblast cells have a rapid cell cycle. However, they eventually differentiate at which time the cell cycle extends. Like the epiblast cells, ESCs have a rapid cell cycle and are pluripotent. However, unlike epiblast cells, ESCs can self-renew indefinitely in the culture dish.

During embryonic development, the epiblast cells differentiate into specialized fetal stem cell populations that have a more limited potency. These include, among others, the fetal neural stem cells and hematopoietic stem cells. These fetal stem cells retain a high proliferative rate but possess a limited potency.[2,3] Eventually, the fetal stem cells are replaced by adult lineage specific stem cells including adult counterparts of the fetal hematopoietic and neural stem cells. The adult stem cells also have a limited potency, but unlike their fetal counterparts, typically have a slow proliferative rate. In fact, adult somatic stem cell populations are largely quiescent, although they generate transient populations of progenitor cells, which typically have a rapid proliferative rate more like that of their fetal stem cell counterparts. Quiescence in adult stem cells may have evolved to reduce the chance of harmful mutations, such as those that cause cancer.[4]

EMBRYONIC STEM CELLS

The molecular basis of the stem cell self-renewal program has been best studied in ESCs. In these cells the self-renewal program is determined by the interaction of numerous factors at the center of which is a distinct transcriptional network.[5] In ESCs, the central transcriptional network includes the transcription factors Oct4, Sox2, Nanog, Tcf3 and the Myc family of proteins (cMyc and nMyc). The coordinated actions of these transcription factors both directly and indirectly determines an epigenetic state that is poised to activate or repress upon differentiation the transcription of genes of any lineage of the three germ layers.[5] In this way the ESC transcriptional network enables its pluripotency. Additionally the ESC transcriptional network drives expression of factors that enable the cell's high proliferative rate by directly and indirectly maintaining the short ESC cell cycle.

With the induction of ESC differentiation, the many components of the self-renewal program must be shut off and a new differentiated program must be activated. Therefore, this cell fate transition is regulated by factors that both silence self-renewal and induce a lineage specific differentiation program. These factors can be classified broadly as those that influence gene expression at the level of chromatin state, transcription, transcript stability, protein translation, protein stability, or protein function.

In this chapter, we will focus on the pro-self-renewal and pro-differentiation functions of miRNAs.

miRNA BIOGENESIS AND FUNCTION

miRNAs are small noncoding RNAs which act to posttranscriptionally silence gene expression through translational inhibition and mRNA destabilization. miRNAs

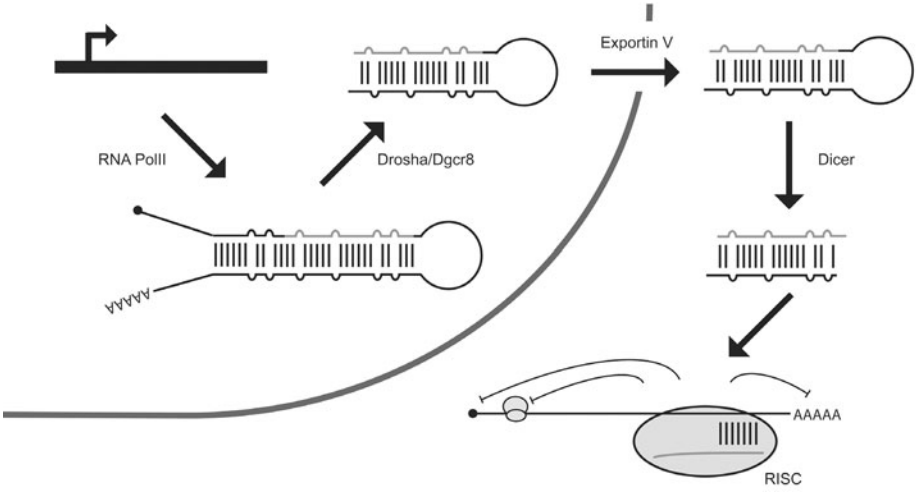

Figure 1. miRNA biogenesis. miRNAs are first transcribed as long RNA polymerase II transcripts. The hairpin structure of these transcripts is recognized by the Microprocessor complex composed of Drosha and Dgcr8 and is cleaved to form a smaller pre-miRNA hairpin. The pre-miRNA is exported from the nucleus and subsequently cleaved by Dicer to form a mature miRNA duplex. A single strand of this duplex is loaded into the RISC complex. The miRNA loaded complex destabilizes and inhibits translation of its target mRNAs.

are generated through the sequential processing of RNA transcripts (Fig. 1). miRNAs are first transcribed as long RNA polymerase II transcripts termed primary miRNAs (pri-miRNAs).[6,7] These pri-miRNAs can be either noncoding or coding. In the latter case, miRNAs will often reside within the intron of a coding gene.[8] In the nucleus, the pri-miRNA is recognized and cleaved by the microprocessor complex, which consists of the RNA binding protein DGCR8 and the RNAse III enzyme DROSHA.[9-13] This complex recognizes a stem loop structure of approximately 33 base pairs in length and posses an enzymatic activity that cleaves the loop 11 base pairs from its base leaving a characteristic 2 nucleotide 3′ overhang.[14] The processed RNA, now termed pre-miRNA, is exported from the nucleus to the cytoplasm by Exportin V where it is recognized by a second complex containing the RNAse III enzyme DICER.[15-18] This complex recognizes the pre-miRNA hairpin and cleaves it at the base of the hairpin loop again to form a 2 nucleotide 3′ overhang to generate an approximately 22 nucleotide mature miRNA duplex.[18] This mature duplex remains double-stranded until it is incorporated into the RNA-induced silencing complex (RISC). Only a single strand of the small RNA duplex is incorporated, typically the strand with the less stable 5′ end.[19]

miRNAs which are loaded into the RISC complex directly interact with their mRNA targets through base pairing to sites in the open reading frame and 3′ untranslated region. These interactions depend on base pairing of a 6-8 nucleotide seed sequence of the miRNA (nucleotides 2-8 on the 5′ end) with the mRNA target.[20] The RISC complex which is bound to target mRNAs disrupts protein production through a variety of mechanisms including disruption of ribosome initiation via interacting with the 5′ cap, prevention of ribosome elongation and promotion of RNA degradation by shortening of the polyA tail.[21]

ESCC miRNAs PROMOTE SELF-RENEWAL

Many miRNAs are co-expressed from a single transcript. One such group is the miR-290 cluster, which consists of 7 miRNAs and is highly expressed in mouse ESCs. A subgroup of the miR-290 cluster miRNAs share a common seed sequence and regulate the ESC cell cycle and, therefore, have been coined the ESCC family (ESC cell cycle promoting miRNAs).[22] Related families to the ESCC miRNAs include the miR-302 family and the mir-17/20/106 family, although the later family has a slightly different seed sequence. The ortholog of the miR-290 cluster in humans is the slightly diverged miR-370 cluster while the miR-302 clusters in mouse and human are very similar.[23,24]

The common expression of similar miRNAs in pluripotent stem cells in mouse and human suggests an important functional role in ESC self-renewal. Indeed, the first evidence for such a function was uncovered in ESC miRNA knockout models through deletion of either *Dicer* or *Dgcr8*.[25-27] These ESCs have a slowed proliferation rate and an altered cell cycle profile with an extended G1 phase.[27] These findings are particularly interesting considering that wild-type mouse ESCs are characterized by an atypical cell cycle with a abbreviated G1 phase compared to somatic cells.[28] That is, these initial findings suggested that the ESC expressed miRNAs suppress the somatic cell cycle structure.

The abbreviated G1 phase of ESCs promotes their rapid proliferation and is, at least in part, secondary to an alleviation of the G1/S restriction point.[28] In a typical somatic cell, the G1/S restriction point prevents the initiation of S phase and DNA replication. The G1/S restriction point includes a complex series of signaling events, which must reach a threshold before transitioning into S phase. Key molecular components of this reaction include, but are not limited to, the cyclins, the cyclin dependent kinases (CDKs), cdk inhibitors (CKIs), the Rb family of proteins and the E2F family of proteins.[29]

D and E type cyclins in complex with CDKs drive phosphorylation of the Rb family of proteins.[30] In mouse ESCs, CyclinE is expressed at high levels independent of cell cycle phase whereas CyclinD is not expressed.[31] CyclinE complexes with CDK2, to initiate the phosphorylation and subsequenct inactivation of the Rb family of proteins (pRb, P107 and P130). The Rb family of proteins, when in a hypophosphorylated active state, sequester activating E2Fs (E2F1-3) as well as activate repressive E2F proteins (E2F4 and 5) preventing transcription of S phase genes.[30] When Rb proteins are hyperphosphorylated and inactivated, they no longer activate the repressive E2Fs. Simultaneously, the suppression of the activating E2Fs is relieved, which allows them to drive transcription of S phase genes. Progression to S phase can be blocked by CDK inhibitors, which include members of the CIP and INK families. These inhibitors block activity of CDK/Cyclin complexes.[32] INK family inhibitors are nonfunctional in mouse ESCs as they act through CyclinD, which is not expressed at high levels. CIP family inhibitors, however, are more promiscuous in their inhibitory effects on CDK/Cyclin complexes and are able to bind and inactivate CDK2/CyclinE complexes.[32] In mouse ESCs, CIP family inhibitors are expressed at low levels, as are the Rb proteins.[28,31]

By screening miRNAs, which enhance proliferation in a *Dgcr8* knockout (–/–) ESC background, the role of ESCC miRNAs in cell cycle control was uncovered. These miRNAs not only accelerate proliferation of *Dgcr8* $^{-/-}$ ESCs, but also decrease the number of cells in the G1 phase of the cell cycle. This effect on the G1 phase is in part through direct miRNA targeting of the CIP family CDK inhibitor P21, LATS2 and some of the Rb family of proteins including pRb and P130. Through inhibition of these and other

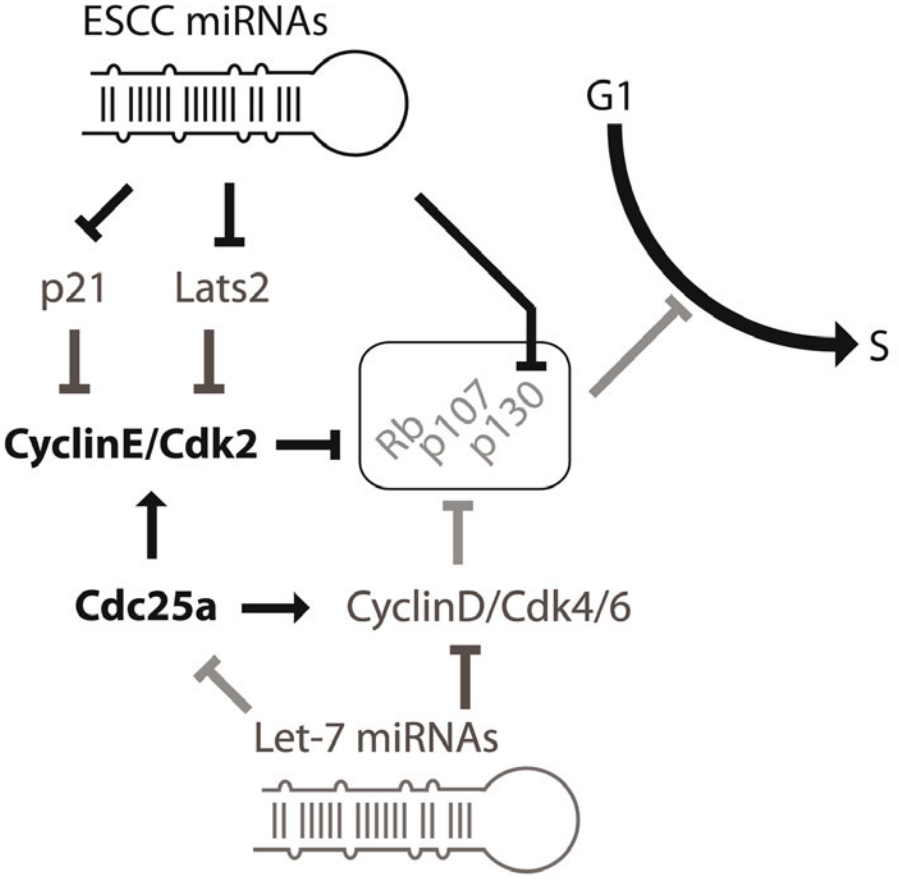

Figure 2. Let-7 and ESCC miRNAs have opposing effects on the G1-S transition. This figure represents a model of the direct inhibitory effects of the ESCC and let-7 miRNAs on factors involved in the ESC G1-S transition. As ESCs transition from a self-renewing to a differentiated state, the ESCC miRNAs are down-regulated and the let-7 miRNAs are upregulated. These changes have direct consequences on the cell cycle. Dark/bold arrows, lines and text indicate interactions, miRNAs and proteins that are up-regulated in the ESC state. Grey arrows, lines and text indicate interactions, miRNAs and proteins that are down-regulated in the ESC state. Note the interactions and functional consequences of the let-7 miRNAs on cell cycle have been tested in various somatic cell populations, but not ESCs.

predicted miRNA targets involved in the G1 phase, the ESCC miRNAs promote the ESC cell cycle (Fig. 2).[22]

Recently the impact of the ESCC miRNAs on the ESC transcriptome was analyzed in depth. It was discovered that the ESCC miRNAs indirectly activate cMyc expression.[33] Myc is a transcription factor that both promotes proliferation and is required for ESC self-renewal.[34,35] Additionally, in ESCs inhibition of Myc proteins promotes loss of ESC self-renewal, while enforced expression of cMyc prevents loss of self-renewal in the absence of LIF.[35] Lin et al recently sought to identify the mechanisms by which Myc proteins promote ESC self-renewal. In particular they found that cMyc drives transcription

of numerous pro-self-renewal miRNAs including miR-141, miR-200 and miR-429. These miRNAs promote the maintenance of self-renewal in the absence of LIF although the biological mechanisms underlying these effects remain unknown.[36] Furthermore, cMyc regulates expression of the ESCC miRNAs forming a positive feedback loop as described below.

A number of other factors have been identified as indirectly upregulated by the ESCC miRNAs including the DNA methyl transferases (DNMT3a and b).[37,38] The increase in expression of these DNA methyl transferases is required to maintain appropriate DNA methylation in sub-telomeric regions, which in turn is required to prevent abnormal telomere elongation.[37] The regulation of DNMT3a and b occurs via ESCC targeting of P130—a negative regulator of DNMT3a and b transcription.[37,38] In addition to the DNA methyl transferases, a number of other pluripotency associated transcripts are indirectly upregulated by the ESCC miRNAs. These include Lin28, Trim71 and Sall4.[33] Together these numerous molecular changes induced by the ESCC miRNAs have a profound effect on promoting the cell cycle and preserving faithful maintenance of telomeres to ensure proper ESC self-renewal and maintenance of pluripotency.

miRNAs INDUCED DURING ESC DIFFERENTIATION SUPPRESS THE SELF-RENEWAL PROGRAM

As miRNAs are suited to stabilizing the self-renewing state, so are they well situated to promote the transition from self-renewal to differentiation. MicroRNAs, which silence self-renewal, can be categorized by their targets and by their expression patterns. A small number of miRNAs have been found to directly target components of the central ESC transcriptional network.[39-41] These same miRNAs are induced rapidly during ESC differentiation down specific lineages. A second class of microRNAs is induced during differentiation down a broad set of lineages and broadly suppress ESC associated genes but not the central ESC transcription factors themselves.[33] They also promote a somatic cell cycle.[42-44] These two classes of pro-differentiation miRNAs likely play distinct roles in the differentiation process. The first class of microRNAs directly suppress ESC self-renewal state, while the second class of microRNAs predominantly stabilize the differentiated state—much like the ESC microRNAs stabilize the ESC state.

MiRNAs miR-134, miR-296 and miR-470 have been discovered to directly suppress Nanog, Pou5f1 (also known as Oct4) and Sox2 in mouse ESCs.[40,41] These miRNA-target interactions occur predominantly through interactions in the open reading frame. These miRNAs are highly upregulated during retinoic acid (RA) induced differentiation, which induces predominantly neural differentiation suggesting that these miRNAs may be involved in lineage specific silencing of ESC self-renewal. In human ESCs, miR-145 was found to directly suppress ESC self-renewal via targeting Oct4, Sox2 and Klf4.[39] Understanding the biological functions and relative in vivo contributions of various direct miRNA suppressors of self-renewal will be an important area of future pursuit.

In contrast to the miRNAs which directly suppress ESC self-renewal, the let-7 family of miRNAs are stabilizers of the differentiated cell fate.[33] Mutations in let-7 were first discovered in C. elegans in a mutagenesis screen for genes that prevented terminal differentiation of seam cells in the hypodermis.[45] Since the discovery of let-7 in C. elegans, homologues of let-7 have been found in all metazoans studied.[46] In mouse and human there are 9 distinct let-7 family members with varied tissues specific expression

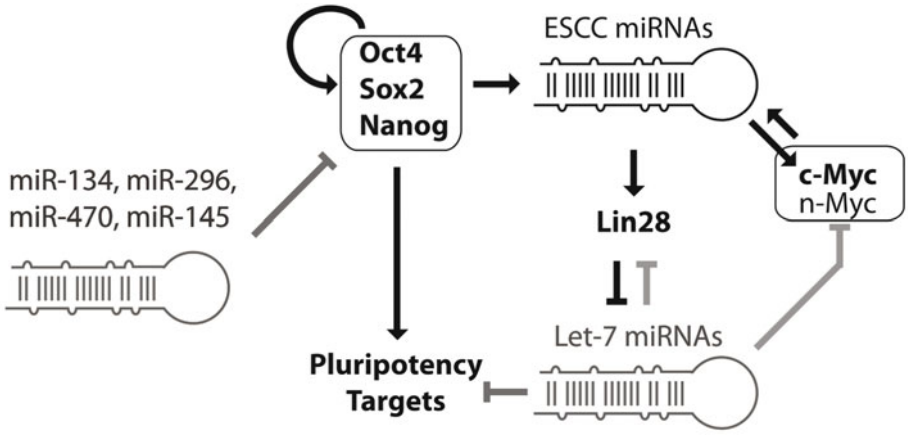

Figure 3. miRNA interactions in the ESC self-renewal network. This figure represents a model of the direct inhibitory and indirect activating effects of the ESCC, let-7 and miR-134, miR-296, miR-470 and miR-145 miRNAs. Dark/bold arrows, lines and text indicate interactions, miRNAs and proteins that are up-regulated in the ESC state. Grey arrows, lines and text indicate interactions, miRNAs and proteins that are down-regulated in the ESC state. As ESCs differentiate, the miR-134, miR-296, miR-470 and miR-145 miRNAs destabilize the Oct4/Sox2/Nanog transcriptional network to promote differentiation, whereas the let-7 miRNAs inhibit Myc and downstream targets of the Oct4/Sox2/Nanog network to stabilize the differentiated state.text.

patterns.[47-50] In ESCs an elegant mechanism exists which allows for the post-transcriptional silencing of let-7 transcripts. A complex of the RNA binding protein, Lin28 and the terminal uridyl-transferase, TUT4, binds to and induces the degradation of pre-let-7 transcripts.[51-56] Lin28 expression is quickly lost during ESC differentiation,[57,58] which allows for the rapid increase in let-7 expression.[55]

Recently, it was discovered that let-7 family members could induce silencing of self-renewal in the miRNA deficient *Dgcr8* $^{-/-}$ ESCs but not in wild-type ESCs.[33] This observation suggested that miRNAs expressed in ESCs normally prevent let-7 from silencing ESC self-renewal. Indeed, the ESCC miRNAs that predominate in ESCs, are able to prevent loss of self-renewal induced by the let-7 miRNAs. Let-7 preferentially targets transcripts that are enriched in ESCs, including many transcripts that are regulated by the pluripotency transcription factors Oct4, Sox2, Nanog and Tcf3. Additionally, a number of direct targets of let-7 are indirectly upregulated by the ESCC miRNAs, which can explain how the ESCCs antagonize let-7. Among the targets with opposing regulation by let-7 and the ESCCs are the Myc proteins, Sall4, Lin28 and Trim71.[33]

The antagonism observed between the ESCC and let-7 miRNAs and the targets which are regulated in opposing fashion by these miRNAs, suggest a network in which ESCC miRNAs and let-7 miRNAs have mutually exclusive expression and function (Fig. 3). In ESCs, the ESCC miRNAs lead to upregulation of Lin28, which directly suppresses let-7 maturation. Additionally, ESCCs indirectly upregulate cMyc and other direct let-7 targets that promote ESC self-renewal. By these mechanisms ESCC miRNAs counteract the effects of let-7. ESCC miRNA expression is promoted by Oct4, Sox2 and Nanog.[59] As ESCs differentiate, Oct4, Sox2 and Nanog expression decrease resulting in a corresponding decrease in ESCC expression. In the absence of ESCCs, Lin28 levels also decrease. In

this differentiated state, let-7 is no longer inhibited and feeds back to directly target Lin28 thereby reinforcing its own expression. Furthermore, let-7 now stabilizes the differentiated state by limiting expression of factors required for the ESC fate including transcripts that were previously activated by the pluripotency transcription factors Nanog, Oct4 and Sox2.

The let-7 miRNAs in addition to suppressing the ESC transcriptional program also promote the somatic cell cycle (Fig. 2). Let-7 miRNAs target both directly and indirectly multiple activators of the G1-S transition including cdc25a, cdk6, cyclinD1 and cyclinD2.[42,44] These interactions and others contribute to the overall effect of the let-7 miRNAs on increasing the number of cells in the G1 phase of the cell cycle.[42-44] It remains unclear how or if the cell cycle directly influences ESC self-renewal. It has been postulated that in the G1 phase cells are most susceptible to pro-differentiation signaling cascades including MAPK signaling.[60] It will be important to understand in more detail the interactions between the cell cycle and the ESC transcriptional network and to understand the impact of miRNAs on these interactions.

REGULATORY NETWORKS CONTROLLING miRNA EXPRESSION

In ESCs, ESCC miRNA expression from the miR-290 cluster is controlled by the pluripotency transcription factors Nanog, Oct4, Sox2 and Tcf3 as well as by the Myc transcription factors nMyc and cMyc.[59,61] ESCC miRNAs indirectly upregulate cMyc to form a positive feedback loop which likely reinforces their own expression. When ESCs differentiate, pluripotency transcription factors are downregulated and in turn so are the ESCC miRNAs.[59]

Transcriptional control of expression of direct miRNA suppressors of ESC self-renewal remains an open area of research; however, high-throughput sequencing of chromatin immuno-precipated factors (ChIP seq) data in ESCs give us some insight into their regulation. In ESCs the miR-296 promoter is bound by Oct4, Sox2, Nanog and Tcf3; however, it is also marked by repressive H3K27 methylation and is bound by the polycomb group protein Suz12.[59] These data suggest a mechanism by which miR-296 is poised to be activated in ESCs. If upon differentiation the repressive H3K27 histone mark is rapidly lost prior to loss of Oct4, Sox2 and Nanog, these transcription factors could transiently drive transcription of miR-296. This regulation would form a negative feedback loop leading to more robust loss of ESC self-renewal. How H3K27 methylation is maintained at the miR-296 promoter in ES cells and lost with differentiation is unclear. Regulation of miR-134 and miR-470 promoters is even less well understood.

Likewise transcriptional control of let-7 expression remains relatively unclear. Different let-7 transcripts are expressed in the various differentiated tissues and thus likely diverse transcription factors are able to induce let-7 expression.[47] In ESCs, Oct4, Sox2 and Nanog drive expression of the let-7g primary transcript.[59] The primary transcripts are processed to pre-miRNAs in ESCs where they are degraded by the Lin28/Tut4 complex.[51-56] As ESCs differentiate, suppression by Lin28/Tut4 is lost and mature let-7 is produced.[55,57,58] Additional miRNAs are regulated in this way in ESCs.[53]

Recently, a new class of regulatory RNA binding proteins, the Trim-NHL proteins, has been discovered. In neural stem cells, Schwamborn et al showed that expression of Trim32 potentiates let-7 inhibition of targets and is associated with the differentiation of NSCs.[62] In ESCs, the ESCC miRNAs promote expression of Trim71 (also known as Mlin-41). Trim71 is a let-7 target essential for mouse development.[63] Rybak et al demonstrated

that Trim71 acts as an ESC expressed E3 ubiquitin ligase that functions to degrade Ago2 protein, a component of the RISC complex.[64] Both Trim32 and Trim71 are members of a larger family of Trim-NHL proteins, which also include the Drosophila proteins Brat and Mei-P26. These Drosophila proteins also function to modulate the miRNA pathway through interactions with Ago1.[65] It will be important to understand if Trim71 simply functions to modulate activity of the entire miRNA pathway via degradation of Ago2 or if like Trim32 it can associate and increase the activity of specific miRNA subtypes.

miRNAs CAN PROMOTE OR INHIBIT DEDIFFERENTIATION TO IPS CELLS

ESCC miRNAs promote self-renewal in ESCs while the let-7 miRNAs promote silencing of ESC self-renewal. Reprogramming of somatic cells to induced pluripotent stem (iPS) cells can be achieved by nuclear transfer or by directed reprogramming with exogenously introduced transcription.[66] Consistent with the role of ESCC miRNAs in promoting ESC self-renewal, addition of these miRNAs to directed reprogramming assays enhances reprogramming efficiency.[61] Likewise inhibition of the let-7 miRNAs enhances reprogramming.[33] The effects of inhibiting the direct miRNA suppressors of ESC self-renewal on reprogramming remain unknown. Together, these findings demonstrate that the same mechanisms that control ESC self-renewal and differentiation also govern the dedifferentiation process.

Additionally, the ability to reprogram with cocktails of transcriptions factors with and without Myc (either Sox2, Oct4, Klf4, cMyc or Sox2, Oct4, Klf4, no cMyc) has allowed for interrogation of the function of miRNAs and miRNA inhibitors in regard to whether they function in the same or alternate pathways to each of these factors. For example, the ESCC miRNAs were shown to enhance reprogramming in the absence, but not in the presence of cMyc.[61] These findings suggest that ESCCs and Myc have redundant roles. Indeed it is now known that ESCCs induce the indirect upregulation of cMyc and that both cMyc and nMyc promote transcription of ESCC miRNAs.[33,61] Additionally, it has been discovered that inhibition of let-7 promotes reprogramming more so in the absence than in the presence of Myc.[33] This finding suggests that let-7 in somatic cells in part acts to suppress ESC self-renewal through Myc. Indeed, both cMyc and nMyc are direct targets of let-7.[33,67] It will be important and interesting to understand if there exist miRNAs, which operate in the same pathways as the other pluripotency transcription factors and whether these miRNAs can replace these transcription factors in iPS cell reprogramming.

miRNAs IN SOMATIC STEM CELLS

miRNA function in somatic stem cells remains poorly studied. Indeed, aside from ESC derived neural progenitor cells (NPCs) no detailed analysis of the miRNA repertoires of pure somatic stem cell populations has been performed. In NPCs the let-7 miRNAs are the dominant miRNA species.[59] Interestingly, recent data suggest that the let-7 miRNAs are not required for the propagation but rather the differentiation of neural stem cells in the embryonic mouse brain.[62] In this model, asymmetric divisions in neural stem cells segregates the RNA binding protein Trim32 into the daughter cell committed

to differentiate further. Trim32, among other functions, increases the activity of let-7 in this cell to promote differentiation.[62]

miRNAS IN CANCER CELLS

ESCC miRNAs and the miR-17/20/106 family share a similar seed sequence. The miR-17/20/106 family has been shown to have important roles in cancer. For example, miR-93 and miR-106 miRNAs target p21 to deregulate the G1/S checkpoint and promote rapid cell proliferation in multiple tumor types.[68,69] Additionally, in vivo studies have shown important roles for these miRNAs in tumorigenesis. In particular, enforced expression of the miR-17-19b polycistron accelerates tumor formation and decreases apoptosis in an Eμ-Myc B cell lymphoma mouse.[70] The decreased apoptosis in this model is likely, at least in part, due to miR-17 family miRNAs targeting the pro-apoptotic protein Bim.[71] The miR-17/92 cluster also contributes to tumorigenesis by increasing angiogenesis in tumors.[72] The human miRNAs miR-372 and miR-373 share the ESCC seed sequence. These miRNAs cooperate with oncogenic Ras to promote tumor formation in primary human fibroblasts and are highly expressed in germ cell tumors.[73] Collectively, these data demonstrate that miRNAs that share a similar seed sequence to the ESCC miRNAs, function as potent oncogenes often by acting through similar pathways normally seen in ESCs.

In contrast to the ESCC and related miRNAs, the let-7 miRNAs act as tumor suppressors. In a model of breast cancer, a subpopulation of the cancer cells, the tumor initiating cells (TICs), can regenerate the tumor. When the TICs differentiate they are no longer capable of forming a full tumor. The let-7 miRNAs are sufficient for differentiation of these cells. In this setting, let-7 acts in part by suppressing Ras, to suppress proliferation and HMGA2, to promote differentiation of the cancer cells.[74] Likewise, in a mouse models of K-Ras induced lung cancer and in xenograft models of established cancer cell lines, addition of exogenous let-7 miRNAs suppresses while inhibition of let-7 activity promotes tumorigenesis.[75-77] Furthermore, recent evidence suggests that Lin28 through inhibition of let-7 activity can promote tumor formation.[78-81] Let-7 has been shown to target multiple oncogenes including K-Ras, N-Ras, Hmga2, cMyc, nMyc and additional factors that collectively reduce cell proliferation.[82] Together, these data strongly support a functional role for let-7 as a tumor suppressor.

CONCLUSION

The data summarized in this chapter support an important role for various miRNA species in either stabilizing the self-renewing state of stem cells or in promoting their differentiation. These miRNAs are similar to other global regulators of gene expression as different subclasses of these miRNAs can either promote or inhibit stem cell self-renewal. These impacts on self-renewal occur both through regulation of the cell cycle and the stem cell transcriptional program. As we learn more about the miRNAs that influence stem cell self-renewal it is becoming clear these miRNAs are tightly regulated in complex molecular networks. This regulation can occur at various levels both transcriptional and post-transcriptional. Furthermore, different classes of miRNAs can inhibit or activate each other's expression. Understanding the extent and function of these networks in development will greatly enhance our knowledge of both developmental and disease states.

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