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REVIEW

The Functions of MicroRNAs and Long Non-coding RNAs in Embryonic and Induced Pluripotent Stem Cells

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Abstract Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) hold immense promise for regenerative medicine due to their abilities to self-renew and to differentiate into all cell types. This unique property is controlled by a complex interplay between transcriptional factors and epigenetic regulators. Recent research indicates that the epigenetic role of non-coding RNAs (ncRNAs) is an integral component of this regulatory network. This report will summarize findings that focus on two classes of regulatory ncRNAs, microRNAs (miRNAs) and long ncRNAs (lncRNAs), in the induction, maintenance and directed differentiation of ESCs and iPSCs. Manipulating these two important types of ncRNAs would be crucial to unlock the therapeutic and research potential of pluripotent stem cells.

Introduction

Embryonic stem cells (ESCs) derived from the inner cell mass, which possess the potential for unlimited proliferation and differentiation into three germ layers, are the ideal cell source for cell therapy [1–3]. The acquisition of human ESCs (hESCs), however, requires the destruction of human embryos. Therefore, possible immunological rejection or religious and ethical concerns greatly hinder the pace of ESCs in basic and clinical applications. In 2006, the Yamanaka group obtained induced pluripotent stem cells (iPSCs) with characteristics similar to those of ESCs by overexpressing four exogenous factors (Oct4, Sox2, c-Myc and Klf4) in fibroblasts [4]. This method of deriving patient-specific iPSCs from donor somatic cells removes many of these medical, ethical and political obstacles, creates disease-specific stem cells and provides a platform to study molecular mechanisms of genetic diseases. Understanding how these regulatory processes function in iPSCs would help to accelerate the basic research and clinical applications of iPSCs [5].

ESCs and iPSCs are characterized by their self-renewal and differentiation into any cell type. Transcription factor networks and epigenetics (including DNA methylation, histone modifications and ncRNAs) undergo a tremendous change during this process [6–8]. ncRNAs can be classified into either housekeeping or regulatory ncRNAs. Housekeeping ncRNAs are most often constitutively expressed, which include transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs).

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Regulatory ncRNAs can be broadly classified by size as lncRNAs (>200 bp) and small ncRNAs (<200 bp) such as miRNAs, endogenous small interfering RNAs (endo-siRNAs) and PIWI-interacting RNAs (piRNAs) [9]. lncRNAs originate from intronic, exonic, intergenic, intragenic, promoter regions, 3′- and 5′-untranslated regions (UTR) and enhancer sequences. lncRNAs sometimes are bidirectional transcripts [10]. lncRNAs consists of intergenic ncRNAs, intronic ncRNAs, natural antisense transcripts (NATs), pseudogene transcripts, *etc*. Long intergenic ncRNAs (lincRNAs) are derived from non-coding DNA sequences between protein-coding genes, whereas intronic lncRNAs are transcribed from within introns of protein-coding genes and NATs are transcribed from the opposite strand of protein-coding sense transcripts [11,12]. Pseudogene transcripts can modulate the expression of their counterpart genes through competing for endogenous RNA (ceRNA) [13]. This review will focus on miRNAs and lncRNAs.

miRNAs are hairpin-derived RNAs that are 20–24 nucleotides (nt) long. They act at the RNA level by destabilizing and repressing target RNAs via binding to the 3′ UTRs, 5′ UTRs and coding sequences of the transcripts [14–17]. Nonetheless, miRNAs can also enhance mRNA translation by binding to the 5′ UTRs [18]. Some miRNA genes are distributed as clusters in the genome and thus these closely distributed miRNAs are termed as the miRNA cluster. miRNA-coding genes are transcribed into long primary miRNAs (pri-miRNAs) by RNA polymerase II in the nucleus, and then the Drosha-DiGeorge critical region-8 (DGCR8) complex processed pri-miRNAs into precursors (pre-miRNAs) of 60–70 nt in length. Drosha is a member of the ribonuclease III family (RNase III) [19]. Drosha and its cofactor, DGCR8, form a multiprotein complex called Microprocessor to mediate the nuclear export. pre-miRNAs possess a short stem plus a 2-nt 3′ overhang (also known as the nuclear cropping step) [20]. After being exported from the nucleus to the cytoplasm, pre-miRNAs are processed by Dicer (an RNase III enzyme) to produce mature miRNAs, which are incorporated into the RNA-induced silencing complex (RISC) to repress the expression of the target genes or bind directly to DNA preventing transcription [21–24].

In contrast to miRNAs, some lncRNAs are remarkably similar to messenger RNAs (mRNAs). lncRNAs are transcribed by RNA polymerase II, capped, spliced and get polyadenylated like mRNAs, although they cannot act as templates for protein synthesis [25]. lncRNAs are able to activate or repress gene expression at multiple levels through diverse mechanisms. For example, lncRNAs can recruit repressive (*e.g.*, PRC2) and activating (*e.g.*, the Trithorax group) chromatin modifiers at the DNA level much like molecular scaffolds, leading to regulation of target gene expression [26–29]. At the RNA level, lncRNAs play a role in post-transcriptional events during gene expression and contribute to splicing, mRNA translation and mRNA degradation [30–33]. In addition, certain lncRNAs can inhibit miRNA function, which indirectly enhances protein expression of miRNA targets [34]. Along with the growing understanding of the significance of ncRNAs in mammalian cell differentiation and human diseases [35,36], accumulating examples are being identified that illustrate the specific importance of short and long ncRNAs in PSCs. In this review, we focus on the recent advances in our understanding of miRNAs and lncRNAs, in the induction, maintenance and differentiation of PSCs.

ESC-specific miRNA family

Numerous studies identified a set of ESC-specific miRNAs that were preferentially expressed in ESCs and downregulated during differentiation into embryoid bodies [37,38]. The expression signature of ESCs has been characterized (**Table 1** and **Figure 1**). The requirement of miRNAs in ESC self-renewal and differentiation was demonstrated by ESCs lacking the miRNA-processing enzymes, Dicer or DGCR8. Dicer- or Dgcr8-null ESCs exhibited reduced cell proliferation due to G1 cell cycle arrest and resistance to differentiation through embryoid body formation or retinoic acid induction [39,40]. miRNAs, miR-195 and miR-372 participate in hESC cell cycle control by depleting the two main miRNA processing enzymes, Dicer and Drosha [41].

In addition, two clusters of miRNAs—miR-302 cluster and mouse miR-290-295/human miR-371-373 cluster—were

Table 1 Summary of pluripotency or differentiation associated miRNAs and lncRNAs

Name	Validated targets	Cell type	Function	Ref
miR-302a	CyclinD1	hESCs	Regulate cell cycle, promote self-renewal	[42]
miR-302	NR2F2	hESCs	Maintain pluripotency	[43]
miR-302b, miR-372	TGFBR2, RHOC	hiPSCs	Accelerate mesenchymal to epithelial transition	[57]
miR-291b-5p, miR-293	P65	mESCs	Maintain pluripotency and self-renewal	[46]
miR-138	P53	miPSCs	Promote reprogramming	[63]
miR-145	Oct4, Sox2, Klf4	hESCs	Induce differentiation	[77]
miR-125, miR-181	Cbx7	mESCs	PRC1-mediated differentiation	[78]
Let-7b	TLX, CyclinD1	Neural stem cells	Reduce proliferation and differentiation	[79]
miR-9	Stathmin	hNPCs	Coordinate proliferation and migration	[81]
miR-18, let-7	Smad2, Acvr1b, Lin28	mESCs	Mesoderm differentiation	[71,84]
miR-27b	Pax3	Muscle stem cells	Myogenic differentiation	[88]
miR-375	Hnf1β, Sox9	hESCs	Endoderm differentiation	[92]
Panct1-3	Oct4	mESCs	Maintain pluripotency	[97]
lincRNA-RoR	P53	hiPSCs	Promote reprogram	[99]
Tsix	PRC2	mESCs	X-chromosome inactivation	[103]
Mistral	MLL1	mESC	Germ layer differentiation	[104]
lncRNA_N1, lncRNA_N3	SUZ12, REST	hESCs	Neurogenesis	[105]

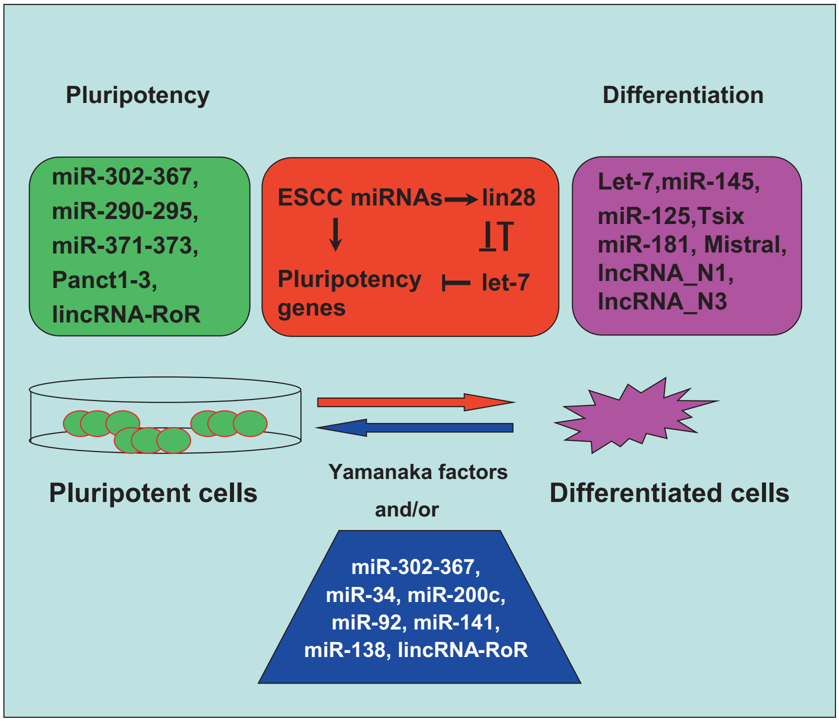

Figure 1 Summary of the published interactions between pluripotency and differentiation associated miRNAs and lncRNAs
miRNAs and lncRNAs that are upregulated in pluripotency are indicated in green, miRNAs that are downregulated in pluripotency are in purple, miRNAs and lncRNAs regulating the somatic reprogramming are in blue. ESCC miRNAs and let-7 miRNAs that form a feedback loop in regulating ESC pluripotency and differentiation are showed in red.

strongly expressed in ESCs. The miR-302 cluster (containing 8 miRNAs on chromosome 3/4 in human), consists of highly expressed ESC-specific miRNAs including some of the most commonly studied miRNAs [42]. Pluripotency factors Oct4 and Sox2 can bind to a conserved promoter region of the miR-302 to initiate its expression [42]. miR-302a can inhibit the expression of G1 regulator cyclin D1, which contributes to the increased population of hESCs in S phase and regulating the cell cycle of ESCs, thereby promoting self-renewal and pluripotency [42]. A recent study found that in undifferentiated ESCs, Oct4 and miR-302, directly inhibit the expression of nuclear receptor subfamily 2, group F, member 2 (NR2F2; COUP-TFII) at the transcriptional and post-transcriptional levels, respectively. As a positive feedback loop, NR2F2 directly inhibits *Oct4* expression [43].

The human homolog of the mouse miR-290-295 cluster, which also forms a tight genomic cluster (miR-371-373 cluster), is specifically expressed in hESCs, which is upregulated in several human tumors [44]. A recent study indicated that several Wnt-signaling pathway genes, including Dickkopf-1 (DKK1), TGF-beta type II receptor (TGFBR2), B cell translocation gene 1 (BTG1), and left right determination factor 1 (LEFTY1), were direct targets of miR-372 and -373 [45]. The expression of the miR-371-373 cluster was transactivated via the Wnt/β-catenin pathway by directly binding β-catenin/LEF1 to the *miR-371-373* promoter. These findings elucidate a novel beta-catenin /LEF1 - miR-372 and -373-DKK1 regulatory feedback loop, which likely plays a crucial role in ESC maintenance [45]. Luningschror et al. reported that overexpression of the NF-kB subunit p65 results in the loss of pluripotency and differentiation of ESCs, as well as the epithelial to mesenchymal transition [46]. Interestingly, the miR-290 cluster, specifically miR-291b-5p and miR-293, targets the p65 coding sequence to repress its translation, which may also contribute to regulatory networks in pluripotency [46].

The seed sequence of miRNAs is about 6–8 nt in length, which is thought to be the most important feature for miRNA target specificity [47–49]. Interestingly, previous studies showed that several miRNAs from different clusters including miR-106, miR-302-367 and miR-290 have similar seed sequences (*AAGUGCU*) and are all upregulated in ESCs, suggesting that they may repress similar pools of mRNAs to maintain the stem cell state [50].

miRNAs function during somatic cell reprogramming

Since the Yamanaka's group claimed that somatic cells can be reprogrammed into iPSCs by expressing four transcription factors Oct4, Sox2, c-Myc and Klf4 (also known as the Yamanaka factors), scientists have tried different methods to obtain the iPSCs [4,51–55].

Several miRNAs have been shown to increase the efficiency of reprogramming when expressed along with a combination of the four or fewer Yamanaka factors [56,57]. For example, MYC can be regulated by the miR-17-92 cluster, and overexpression of MYC leads to increased levels of miR-92 [58]. miR-92 belongs to the miR-17-92 cluster, which is upregulated in cancer [58]. Studies have shown that there are differences in the miRNA patterns between human iPSCs (hiPSCs) and hESCs, suggesting that fibroblasts may not be induced to a state identical to that of ESCs [59,60]. To better appreciate such differences in the expression pattern of miRNAs, Neveu and his colleagues profiled the miRNA expression in different cell types, including hESCs, iPSCs, differentiated cells, cancer cells and glioma biopsies [61]. These researchers identified two distinct categories of miRNA patterns in pluripotent cells, regardless the reprogrammed cells were derived from somatic or embryonic cells. The results are surprising, since these two cell categories differ in the status of their p53 network. The overexpression of miR-92 and miR-141, the p53 regulatory miRNAs, in iPSCs conferred alterations in the miRNA profile [61]. As p53 targets, miR-34 (a, b and c) can cooperate with p21, another target of p53, to restrain somatic reprogramming [62]. Numerous studies reported that as a tumor suppressor, p53 prevents pluripotency during somatic reprogramming [61,62]. In our study, we demonstrated that the ectopic expression of miR-138 dramatically increased the efficiency of iPSC formation by targeting the 3′ UTR of p53 [63]. Surprisingly, overexpression of the miR-302-367 cluster can directly reprogram human and mouse somatic cells into a PSC state without exogenous transcription factors, and the reprogramming efficiency is greatly increased compared to that induced by the Yamanaka factors [64]. Further study showed that the miR-302-367 cluster can activate *Oct4* gene expression and suppress HDAC2 activity, which may cooperate to reprogram somatic cells to pluripotency [64]. Meanwhile, mature double-stranded miRNAs (combination of miR-200c, miR-302s and miR-369s family) can also reprogram mouse and human cells to a pluripotent state by using transfection reagents, which may be safer for biomedical research by avoiding the vector-based gene transfer system [65].

Furthermore, other reports confirm that these miRNAs function in part through increasing the mesenchymal-epithelial transition (MET) by targeting at least the TGFBR2 and Ras homolog gene family, member C (RHOC), to enhance reprogramming [57]. MET occurs during organ development and also at an early stage during the reprogramming of fibroblasts [57,66,67]. Further study revealed that miR-302 significantly decreased the activities of amine oxidase flavin-containing domain protein 2 (AOF2) and DNA methyltransferase 1 (DNMT1). In addition, in conjunction with the co-suppression of methyl-specific proteins (MECP1/2), miR-302 resulted in global genomic DNA demethylation and histone H3 lysine 4(H3K4) modification [68]. Modification of chromosomal histones can either activate or silence genes; in particular, the methylation level of H3K4 is likely to be important for the efficient reprogramming of pluripotency genes [69].

One miRNA can have many target genes. Therefore, the mechanisms of miRNA-mediated gene regulation are particularly complex during the somatic cell reprogramming process. The studies described above found that various miRNAs can improve or restrain the efficiency of induction during somatic cell reprogramming. However, the mechanism by which genes are targeted by miRNAs remains largely unknown.

miRNAs act as suppressors of the pluripotent state

miRNAs are critical for embryonic development and pluripotency maintenance and are involved in cell fate decisions as well. ESC-specific miRNAs have been described previously [37,38]. Nonetheless, miRNAs can also promote the differentiation of ESCs into the three germ layers—ectoderm, mesoderm and endoderm.

The let-7 miRNAs are broadly expressed in differentiated tissues and are increased during ES cell differentiation [70,71] (Table 1 and Figure 1). At the early differentiation stage of ESCs, expression of *Oct4*, *Sox2*, *Nanog* and other pluripotency genes are downregulated, which leads to the downregulation of the ES cell-specific cell cycle-regulating (ESCC) miRNAs and Lin28. Lin28, an RNA-binding protein, is a posttranscriptional repressor of let-7 miRNA biogenesis [72]. Therefore, the downregulation of Lin28 dramatically increases the expression of let-7 miRNAs. By targeting the 3′ UTR of *Lin28*, let-7 may inhibit the translational initiation of the genes downstream of Oct4, Sox2, Nanog and transcription factor 3 (Tcf3), thereby accelerating the differentiation of ESCs [73,74]. Further study demonstrated that TUT4 is the uridylyl transferase for the let-7 precursor, which adds an oligouridine tail to downstream targets of the let-7 miRNAs, blocking the biogenesis of let-7 miRNA at the dicing step [75].

Expression of miR-145 is low in self-renewing hESCs, which is highly upregulated during differentiation [76]. A recent study reported that increased miR-145 expression inhibits hESC self-renewal and induces lineage-restricted differentiation [76]. Furthermore, Xu et al. demonstrated that endogenous miR-145 binds to the 3’ UTR of the pluripotency genes *Oct4*, *Sox2* and *Klf4*. Interestingly, as part of a double-negative feedback loop, the *miR-145* promoter, is bound and repressed by Oct4 in hESCs [77].

The polycomb group (PcG) contains multiple homologs of the polycomb repressive complex 1 (PRC1) components including five orthologs of the Drosophila polycomb protein (Cbx2, Cbx4, Cbx6, Cbx7 and Cbx8), and is critical for ES pluripotency and differentiation. A recent study demonstrated that Cbx7 is the primary polycomb ortholog of the PRC1 complexes in ESCs and knockdown of Cbx7 expression in ESCs can induce differentiation and increase expression of lineage-specific markers [78]. The miR-125 and miR-181 families are regulators of Cbx7, and overexpression of these miRNAs accelerates ESC differentiation [78].

Studying ESCs can help us understand how miRNAs play a role in suppressing the pluripotent gene expression. However, to clarify the specific role of microRNA in ESC differentiation, further study is needed.

Role of miRNAs in stem cell lineage determination

The iPSC technology provides an unlimited source of stem cells to promote the clinical applications of cell therapy. However, one of the biggest challenges to such clinical application is differentiating these pluripotent cells into the final functional cells of a specific organ. A further understanding of miRNAs demonstrated that the function of different cell types is associated with a unique miRNA expression pattern.

The let-7 family plays an important role in the ectoderm lineage differentiation of ESCs. Further study demonstrated that the let-7b miRNA regulates neural stem cell proliferation and differentiation by targeting the stem cell regulator TLX and the cell cycle regulator cyclin D1 [79]. Expression of musashi 1 (Msi1), an RNA-binding protein, is increased during the early neural differentiation of ESCs. Msi1 can enhance Lin28 localization to the nucleus and block let-7 family member miR-98 in the nuclear cropping step, thus affecting early neural differentiation of ESCs [80]. miR-9, a brain-specific miRNA, is expressed in human neural progenitor cells (hNPCs) that are derived from hESCs. Further results suggest that miR-9 regulates the proliferation and migration of hNPCs by directly targeting the microtubule-related gene stathmin [81]. The highly expressed miR-371-373 cluster in PSCs has also been reported to play a critical role in human PSC neurogenic differentiation behavior [82,83].

Another group found that let-7 and miR-18 downregulated Acvr1b and Smad2, respectively, to increase the mesoderm at the expense of endoderm in mouse ESCs (mESCs) [84]. Expression of miR-125b is upregulated in patients with leukemia and can regulate hematopoiesis by targeting Lin28 in mouse hematopoietic stem cells and progenitor cells [85]. Ivey and colleagues demonstrated that miR-1 and miR-133 can regulate mesoderm formation and cardiac muscle differentiation by suppressing the gene expression in desired lineages [86,87]. Pax3, a regulator of skeletal muscle stem cells, is required for the maintenance of muscle cell differentiation. miR-27b downregulates the Pax3 protein levels by directly targeting the 3′ UTR of *Pax3* and accelerates myogenic differentiation in muscle stem cells [88]. However, a recent study demonstrated that miR-489 is a quiescence-specific miRNA in the satellite cell lineage. The highly expressed miR-489 in quiescent satellite cells decreased quickly during satellite cell activation. Further results have shown that miR-489 suppresses the oncogene *Dek* at the posttranscriptional level, which may be associated with the mechanism for maintaining the quiescent state of a stem cell population [89].

miR-24 and miR-10a were upregulated to inhibit endodermal differentiation during NaButyrate induction of hESCs [90]. Joglekar et al. showed that the expression of four islet-specific miRNAs including miR-7, miR-9, miR-375 and miR-376 was high during human pancreatic islet development [91]. Overexpression of miR-375 can downregulate the expression of gut-endoderm/pancreatic progenitor-specific markers, hepatocyte nuclear factor 1 beta(Hnf1β) and Sox9, during endodermal differentiation of hESCs. These data indicate that miR-375 may regulate hESC differentiation toward pancreatic islet cells [92].

Taken together, as one miRNA can target more than one gene, the role of miRNAs in cell differentiation is not only related to the level of its own expression but also has a close relationship with factors like the differentiation system, cell type and microenvironment.

lncRNAs in ESC pluripotency and somatic cell reprogramming

Recent studies have identified over 900 so-called lincRNAs in mESCs and hESCs, which potentially control the self-renewal and pluripotency of ESCs [93,94]. Intriguingly, more than 100 lincRNAs (with proximal genomic targets located less than 10 kb genomic distance from a gene to the binding site) in mESCs appear to be directly bound by ESC-specific transcription factors, such as Sox2, Oct4 and Nanog [95]. Lipovich's group observed two lincRNAs that are regulated by Oct4 and Nanog and are essential for maintaining pluripotency. The inhibition or misexpression of these two lincRNAs leads to dramatic changes in the expression of *Oct4* and *Nanog*, indicating the involvement of a feedback loop in the regulatory mechanism [95]. To further examine the role of lincRNAs in pluripotency, Guttman et al. performed loss-of-function studies on 147 lincRNAs using lentiviral-based shRNAs in mESCs [96]. Of these lincRNAs, 26 showed involvement in the maintenance of pluripotency. After deleting these lincRNAs, a reduced *Nanog* promoter activity was discovered and expression pattern in mESCs was similar to that in the differentiated cell types, suggesting that these lincRNAs repress differentiation programs in mESCs. Another large-scale screen of functional lincRNAs in mESCs was achieved by using RNA interference (RNAi) with transcript localization. Consequently, three non-coding transcripts, Panct1-3, were identified as modulators of mESC pluripotency based on reduced *Oct4* promoter activity [97]. Recent findings showed that lincRNA-RoR (regulator of reprogramming, formerly called lincRNA-ST8SIA3) shares miRNA response elements with *Oct4*, *Sox2* and *Nanog*, and that lincRNA-RoR prevents these core transcription factors from miRNA-mediated suppression in self-renewing hESCs [98]. Together, these findings connect lincRNAs to the regulatory networks that maintain ESC identity.

In addition to maintaining ESC pluripotency, lncRNAs are involved in the generation of iPSCs (Table 1 and Figure 1). This cellular reprogramming is accompanied by an extensive global remodeling of the epigenome. The research group, led by Loewer et al., found that the expression profiles of lincRNAs in iPSCs were similar to those in ESCs but not to those in the somatic cells of origin, such as fibroblasts and hematopoietic stem cells [99]. Further study showed that expression of 10 lincRNAs was elevated in iPSCs compared with ESCs, suggesting that their increased expression may promote reprogramming [99]. Promoter loci of 3 iPSC-enriched lincRNAs, including lincRNA-SFMBT2, lincRNA-VLDLR and lincRNA-RoR, are bound by Oct4, Sox2 and Nanog. In addition, knockdown of Oct4 led to downregulation of these lincRNAs, suggesting that their expression is directly regulated by the key pluripotency transcription factors. The depletion of lincRNA-RoR resulted in a 2–8-fold decrease in the number of emerging iPSC colonies. Conversely, overexpression of lincRNA-RoR increased the efficiency of iPSC colony formation. Microarray gene expression analysis demonstrated that knockdown of lincRNA-RoR led to p53 upregulation, which induces oxidative stress, DNA damage and cell death, confirming the role of lincRNAs in the induction of pluripotency by promoting the survival of iPSCs [99].

lncRNAs are implicated in the differentiation of PSCs

lncRNAs can regulate the differentiation of ESCs as well. For example, X-inactive specific transcript (Xist) plays a role in X-chromosome inactivation (XCI) during female ESC differentiation. In placental mammals, XCI randomly inactivates one of the two female X chromosomes to obtain the proper gene dosage of X-linked genes in females as compared with males [100]. In female ESCs, Oct4, Sox2 and Nanog bind to intron 1 of Xist to suppress its expression, whereas the antagonizing lncRNA Tsix is activated by the pluripotency factors Oct4, Sox2, Rex1, c-Myc and Klf4 [101,102]. Upon differentiation, downregulation of the pluripotency factors initiates the expression of Xist, which later recruits chromatin regulators such as PRC2 to mediate XCI [103]. In addition to Xist in the early step of differentiation, certain other lncRNAs play important roles in the later lineage commitment. Mixed lineage leukemia 1 (MLL1) is an epigenetic activator involved in embryonic development and hematopoiesis [104]. Bertani et al. found that the lncRNA Mistral is able to recruit MLL1 to chromatin and subsequently induce the expression of the homeotic genes Hoxa6 and Hoxa7 during mESC germ layer differentiation [104]. Another study reported an essential role for lncRNAs in neurogenesis. Cytoplasmic lncRNA_N2 promoted neurogenesis possibly by maintaining the expression of neurogenic miRNAs, miR-125b and let-7a, since both of them are located within the introns of lncRNA_N2. Additionally, nuclear lncRNA_N1 and lncRNA_N3 were identified to physically interact with nuclear factors REST and SUZ12, respectively, suggesting their potential roles in regulating neuronal differentiation [105].

Expression of lncRNAs is correlated with the full development potential of iPSCs. In most of the iPSC clones, the lncRNA Gtl2, which belongs to the *Dlk1–Dio3* imprinted locus, is aberrantly silenced by DNA hypermethylation and histone hypoacetylation [106,107]. The *Gtl2* gene is maternally expressed and its expression is thought to negatively regulate the expression of paternal *Dlk1* gene, which is located within the same gene cluster and gets involved in fetal growth. In addition, a total of 26 miRNAs all localized to the *Dlk1–Dio3* cluster are differentially expressed in non-4n complementation-competent and 4n complementation-competent iPSC lines [106]. The silenced status of this cluster in iPSCs is closely correlated with the developmental failure of these iPSCs. In contrast, iPSC clones with normal *Dlk1–Dio3* cluster expression contributed to high-grade chimaeras and yielded viable all-iPSC mice. Interestingly, when an iPSC clone with silenced *Dlk1–Dio3* was treated with a histone deacetylase inhibitor valproic acid (VPA), the locus that includes Gtl2 got reactivated, thus recovering the capability of this clone to support full-term development of all-iPSC mice.

Future perspectives

In recent years, the miRNAs and lncRNAs have been emerging as important components of gene regulation and have become the new hotspot of current molecular biology. Somatic cell reprogramming technology renders terminally-differentiated cells to revert to a pluripotent state, thus injecting new vitality into the field of stem cell research. Studies have shown that the regulatory interactions between ESC-specific miRNAs and their targets in the cell cycle, DNA methylation, mesenchymal to epithelial transition and apoptosis pathways influence stem cell pluripotency and somatic cell reprogramming and differentiation. A large number of miRNA and lncRNA sequences have been obtained via high-throughput sequencing technologies. However, the underlying molecular mechanism of ESC differentiation and pluripotency maintenance and somatic cell reprogramming still remains elusive. Better understanding of the new functions and mechanisms of miRNAs and lncRNAs in these processes would be conducive to achieving better appreciation of epigenetics and even more extensive impact on life sciences and biomedical research.

Competing interests

The authors have declared that no competing interests exist.

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References

[1] Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature 1981;292:154–6.

[2] Kim JH, Auerbach JM, Rodriguez-Gomez JA, Velasco I, Gavin D, Lumelsky N, et al. Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. Nature 2002;418:50–6.

[3] Antonica F, Kasprzyk DF, Opitz R, Iacovino M, Liao XH, Dumitrescu AM, et al. Generation of functional thyroid from embryonic stem cells. Nature 2012;491:66–71.

[4] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–76.

[5] Robinton DA, Daley GQ. The promise of induced pluripotent stem cells in research and therapy. Nature 2012;481:295–305.

[6] Ang YS, Tsai SY, Lee DF, Monk J, Su J, Ratnakumar K, et al. Wdr5 mediates self-renewal and reprogramming via the embryonic stem cell core transcriptional network. Cell 2011;145:183–97.

[7] Bar-Nur O, Russ HA, Efrat S, Benvenisty N. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. Cell Stem Cell 2011;9:17–23.

[8] Hirai H, Tani T, Katoku-Kikyo N, Kellner S, Karian P, Firpo M, et al. Radical acceleration of nuclear reprogramming by chromatin remodeling with the transactivation domain of MyoD. Stem Cells 2011;29:1349–61.

[9] Kawaji H, Nakamura M, Takahashi Y, Sandelin A, Katayama S, Fukuda S, et al. Hidden layers of human small RNAs. BMC Genomics 2008;9:157.

[10] Ahmed RP, Haider HK, Buccini S, Li L, Jiang S, Ashraf M. Reprogramming of skeletal myoblasts for induction of pluripotency for tumor-free cardiomyogenesis in the infracted heart. Circ Res 2011;109:60–70.

[11] Moran VA, Perera RJ, Khalil AM. Emerging functional and mechanistic paradigms of mammalian long non-coding RNAs. Nucleic Acids Res 2012;40:6391–400.

[12] Beiter T, Reich E, Williams RW, Simon P. Antisense transcription: a critical look in both directions. Cell Mol Life Sci 2009;66:94–112.

[13] Tay Y, Kats L, Salmena L, Weiss D, Tan SM, Ala U, et al. Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. Cell 2011;147:344–57.

[14] Qin W, Shi Y, Zhao B, Yao C, Jin L, Ma J, et al. MiR-24 regulates apoptosis by targeting the open reading frame (ORF) region of FAF1 in cancer cells. PLoS One 2010;5:e9429.
[15] Lytle JR, Yario TA, Steitz JA. Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5′ UTR as in the 3′ UTR. Proc Natl Acad Sci U S A 2007;104:9667–72.
[16] 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;455:1124–8.
[17] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009;136:215–33.
[18] Orom UA, Nielsen FC, Lund AH. MicroRNA-10a binds the 5′ UTR of ribosomal protein mRNAs and enhances their translation. Mol Cell 2008;30:460–71.
[19] Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, et al. The nuclear RNase III Drosha initiates microRNA processing. Nature 2003;425:415–9.
[20] Kim VN. MicroRNA biogenesis: coordinated cropping and dicing. Nat Rev Mol Cell Biol 2005;6:376–85.
[21] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97.
[22] Han J, Lee Y, Yeom KH, Nam JW, Heo I, Rhee JK, et al. Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. Cell 2006;125:887–901.
[23] Iorio MV, Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. EMBO Mol Med 2012;4:143–59.
[24] Kim DH, Saetrom P, Snøve Jr O, Rossi JJ. MicroRNA-directed transcriptional gene silencing in mammalian cells. Proc Natl Acad Sci U S A 2008;105:16230–5.
[25] Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet 2009;10:155–9.
[26] Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, et al. Long noncoding RNA as modular scaffold of histone modification complexes. Science 2010;329:689–93.
[27] Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, et al. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 2007;129:1311–23.
[28] Flynn RA, Chang HY. Active chromatin and noncoding RNAs: an intimate relationship. Curr Opin Genet Dev 2011;22:172–8.
[29] Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Mol Cell 2011;43:904–14.
[30] Gong C, Maquat LE. lncRNAs transactivate STAU1-mediated mRNA decay by duplexing with 3′ UTRs via Alu elements. Nature 2011;470:284–8.
[31] Tripathi V, Ellis JD, Shen Z, Song DY, Pan Q, Watt AT, et al. The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. Mol Cell 2010;39:925–38.
[32] Yoon JH, Abdelmohsen K, Srikantan S, Yang X, Martindale JL, De S, et al. LincRNA-p21 suppresses target mRNA translation. Mol Cell 2012;47:648–55.
[33] Guttman M, Rinn JL. Modular regulatory principles of large non-coding RNAs. Nature 2012;482:339–46.
[34] Cesana M, Cacchiarelli D, Legnini I, Santini T, Sthandier O, Chinappi M, et al. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. Cell 2011;147:358–69.
[35] Gibb EA, Brown CJ, Lam WL. The functional role of long non-coding RNA in human carcinomas. Mol Cancer 2011;10:38.
[36] Hu W, Alvarez-Dominguez JR, Lodish HF. Regulation of mammalian cell differentiation by long non-coding RNAs. EMBO Rep 2012;13:971–83.
[37] Houbaviy HB, Murray MF, Sharp PA. Embryonic stem cell-specific microRNAs. Dev Cell 2003;5:351–8.
[38] Lee TH, Song SH, Kim KL, Yi JY, Shin GH, Kim JY, et al. Functional recapitulation of smooth muscle cells via induced pluripotent stem cells from human aortic smooth muscle cells. Circ Res 2010;106:120–8.
[39] Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, et al. Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. Genes Dev 2005;19:489–501.
[40] Wang Y, Medvid R, Melton C, Jaenisch R, Blelloch R. DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. Nat Genet 2007;39:380–5.
[41] Qi J, Yu JY, Shcherbata HR, Mathieu J, Wang AJ, Seal S, et al. MicroRNAs regulate human embryonic stem cell division. Cell Cycle 2009;8:3729–41.
[42] Card DA, Hebbar PB, Li L, Trotter KW, Komatsu Y, Mishina Y, et al. Oct4/Sox2-regulated miR-302 targets cyclin D1 in human embryonic stem cells. Mol Cell Biol 2008;28:6426–38.
[43] Rosa A, Brivanlou AH. A regulatory circuitry comprised of miR-302 and the transcription factors OCT4 and NR2F2 regulates human embryonic stem cell differentiation. Embo J 2010;30:237–48.
[44] Stadler B, Ivanovska I, Mehta K, Song S, Nelson A, Tan Y, et al. Characterization of microRNAs involved in embryonic stem cell states. Stem Cells Dev 2010;19:935–50.
[45] Zhou AD, Diao LT, Xu H, Xiao ZD, Li JH, Zhou H, et al. Beta-Catenin/LEF1 transactivates the microRNA-371-373 cluster that modulates the Wnt/beta-catenin-signaling pathway. Oncogene 2011;31:2968–78.
[46] Luningschror P, Stocker B, Kaltschmidt B, Kaltschmidt C. MiR-290 cluster modulates pluripotency by repressing canonical NF-kappaB signaling. Stem Cells 2012;30:655–64.
[47] Ellwanger DC, Buttner FA, Mewes HW, Stumpflen V. The sufficient minimal set of miRNA seed types. Bioinformatics 2011;27:1346–50.
[48] Lambert NJ, Gu SG, Zahler AM. The conformation of microRNA seed regions in native microRNPs is prearranged for presentation to mRNA targets. Nucleic Acids Res 2011;39:4827–35.
[49] 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.
[50] Li MA, He L. MicroRNAs as novel regulators of stem cell pluripotency and somatic cell reprogramming. Bioessays 2012;34:670–80.
[51] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–72.
[52] Li W, Wei W, Zhu S, Zhu J, Shi Y, Lin T, et al. Generation of rat and human induced pluripotent stem cells by combining genetic reprogramming and chemical inhibitors. Cell Stem Cell 2009;4:16–9.
[53] Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, et al. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 2009;4:381–4.
[54] Han J, Yuan P, Yang H, Zhang J, Soh BS, Li P, et al. Tbx3 improves the germ-line competency of induced pluripotent stem cells. Nature 2010;463:1096–100.
[55] Huang J, Chen T, Liu X, Jiang J, Li J, Li D, et al. More synergetic cooperation of Yamanaka factors in induced pluripotent stem cells than in embryonic stem cells. Cell Res 2009;19:1127–38.
[56] Judson RL, Babiarz JE, Venere M, Blelloch R. Embryonic stem cell-specific microRNAs promote induced pluripotency. Nat Biotechnol 2009;27:459–61.
[57] Subramanyam D, Lamouille S, Judson RL, Liu JY, Bucay N, Derynck R, et al. Multiple targets of miR-302 and miR-372 promote reprogramming of human fibroblasts to induced pluripotent stem cells. Nat Biotechnol 2011;29:443–8.

[58] He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, et al. A microRNA polycistron as a potential human oncogene. Nature 2005;435:828–33.

[59] Chin MH, Mason MJ, Xie W, Volinia S, Singer M, Peterson C, et al. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. Cell Stem Cell 2009;5:111–23.

[60] Wilson KD, Hu S, Venkatasubrahmanyam S, Fu JD, Sun N, Abilez OJ, et al. Dynamic microRNA expression programs during cardiac differentiation of human embryonic stem cells: role for miR-499. Circ Cardiovasc Genet 2010;3:426–35.

[61] Neveu P, Kye MJ, Qi S, Buchholz DE, Clegg DO, Sahin M, et al. MicroRNA profiling reveals two distinct p53-related human pluripotent stem cell states. Cell Stem Cell 2010;7:671–81.

[62] Choi YJ, Lin CP, Ho JJ, He X, Okada N, Bu P, et al. MiR-34 miRNAs provide a barrier for somatic cell reprogramming. Nat Cell Biol 2011;13:1353–60.

[63] Ye D, Wang G, Liu Y, Huang W, Wu M, Zhu S, et al. MiR-138 promotes induced pluripotent stem cell generation through the regulation of the p53 signaling. Stem Cells 2012;30:1645–54.

[64] Anokye-Danso F, Trivedi CM, Juhr D, Gupta M, Cui Z, Tian Y, et al. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell 2011;8:376–88.

[65] Miyoshi N, Ishii H, Nagano H, Haraguchi N, Dewi DL, Kano Y, et al. Reprogramming of mouse and human cells to pluripotency using mature microRNAs. Cell Stem Cell 2011;8:633–8.

[66] Liao B, Bao X, Liu L, Feng S, Zovoilis A, Liu W, et al. MicroRNA cluster 302–367 enhances somatic cell reprogramming by accelerating a mesenchymal-to-epithelial transition. J Biol Chem 2011;286:17359–64.

[67] Wang G, Guo X, Hong W, Liu Q, Wei T, Lu C, et al. Critical regulation of miR-200/ZEB2 pathway in Oct4/Sox2-induced mesenchymal-to-epithelial transition and induced pluripotent stem cell generation. Proc Natl Acad Sci U S A 2013;110:2858–63.

[68] 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 2011;39:1054–65.

[69] Kim JK, Samaranayake M, Pradhan S. Epigenetic mechanisms in mammals. Cell Mol Life Sci 2009;66:596–612.

[70] Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 2007;129:1401–14.

[71] Melton C, Judson RL, Blelloch R. Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. Nature 2010;463:621–6.

[72] Piskounova E, Viswanathan SR, Janas M, LaPierre RJ, Daley GQ, Sliz P, et al. Determinants of microRNA processing inhibition by the developmentally regulated RNA-binding protein Lin28. J Biol Chem 2008;283:21310–4.

[73] John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS. Human MicroRNA targets. PLoS Biol 2004;2:e363.

[74] Heo I, Joo C, Cho J, Ha M, Han J, Kim VN. Lin28 mediates the terminal uridylation of let-7 precursor microRNA. Mol Cell 2008;32:276–84.

[75] Heo I, Joo C, Kim YK, Ha M, Yoon MJ, Cho J, et al. TUT4 in concert with Lin28 suppresses microRNA biogenesis through pre-microRNA uridylation. Cell 2009;138:696–708.

[76] Yamaguchi S, Yamahara K, Homma K, Suzuki S, Fujii S, Morizane R, et al. The role of microRNA-145 in human embryonic stem cell differentiation into vascular cells. Atherosclerosis 2011;219:468–74.

[77] 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;137:647–58.

[78] O'Loghlen A, Munoz-Cabello AM, Gaspar-Maia A, Wu HA, Banito A, Kunowska N, et al. MicroRNA regulation of Cbx7 mediates a switch of Polycomb orthologs during ESC differentiation. Cell Stem Cell 2012;10:33–46.

[79] Zhao C, Sun G, Li S, Lang MF, Yang S, Li W, et al. MicroRNA let-7b regulates neural stem cell proliferation and differentiation by targeting nuclear receptor TLX signaling. Proc Natl Acad Sci U S A 2010;107:1876–81.

[80] Kawahara H, Okada Y, Imai T, Iwanami A, Mischel PS, Okano H. Musashi1 cooperates in abnormal cell lineage protein 28 (Lin28)-mediated let-7 family microRNA biogenesis in early neural differentiation. J Biol Chem 2011;286:16121–30.

[81] Delaloy C, Liu L, Lee JA, Su H, Shen F, Yang GY, et al. MicroRNA-9 coordinates proliferation and migration of human embryonic stem cell-derived neural progenitors. Cell Stem Cell 2010;6:323–35.

[82] Wilson KD, Venkatasubrahmanyam S, Jia F, Sun N, Butte AJ, Wu JC. MicroRNA profiling of human-induced pluripotent stem cells. Stem Cells Dev 2009;18:749–58.

[83] Kim H, Lee G, Ganat Y, Papapetrou EP, Lipchina I, Socci ND, et al. MiR-371-3 expression predicts neural differentiation propensity in human pluripotent stem cells. Cell Stem Cell 2011;8:695–706.

[84] Colas AR, McKeithan WL, Cunningham TJ, Bushway PJ, Garmire LX, Duester G, et al. Whole-genome microRNA screening identifies let-7 and mir-18 as regulators of germ layer formation during early embryogenesis. Genes Dev 2012;26:2567–79.

[85] Chaudhuri AA, So AY, Mehta A, Minisandram A, Sinha N, Jonsson VD, et al. Oncomir miR-125b regulates hematopoiesis by targeting the gene Lin28A. Proc Natl Acad Sci U S A 2012;109:4233–8.

[86] Martinez NJ, Gregory RI. MicroRNA gene regulatory pathways in the establishment and maintenance of ESC identity. Cell Stem Cell 2010;7:31–5.

[87] Ivey KN, Muth A, Arnold J, King FW, Yeh RF, Fish JE, et al. MicroRNA regulation of cell lineages in mouse and human embryonic stem cells. Cell Stem Cell 2008;2:219–29.

[88] Crist CG, Montarras D, Pallafacchina G, Rocancourt D, Cumano A, Conway SJ, et al. Muscle stem cell behavior is modified by microRNA-27 regulation of Pax3 expression. Proc Natl Acad Sci U S A 2009;106:13383–7.

[89] Cheung TH, Quach NL, Charville GW, Liu L, Park L, Edalati A, et al. Maintenance of muscle stem-cell quiescence by microRNA-489. Nature 2012;482:524–8.

[90] Tzur G, Levy A, Meiri E, Barad O, Spector Y, Bentwich Z, et al. MicroRNA expression patterns and function in endodermal differentiation of human embryonic stem cells. PLoS One 2008;3:e3726.

[91] Joglekar MV, Joglekar VM, Hardikar AA. Expression of islet-specific microRNAs during human pancreatic development. Gene Expr Patterns 2009;9:109–13.

[92] Wei R, Yang J, Liu GQ, Gao MJ, Hou WF, Zhang L, et al. Dynamic expression of microRNAs during the differentiation of human embryonic stem cells into insulin-producing cells. Gene 2013;518:246–55.

[93] Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, et al. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 2009;458:223–7.

[94] Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, et al. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci U S A 2009;106:11667–72.

[95] Sheik Mohamed J, Gaughwin PM, Lim B, Robson P, Lipovich L. Conserved long noncoding RNAs transcriptionally regulated by Oct4 and Nanog modulate pluripotency in mouse embryonic stem cells. RNA 2009;16:324–37.

[96] Guttman M, Donaghey J, Carey BW, Garber M, Grenier JK, Munson G, et al. lincRNAs act in the circuitry controlling pluripotency and differentiation. Nature 2011;477:295–300.

[97] Chakraborty D, Kappei D, Theis M, Nitzsche A, Ding L, Paszkowski-Rogacz M, et al. Combined RNAi and localization for functionally dissecting long noncoding RNAs. Nat Methods 2012;9:360–2.

[98] Wang Y, Xu Z, Jiang J, Xu C, Kang J, Xiao L, et al. Endogenous miRNA sponge lincRNA-RoR regulates Oct4, Nanog, and Sox2 in human embryonic stem cell self-renewal. Dev Cell 2013;25:69–80.

[99] Loewer S, Cabili MN, Guttman M, Loh YH, Thomas K, Park IH, et al. Large intergenic non-coding RNA-RoR modulates reprogramming of human induced pluripotent stem cells. Nat Genet 2010;42:1113–7.

[100] Navarro P, Avner P. When X-inactivation meets pluripotency: an intimate rendezvous. FEBS Lett 2009;583:1721–7.

[101] Navarro P, Chambers I, Karwacki-Neisius V, Chureau C, Morey C, Rougeulle C, et al. Molecular coupling of Xist regulation and pluripotency. Science 2008;321:1693–5.

[102] Donohoe ME, Silva SS, Pinter SF, Xu N, Lee JT. The pluripotency factor Oct4 interacts with Ctcf and also controls X-chromosome pairing and counting. Nature 2009;460:128–32.

[103] Zhao J, Sun BK, Erwin JA, Song JJ, Lee JT. Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. Science 2008;322:750–6.

[104] Bertani S, Sauer S, Bolotin E, Sauer F. The noncoding RNA Mistral activates Hoxa6 and Hoxa7 expression and stem cell differentiation by recruiting MLL1 to chromatin. Mol Cell 2011;43:1040–6.

[105] Ng SY, Johnson R, Stanton LW. Human long non-coding RNAs promote pluripotency and neuronal differentiation by association with chromatin modifiers and transcription factors. Embo J 2012;31:522–33.

[106] Liu L, Luo GZ, Yang W, Zhao X, Zheng Q, Lv Z, et al. Activation of the imprinted Dlk1-Dio3 region correlates with pluripotency levels of mouse stem cells. J Biol Chem 2010;285:19483–90.

[107] Stadtfeld M, Apostolou E, Akutsu H, Fukuda A, Follett P, Natesan S, et al. Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. Nature 2010;465:175–81.