

## Review

# Switching cell fate, ncRNAs coming to play

D Guan<sup>1,5</sup>, W Zhang<sup>1,5</sup>, W Zhang<sup>\*,2</sup>, G-H Liu<sup>\*,1</sup> and JC Izpisua Belmonte<sup>\*,3,4</sup>

Cell fate decision is a critical step during physiological development when embryonic stem cells commit to either becoming adult stem cells or somatic cells. Recent advances in reprogramming demonstrate that a similar set of transcription factors (TFs), which are important for maintaining the pluripotent state of stem cells, can also reprogram somatic cells to induced pluripotent stem cells (iPSCs). In addition, trans-differentiation, which entails the use of different sets of defined factors, whereby one type of somatic cell can be directly converted into another and even to cell types from different germ layers has become a parallel widely used approach for switching cell fate. All these progresses have provided powerful tools to manipulate cells for basic science and therapeutic purposes. Besides protein-based factors, non-coding RNAs (ncRNAs), particularly microRNAs and long ncRNAs, are also involved in cell fate determination, including maintaining self-renewal of pluripotent stem cells and directing cell lineage. Targeting specific ncRNAs represents an alternative promising approach to optimize cell-based disease modeling and regenerative therapy. Here we focus on recent advances of ncRNAs in cell fate decision, including ncRNA-induced iPSCs and lineage conversion. We also discuss some underlying mechanisms and implications in molecular pathogenesis of human diseases.

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### Facts

1. Certain non-coding RNAs (ncRNAs) such as some microRNAs (miRNAs) or long ncRNAs (lncRNAs) are critically involved in induced pluripotency or trans-differentiation.
2. Depending on cellular context and species, different miRNAs or lncRNAs can have opposite roles during cell fate transition process.
3. ncRNAs regulate various signaling pathways that are critical for cell fate determination.
4. Studies of ncRNAs on cell fate decision are very important for understanding pathogenesis of genetic diseases and clinical applications, but it is only emerging and more interesting questions are being raised and await answers.

### Open Questions

1. How to improve transfection efficiency of ncRNAs into host cells and how to maintain the effective dose to convert cell fate.

2. How to avoid activation or repression of unwanted targets to initiate lineage-specific programs, and how to activate or shut down certain pathways to obtain temporal gene expression signatures amenable to unique cell fate.
3. How to evaluate potential safety issues caused by ectopic overexpression of ncRNAs such as the off-target effect, cell-type specificity and dose dependency, especially in the context of clinical applications.

### ncRNAs

ncRNAs consist of various RNA species that are not translated and evolutionarily conserved among organisms. One single ncRNA may control hundreds of genes. Based on their length, these regulatory ncRNAs can be further divided into short ncRNAs including small interfering RNAs, miRNAs and PIWI-interacting RNAs, intermediate ncRNAs like small nucleolar RNAs, and lncRNAs. Of particular interest, we will give some brief background of miRNAs and lncRNAs. Mature miRNAs are a group of short ncRNAs with approximate 20

<sup>1</sup>National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China; <sup>2</sup>Department of Pathology, Carver College of Medicine, University of Iowa, Iowa City, IA, USA; <sup>3</sup>Gene Expression Laboratory, Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA, USA and <sup>4</sup>Center for Regenerative Medicine in Barcelona, Dr Aiguader 88, 08003 Barcelona, Spain

\*Corresponding authors: G-H Liu, National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China. Tel: +86 10 64 888315; Fax: +86 10 648 89970. E-mail: ghliu@ibp.ac.cn

or W Zhang, Department of Pathology, Carver College of Medicine, University of Iowa, Iowa City, IA, USA. Tel: +1 3193358214; Fax: +1 3193358453. E-mail: weizhou-zhang@uiowa.edu

or JCI Belmonte, Gene Expression Laboratory, Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA. Tel: +858 453 4100; Fax: +858 453 2573. E-mail: belmonte@salk.edu

<sup>5</sup>These authors contributed equally to this work.

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**Abbreviations:** ncRNAs, non-coding RNAs; miRNAs, microRNAs; lncRNAs, long ncRNAs; lincRNAs, long intergenic non-coding RNAs; MET, mesenchymal-epithelial transition; TGF- $\beta$ , transforming growth factor- $\beta$ ; NF- $\kappa$ B, nuclear factor kappa-light-chain-enhancer of activated B cells; TFs, transcription factors; ESC, embryonic stem cell; iPSCs, induced pluripotent stem cells; UTRs, untranslated regions; BMP, bone morphogenic protein; XCI, X-chromosome inactivation; PRC2, polycomb repressive complex 2; REST, repressor element-1 silencing transcription factor; lincRNA-RoR, lincRNA-regulator of reprogramming; BAF53b, a 53KD subunit of BRG1/brm-associated factor complex; OSKM, the combination of Oct4, Sox2, Klf4 and c-Myc

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nucleotides that target specific mRNA motifs, which may be located within the coding regions or untranslated regions (UTRs). Most miRNAs target hundreds of genes and mainly repress post-transcriptional protein expression. Processing miRNAs require specific factors including Drosha and Dicer, deletion of which abolishes miRNA maturation and critical for embryonic development.<sup>1</sup> LncRNAs are a group of ncRNAs > 200 nucleotides and widely distributed,<sup>1</sup> among which long intergenic ncRNAs represent one particular class located within intergenic regions in the genome with a specific chromatin signature, usually lysine methylation on histones.<sup>2,3</sup> They contain some characteristics of mRNA, including 5' capping and splicing, but have no peptide-encoding open reading frames.<sup>4,5</sup> LncRNAs are able to regulate protein expression at transcriptional or post-transcriptional level by targeting modifiers to a specific genomic position or working as an enhancer. Below we summarized recent studies that focused on elucidating the essential roles of miRNAs (Table 1) and lncRNAs (Table 2) in somatic cell reprogramming and trans-differentiation.

## ncRNAs and Reprogramming

**miRNAs and reprogramming.** The idea that miRNAs might be involved in pluripotency of stem cells came from the initial discovery of embryonic stem cell (ESC)-specific miRNAs.<sup>6,7</sup> Follow-up studies demonstrated that a subset of these miRNAs have essential roles in cell cycle regulation and self-renewal of ESCs.<sup>1,8–11</sup> Of note, transcription factors

(TFs) could be completely replaced by certain ESC-specific miRNAs to efficiently reprogram human or mouse somatic cells to induced pluripotent stem cells (iPSCs).<sup>11</sup> The role of miRNAs in reprogramming is indispensable and irreplaceable because the same set of reprogramming factors fail to reprogram somatic cells when certain miRNA expression is defective.<sup>12</sup> One good example of ESC-specific miRNAs is the miR-302 family that is shown to drive the initiation of a pluripotent state.<sup>13–17</sup>

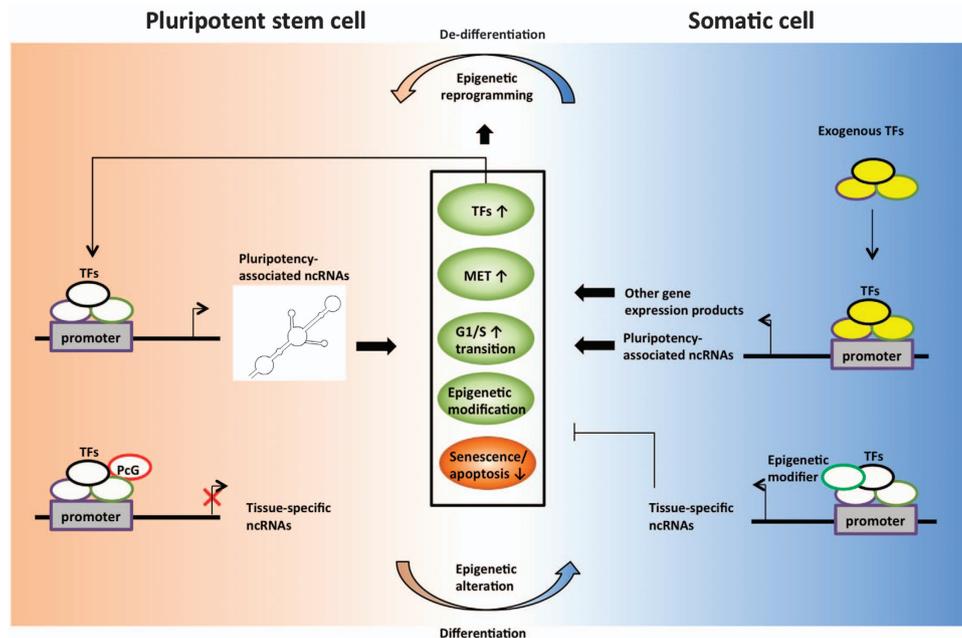
Many signaling pathways have been implicated in mediating miRNA-induced reprogramming, including those involved in mesenchymal–epithelial transition (MET), cell cycle regulation, epigenetic modification, and others like nuclear factor kappa-light-chain-enhancer of activated B cell (NF- $\kappa$ B) and transforming growth factor (TGF)- $\beta$  pathways<sup>18</sup> (Figure 1). The miR-302/367 family are able to facilitate pluripotency by regulating all above pathways, which may explain why miR302/367 are sufficient to induce somatic cell reprogramming.<sup>14,15,17,19,20</sup> MET has been proposed to be required for the initial phase of reprogramming and thus could be a preferred target for various miRNAs.<sup>21,22</sup> Some of those miRNAs regulate TGF- $\beta$  signaling, leading to increased E-cadherin expression, a hallmark of epithelial cells. For example, miR-205 and miR-200 are induced at the initial stage of reprogramming and promote MET in a bone morphogenic protein (BMP, TGF- $\beta$  superfamily member)-dependent manner, likely through inhibiting Zeb1 and Zeb2, two transcriptional repressors for E-cadherin expression.<sup>22,23</sup> miR-93 and miR-106b regulate TGF- $\beta$  receptor 2 during MET process.<sup>24</sup> miRNAs in the miR-290 cluster share a similar

**Table 1** miRNAs and cell fate decision

	Negative/ positive	Functions	Species	Co-operators	Target genes	Reference
miR-302/367 family	Positive	iPSCs generation, maintenance of hESCs pluripotency, regulation of hESCs differentiation.	Mouse/ human	Alone or with other ESC-specific TFs or miRNAs	CDKN1A, RBL2, AOF1/2, MECP1/2, MR2F2, RHOC, TGF- $\beta$ signaling, BMP signaling	13–16,19
miR-291-3p, miR-294, miR-295	Positive	iPSCs generation	Mouse	OSK	NF- $\kappa$ B subunit p65	11,25
miR-108a*	Positive	Inhibit somatic differentiation	Chicken			74
miR-302, miR-456	Positive	Maintain pluripotency	Chicken			74
miR-17-92, miR-106b-25, miR-106a-363	Positive	iPSCs generation	Mouse	OSK/OSKM	TGF- $\beta$ receptor II, P21	55
miR-138	Positive	iPSCs generation	Mouse	OSK/OSKM	p53	32
Combination of miR-200c, miR-302 s and miR-369 s	Positive	iPSCs generation	Human			75
miR-199a-3p	Negative	Barrier to iPSCs generation, downstream mediator of p53	Mouse	OSK/OSKM		34
miR-34	Negative/ positive	Barrier to iPSCs generation, downstream mediator of p53; leading to coronary artery disease	Mouse	OSM/OSKM	SIRT1	33,76
miR-21, miR-29a	Negative	iPSCs generation	Mouse	OSKM	p53, ERK1/2	35
miR-145	Negative	Repressing self-renewal capability and pluripotency in hESCs; inducing differentiation	Human		OCT4, SOX2 and KLF4	77
Let-7	Negative	ESCs self-renewal	Mouse		Lin28	78
miR-124, miR-9/9*	Positive	Direct induction of reprogramming to neuron cells	Human	MYE1L, BRN2	REST, CoREST, PTBP-1, npBAF complex	55,56

**Table 2** LncRNAs and cell fate decision

	Negative/positive	Functions	Species	Co-operators	Target genes	Reference
LincRNA-RoR (ST8SIA3)	Positive	iPSCs generation	Human	OSKM		38
AK028326 AK141205	Positive	ESCs pluripotency maintenance	Mouse		OCT4	41



**Figure 1** Scheme describing how ncRNAs modulate induction of somatic cells to iPSCs. Multiple mechanisms are involved: (I) activating pluripotency-associated TFs; (II) activating MET in the context of iPSC formation; (III) promoting cell cycle progression and/or inhibiting apoptosis; and (IV) modulating chromatin-modifying enzymes to affect epigenetic reprogramming of somatic cells. Of note, conventional TFs are able to activate ncRNAs targeting various signaling pathways to facilitate reprogramming and PcG components block the transcription of tissue-specific ncRNAs by co-occupying their promoters with TFs

seeding sequence to activate NF- $\kappa$ B signaling pathway.<sup>24,25</sup> p53, a well-studied tumor-suppressor gene and whose activation has been known to be a roadblock for reprogramming, is proved to be a good target for reprogramming-inducing miRNAs.<sup>26–31</sup> miR-138 directly targets 3'-UTR of p53 mRNA and significantly increases reprogramming efficiency.<sup>32</sup> In addition to regulate specific pathways mentioned above, miRNAs could modify global gene expression profile by controlling epigenetic factors to induce pluripotency.<sup>15,19</sup> miR-302 represses at least four different epigenetic regulators including lysine-specific histone demethylase 1 and 2, and methyl-CpG-binding proteins 1 and 2, which in turn leads to global demethylation and activation of pluripotency-associated genes.<sup>15,19</sup> Another set of tissue-specific miRNAs, including miR-21, miR-29a, miR-34 and miR-199a-3p, have a suppressive role during reprogramming<sup>16,33–35</sup> (Figure 1). Such miRNAs use various strategies to inhibit reprogramming. miR-21 and miR-29a target pluripotent factors involved in p53 and Erk1/2 pathways to build tissue-specific barriers; miR-34 and miR-199a-3p repress proliferation;<sup>33–35</sup> miR-34 and miR-199a-3p are also involved in p53-associated inhibition of reprogramming in synergy with p21, another p53 downstream effector.<sup>27,33,34</sup>

**LncRNAs and reprogramming.** LncRNAs represent another group of ncRNAs that are involved in cell fate decision. Loss of function studies demonstrate that lncRNAs regulate genetic and epigenetic activities primarily in a *trans*-manner at transcription level, a mechanism that differs from siRNA/miRNA pathway.<sup>36,37</sup> The first direct evidence of lncRNA in reprogramming came from the Rinn lab who demonstrated that lincRNA-regulator of reprogramming bears the ability to modulate reprogramming.<sup>38</sup> Another example is Xist, a marker of X-chromosome inactivation (XCI) and identified as a molecular signature of human iPSCs.<sup>39</sup> Xist-deficient iPSCs exhibit increased expression of some X-linked oncogenes, abnormal growth rates and deficient differentiation potential relative to normal iPSCs.<sup>39</sup> LncRNAs may serve as a good benchmark to evaluate certain aspects of stem cell quality. In addition, both Xist lncRNA and its target polycomb repressive complex 2 (PRC2) are required for XCI.<sup>40</sup> A positive feedback loop is identified between lncRNAs and TFs in ESCs, probably through epigenetic activation.<sup>2,36,37,41–43</sup> AK028326 and AK141205 are two lncRNAs that were identified as direct targets of the key pluripotent factors Oct4 and Nanog in mouse ESCs, respectively. Although a

direct connection between lncRNAs and reprogramming is still missing, owing to the critical role of Oct4 in pluripotency and ES lineage-specific differentiation, these results strongly imply a role of these lncRNAs in controlling stem cell fate.<sup>41</sup>

### ncRNAs and Trans-Differentiation

Trans-differentiation refers to direct conversion of one somatic cell type into another. This approach could avoid the induced pluripotent state that bears perceivable higher oncogenic potential than somatic cells and directly generate patient-specific progenitors or somatic cells for disease modeling and personalized regenerative therapy.<sup>44</sup> The capability of TFs to trigger trans-differentiation was initially unveiled by Davis *et al.*<sup>45</sup> More recently, the Wernig group<sup>46</sup> demonstrated that mesoderm cells (e.g., fibroblasts) can be directly converted into functional neurons. During the process of trans-differentiation into neurons or their precursors, combinations of multiple TFs have been used.<sup>47–53</sup> Besides TFs, ncRNAs are also involved in trans-differentiation, either alone or in combination with TFs. As recently summarized by Shenoy and Blalock,<sup>54</sup> miRNAs seem to inhibit lineage suppressors to lower the threshold for commitment. One example is miR-124, when combined with MYT1L and BRN2 or miR-9/9\*, is able to convert human fibroblasts to functional neurons.<sup>55,56</sup> However, at this stage it is not clear how miRNAs manage to activate neuronal-specific pathways. It will be logical to hypothesize that miR-124 and miR-9/9\* target components of chromatin-remodeling complexes, such as BAF53a, PTBP-1 and components of the repressor element-1 silencing transcription factor (REST) complex, which in turn remodels chromatin structure and turns on the neuron-specific epigenetic switch. Yoo *et al.*<sup>56</sup> demonstrated that miR-124 and miR-9/9\* suppress fibroblast-expressing BAF53a and activated neurogenesis-essential BAF53b (a 53KD subunit of BRG1/brm-associated factor complex), serving as a potential explanation for miRNA-induced neuronal commitment.<sup>54</sup>

Although direct evidence for a role of lncRNAs in trans-differentiation is yet-to-be established, lncRNAs have been shown to be critical for regulating the expression of Malat1, Gomafu, Neat1 and RMST, factors that are involved in neurogenesis and neural cell fate specification.<sup>57–60</sup> Some lncRNAs physically associate with neural TFs such as REST or epigenetic modulators PRC2, implying that lncRNAs may critically regulate neural trans-differentiation.<sup>58–60</sup>

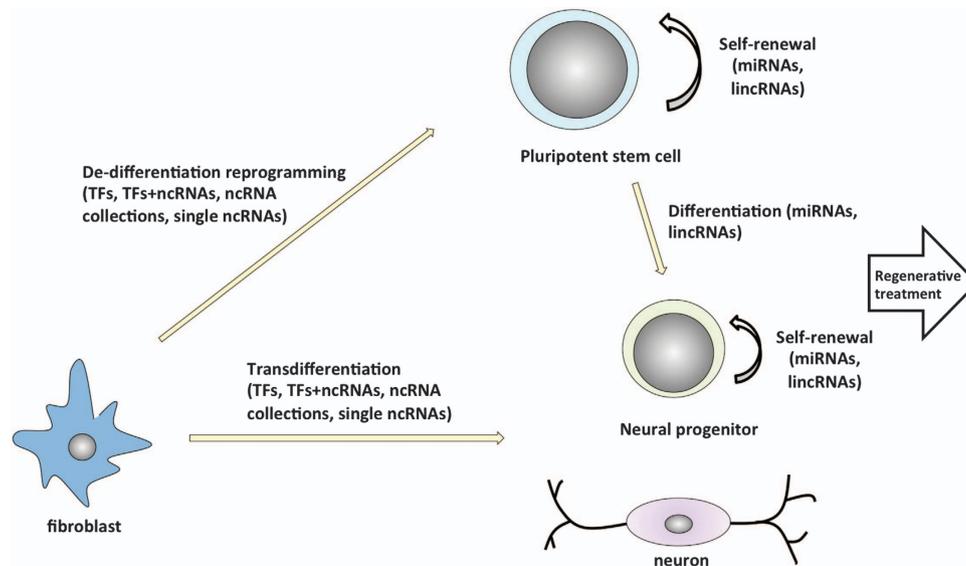
Although miRNAs and lncRNAs possess distinct regulatory mechanisms, they can interplay with each other during cell fate determination.<sup>61</sup> Certain lncRNAs share the same imprinted genomic region with miRNAs, as miRNAs are also mapped in non-coding genomic regions and sometimes share genomic regions with lncRNAs.<sup>3,62,63</sup> Several studies identified active *Dlk1-Dio3* region as a marker to distinguish iPSCs with full pluripotency from those that are partially reprogrammed.<sup>64–66</sup> Liu *et al.*<sup>63</sup> demonstrated that miRNA cluster transcribed from active *Dlk1-Dio3* region may in turn attenuate imprinting and promote expression of genes and lncRNAs located within the *Dlk1-Dio3* region in an epigenetic-dependent pattern by physically targeting

constituent parts of PRC2. Therefore, transcription of these lncRNAs is predicted to be under the control of the neighbor miRNAs in fully pluripotent stem cells.<sup>63</sup> Meanwhile, miRNAs could be transcriptionally adjusted by lncRNAs in corresponding region.<sup>67</sup> Epigenetic changes including DNA and histone modifications may serve as a switch of reciprocal regulations between miRNAs and lncRNAs.<sup>3,63,68</sup>

### Perspectives

The conversion of terminally differentiated cells to iPSCs or to other lineages entails dramatic transformations of epigenetic remodeling and gene expression, which was initially examined and validated by studying protein-based factors. Accumulating evidence indicates that ncRNAs target diverse cellular processes including epigenetic modifiers, key TFs, MET, as well as cell cycle regulators. Thus, the combined effect of ncRNAs could regulate cell fate decision in a similar way, if no more efficient than protein factors (Figure 2). Compared with protein-mediated reprogramming or lineage conversions, ncRNAs, especially miRNAs, are more easily introduced into primary cells relative to protein-coding vectors or *in vitro* recombinant proteins. They are also easier to be degraded and diluted in cells within several passages. In principle, serial transfection of small ncRNAs together with protein-encoding mRNAs can effectively alter cell fate with minimal toxic effect and alterations in genomic DNA. In addition, ncRNA-mediated cell fate switching appears to be more efficient. The Morrissey team reported that *miR302/367* can induce iPSCs generation approximately two-fold more efficiently than standard protein-based reprogramming.<sup>13</sup> This high efficiency may be partially explained by a coordinated action of more targeting effectors of miRNAs compared with protein factors. Despite these advantages, there are still many barriers before using ncRNAs in basic and therapeutic applications. These barriers include: (1) how to improve the transfection efficiency of miRNAs into host cells, and how to maintain their sustained cellular concentrations; (2) how to avoid activation or repression of unwanted targets of specific ncRNAs to initiate lineage-specific programs, and how to timely activate or shut down certain ncRNAs pathways to obtain spatio-temporal gene expression signatures amenable to unique cell fate; and (3) how to evaluate potential safety issues caused by ectopic over-expression of ncRNAs including off-target effects, cell-type-specific effects and dose-dependent effects, factors to be especially considered in the context of clinical applications. Addressing all these questions will significantly help to advance the development of optimal strategies for basic and therapeutic studies.

Increasing evidence has linked ncRNAs dysregulation to human diseases.<sup>69,70</sup> Particularly, genetic defects in ncRNAs are a common hallmark of human diseases like cancer and neurological disorders. Yin *et al.* found that depletion of one class of small nucleolar long ncRNAs is functionally related to Prader–Willi syndrome.<sup>71</sup> Such disease-causing dysregulation of ncRNAs will provide superior opportunities for studying disease pathophysiology and serve as targets of intervention for therapeutic purposes. Recent progress in iPSC-based gene targeting has established worldwide platforms to study



**Figure 2** Roles of ncRNAs in dedifferentiation and trans-differentiation. Like protein-coding factors, ncRNAs could promote dedifferentiation of somatic cells (e.g., fibroblasts) to iPSCs and other groups of ncRNAs could induce iPSCs to certain functional cells such as neural stem cells or neurons. ncRNAs could also directly convert one type of somatic cells to another type

the cellular and molecular mechanisms involved in various hereditary diseases. These platforms could be extended to manipulate expression of ncRNAs in patient-derived iPSCs.<sup>72,73</sup> Correction of disease-causing events related to dysregulation of ncRNAs may provide alternative strategies for cell replacement therapies of genetic diseases.<sup>73</sup>

### Conflict of Interest

The authors declare no conflict of interest.

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