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Review article

# The expanding horizon of MicroRNAs in cellular reprogramming

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ABSTRACT

Research over the last few years in cellular reprogramming has enlightened the magical potential of microRNAs (miRNAs) in changing the cell fate from somatic to pluripotent. Recent investigations on exploring the role(s) of miRNAs in somatic cell reprogramming revealed that they target a wide range of molecules and refine their protein output. This leads to fine tuning of distinct cellular processes including cell cycle, signalling pathways, transcriptional activation/silencing and epigenetic modelling. The concerted actions of miRNA on different pathways simultaneously strengthen the transition from a differentiated to de-differentiated state. Despite the well characterized transcriptional and epigenetic machinery underlying somatic cell reprogramming, the molecular circuitry for miRNA mediated cellular reprogramming is rather fragmented. This review summarizes recent findings addressing the role of miRNAs in inducing or suppressing reprogramming thus uncovering novel potentials of miRNAs as regulators of induced pluripotency maintenance, establishment and associated signalling pathways. Our bioinformatic analysis sheds light on various unexplored biological processes and pathways associated with reprogramming inducing miRNAs, thus helps in identifying roadblocks to full reprogramming. Specifically, the biological significance of highly conserved and most studied miRNA cluster, i.e. miR-302-367, in reprogramming is also highlighted. Further, roles of miRNAs in the differentiation of neurons from iPSCs are discussed. A recent approach of direct conversion or transdifferentiation of differentiated cells into neurons by miRNAs is also elaborated. This approach is now widely gaining impetus for the generation of neurological patient's brain cells directly from his/her somatic cells in an efficient and safe manner. Thus, decoding the intricate circuitry between miRNAs and other gene regulatory networks will not only uncover novel pathways in the direct reprogramming of somatic cells but will also open new avenues in stem cell biology.



**Contents**



*Abbreviations:* OSK (3 factors), OCT4, SOX2, KLF4; OSKM (4 factors), OCT4, SOX2, KLF4, MYC; ESCs, Embryonic stem cells; MEFs, Mouse embryonic fibroblasts; iPSCs, Induced pluripotent stem cells; MBD2, methyl DNA binding domain protein 2; MECP1/2, methyl-CpG binding proteins 1 and 2; MiRNA, MicroRNA; hHFCs, human hair follicle cells; HDFs, human dermal fibroblasts.

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## 1. Introduction

Stem cell biology is a broad area of life sciences which aims to reveal novel therapeutic regimens using stem cells with an emphatic aspect to study the molecular and genetic machinery and regulatory signalling pathways in stem cells. The embryonic stem cells (ESCs) possess the unique characteristic of self renewal, pluripotency and differentiation as they are derived from inner cell mass of blastocyst. They have unlimited potential of undifferentiated proliferation *in vitro* (Thomson et al., 1998). Nevertheless, due to ethical issues concerning the destruction of human embryos for derivation of ESCs, human embryonic stem cell research often encounters challenges. As an alternative to ESCs and for patient specific and disease specific therapy, another potential source of stem cells in the form of induced pluripotent stem cells (iPSCs) gathered due attention by stem cell researchers. Like ESCs, iPSCs also possess enormous ability to give rise to any type of differentiated cells including neurons. Recently, a new category of small non-coding RNAs, namely microRNAs (miRNAs) has gathered overwhelming attention due to their involvement in a vast array of biological functions. While several physiological processes have shown the critical performance of miRNAs in their fine tuning, functions of miRNAs in cell fate reprogramming and pluripotency require extensive exploration. Therefore, the scientific community has set out to unleash the crucial functions of these small non-coding RNAs in inducing pluripotency and cell fate reprogramming of differentiated cells. More recently, research is geared to direct transition of one lineage of differentiated cells to another lineage of differentiated cells. This is particularly important and espial for the cells of central nervous system where it is of utmost need to obtain patient's sample in dish to explore the neurological diseases at cellular and molecular levels. These transdifferentiated neurons have achieved tremendous specificity to the patient's brain cells and thus will help in better understanding of the neurological disease in a personalized way by displaying the same aetiology and pathophysiology of the disease as compared to animal models, cell lines and post-mortem tissues.

Here, we discuss recent discoveries of the novel functions of miRNAs and their mechanisms in inducing as well as suppressing pluripotency in differentiated cells. Further, our bioinformatic analysis revealed the biological significance of pluripotency inducing miRNAs and of the most studied miRNA cluster (miR–302 ~ 367 cluster) in pluripotency by highlighting the uncharted biological pathways on which future investigations can be spearheaded. We also focused on miRNAs that induce neurons directly-from differentiated cells using transdifferentiation approaches. We have provided some new insights into the mode of action of miRNAs in transdifferentiation of neurons in particular. The review also discusses therapeutic potential of miRNAs with emphasis on areas for future investigations. We believe, understanding the functions of major regulatory elements of cells like miRNAs in pluripotency would not only enhance our basic understanding but also help in setting iPSC translational research.

### 1.1. Induced pluripotent stem cells (iPSCs)

IPSCs belong to a different class of pluripotent stem cells which can be obtained from somatic differentiated cells by reprogramming their genome and epigenome using a cocktail of transcription factors. They are usually considered equivalent to ESCs as they exhibit the crucial characteristics of ESCs in the form of self renewal and differentiation potential. It was proposed quite early on that the somatic cells have the potential of reverting their state of differentiation back to pluripotent (Gurdon, 1962; Tada et al., 1997; Wilmut et al., 1997). The novel method discovered by Takahashi et al. in 2006 has transformed the sphere of regenerative medicine by recapitulating the pluripotent nature of differentiated cells and led to the derivation of iPSCs (Takahashi and Yamanaka, 2006). The first iPSCs were generated from mice using the gain of function technique by overexpressing several pluripotency inducing factors including OCT4, SOX2, c-MYC and KLF4 for the activation of self-renewal program and blockage of differentiation (Takahashi and Yamanaka, 2006). The first human iPSCs came into existence by the viral transduction of OCT4, SOX2, c-MYC and KLF4 (Yamanaka factors) into adult human dermal fibroblasts (HDFs) in 2007 (Takahashi et al., 2007). Another group led by James Thomson used a slightly different cocktail and employed NANOG and LIN28 in place of c-MYC and KLF-4 for the induction of reprogramming in human fibroblasts (Yu et al., 2007). The cocktail of transcription factors including OCT4, SOX2, NANOG and LIN28 was also able to reprogramme the genomic orchestra of somatic differentiated cells into a pluripotent one (Walia et al., 2012). The iPSCs possess several properties like ESCs including morphological characteristics, gene expression profile, surface antigens, epigenetic status and differentiation potential. Thus far, different types of somatic cells have been used, ranging in origin from dermal fibroblast cells to human adipose tissues for the generation of iPSCs (Aoki et al., 2010; Lai et al., 2011; Lowry et al., 2008; Walia et al., 2012).

As reprogramming of somatic cells is a multistep, stochastic, inefficient and gradual process, therefore, in a single population of reprogrammed cells, only a few individual cells display same transcriptional status (Greve et al., 2013). The complete reprogramming process has been hypothesized to take place via two phases i.e. early and late (Samavarchi-Tehrani et al., 2010). In the early phase of reprogramming, somatic cells get reprogrammed to a pre-pluripotent state along with increased proliferation rate and a conversion from mesenchymal to an epithelial-like cell state also occurs. In the late phase of reprogramming, genes associated with pluripotency including Sox2 and Nanog get induced, stabilizing the central pluripotency network and the pre-pluripotent cells subsequently get fully reprogrammed to iPS cells. After the full reprogramming has been attained, the exogenous reprogramming