The role of microRNAs in embryonic stem cell and induced pluripotent stem cell differentiation in male germ cells

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Abstract
New perspectives have been opened by advances in stem cell research for reproductive and regenerative medicine. Several different cell types can be differentiated from stem cells (SCs) under suitable in vitro and in vivo conditions. The differentiation of SCs into male germ cells has been reported by many groups. Due to their unlimited pluripotency and self-renewal, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) can be used as valuable tools for drug delivery, disease modeling, developmental studies, and cell-based therapies in regenerative medicine. The unique features of SCs are controlled by a dynamic interplay between extrinsic signaling pathways, and regulations at epigenetic, transcriptional and posttranscriptional levels. In recent years, significant progress has been made toward better understanding of the functions and expression of specific microRNAs (miRNAs) in the maintenance of SC pluripotency. miRNAs are short noncoding molecules, which play a functional role in the regulation of gene expression. In addition, the important regulatory role of miRNAs in differentiation and dedifferentiation has been recently demonstrated. A balance between differentiation and pluripotency is maintained by miRNAs in the embryo and stem cells. This review summarizes the recent findings about the role of miRNAs in the regulation of self-renewal and pluripotency of iPSCs and ESCs, as well as their impact on cellular reprogramming and stem cell differentiation into male germ cells.

KEYWORDS
embryonic stem cells, induced pluripotent stem cells, male germ cells, miRNAs, reprogramming

1 | INTRODUCTION

Germ cells are able to transfer genetic information to offspring in sexually reproductive species, such as mammals. The emerge of pluripotent stem cells in biology is considered as a breakthrough, which resulted in a new field of science known as regenerative medicine (Medrano, Pera, & Simon, 2013). One of the fields of research, which has had considerable effects in this regard, is regenerative medicine. All forms of cell therapy, including induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs), have played a significant role in some fields of medicine (Bianconi et al., 2018; Hirschli, Li, & Roy, 2014).

The most significant abilities of stem cells include self-renewal and differentiation. Currently, the main sources of stem cells to differentiate into male germ cells are the ESCs, the iPSCs, and spermatogonial stem cells (SSCs; Hou et al., 2014). Scientists have
recently established the iPSCs cells from somatic cells in a breakthrough by overexpressing some transcription factors, including c-Myc, Klf4, Sox2, and Oct4 (Woltjen et al., 2009). Despite the ability of iPSCs to act as sperm cells and express functional genes, there are some safety challenges in their application, which seem more propitious to generate a safe and effective solution for infertility in men (Zhang et al., 2014). Several studies have shown the differentiation ability of ESCs into germ cells in vitro (Jung et al., 2017).

Intercellular and intracellular mechanisms control self-renewing divisions of stem cells (Judson, Babiarz, Venere, & Blelloch, 2009). Differential gene expression is regulated by intracellular mechanisms at epigenetic, transcriptional, translational, and posttranslational levels, whereas the neighboring niche cell signaling is controlled by intercellular mechanisms (Kim et al., 2012). iPSCs have been reported to be fairly similar to their embryonic counterparts. However, it has been suggested by an study of gene expression profiles of human iPSCs (hiPSCs), mouse iPSCs (miPSCs), mouse ESCs (mESCs) and human ESCs (hESCs) that, regardless of the generation method or the origin, a recurrent signature of gene expression appears in iPSCs (Xu, Papagiannakopoulos, Pan, Thomson, & Kosik, 2009). The similarity of hiPSCs’ and hESCs’ gene-expression profiles increases when the culture is extended; however, there is still a unique signature of gene expression that is different from iPSCs and hESCs, extending to microRNA (miRNA) expression (Jia, Chen, & Kang, 2013). The iPSCs signature gene expression differences have been suggested by genome-wide data to be the result of a mechanism, in which the reprogramming factors differentially bind to promoter sequences (Chin et al., 2009).

Noncoding RNAs (ncRNAs) are required to establish the pluripotent network in cell reprogramming. They are also relatively essential for somatic cell reprogramming into pluripotency (Luginbühl, Sivaraman, & Shin, 2017). Long noncoding RNAs (lncRNAs) and miRNAs have recently emerged as important factors, which play critical roles in translational regulation and controlling stem cell behavior and fate (Gangaraju & Lin, 2009).

miRNAs, with the range of 18–23 bp, are considered as significant regulators in gene silencing at transcriptional and posttranscriptional levels in many organisms (Hayashi et al., 2008). miRNAs are able to regulate genetic controlling of differentiation and pluripotency of ESCs and reprogramming of iPSCs (Clancy et al., 2014). They are well-characterized as important elements with the ability of regulating differentiation and development. It has been demonstrated that specific miRNAs are highly expressed in iPSCs and ESCs to control the expression of pluripotency-related genes (Miyoshi et al., 2011). In somatic stem cells and ESCs of vertebrates, the critical role of miRNAs pathway has been reported in maintenance of germ line stem cells. Moreover, their roles have been demonstrated in the maintenance of germ line stem cells and speculation of primordial germ cells (PGCs) in invertebrates (Gangaraju & Lin, 2009). Furthermore, miRNAs are quite essential for spermatogenesis and may play a vital role during stages of mitosis, meiosis, and post-meiotic of germ cells and spermatogenesis by regulating the target gene expression (Wang & Xu, 2015).

Therefore, the present study aimed to study the current rapidly expanding state of understanding of miRNA roles in regulation of embryonic and induced pluripotent states into male germ cells.

## 2 | STEM CELLS

Generally, stem cells are characterized as cells with division ability for an unlimited period of time throughout an individual’s life (self-renewal; Mahabadi, Sabzalipour, Bafrani, Gheibi Hayat, & Nikzad, 2018; Wilson et al., 2009). They can differentiate into several various lineages, with specialized functions and different properties, under specific signals and appropriate differentiation conditions (Jaenisch & Young, 2008). In addition, these undifferentiated cells are able to either differentiate into specialized cells or self-renew. Due to their unique properties, stem cells are highly qualified for both biomedical and basic research in cell biology (Hu & Shan, 2016). Stem cells are classified into five groups of unipotent, oligopotent, multipotent, totipotent, and pluripotent based on their potential of differentiation (Zomer, Vidane, Goncalves, & Ambrosio, 2015).

## 3 | PLURIPOTENT STEM CELLS

Pluripotency is the potential of a cell in developing into all various cell types. This potential is found in an adult and embryonic organism, with the exception of extraembryonic organs, such as umbilical cord and placenta (Robinton & Daley, 2012). Both of these unique properties make pluripotent stem cells attractive sources for regenerative medicine and cell-based therapies (Luginbühl et al., 2017; Tabar & Studer, 2014). However, there are still some unresolved technical problems in the area of cell therapy, such as the best method for manipulation of cells, best delivery system of cells and best source of cells (Bianconi et al., 2017). Pluripotent stem cells are characterized in five types, including ESCs, embryonic carcinoma cells, embryonic germ cells, testis derived germ line stem cells, and iPSCs.

### 3.1 | Embryonic stem cells

According to the literature, ESCs are derived from the inner cell mass of mouse or human blastocysts, possessing the remarkable capacity of differentiation into the cells of all three germ layers as well as male and female germ cells (Qing et al., 2007). Recently, it has been reported that mESCs have the ability to produce sperm-like cells and PGCs in vitro (Pelosi, Forabosco, & Schlessinger, 2011). It is believed that the ability of germ cells production from mESCs present a powerful in vitro model, aiming to study the development of germ cells and to offer new infertility treatment approaches (Miryounesi, Nayernia, Dianatpour, & Mansouri, 2013; Silva et al., 2009; Zhou, Meng, & Li, 2010). To produce hESCs, serum containing medium and
fibroblast feeders were used, whereas differentiation in hESCs was caused by bone morphogenetic protein (BMP) and LIF, both of which support mESCs self-renewal. In contrast to mESCs, activin, TGF-β, and bFGF are the main factors needed for hESCs self-renewal (Greber, Lehrach, & Adjaye, 2007; Nii et al., 2014). However, there are some ethical limitations against the research on use of human embryos. Moreover, the tissue rejection in the recipient is a serious problem.

3.2 | Induced pluripotent stem cells

Any tissue of the body can be used to induce pluripotent stem cells by employing a mixture of reprogramming factors (Takahashi & Yamanaka, 2006). It has been reported that iPSCs or pluripotent ESCs of human, monkey, and rodent are able to differentiate into germ cells (Easley et al., 2012; Kee, Angeles, Flores, Nguyen, & Pera, 2009; Park et al., 2009). More important, the production of germ cell replacement with the patients’ own somatic cells will alleviate some problems associated with using current method to treat infertility (Teramura & Frampton, 2013). Differentiation of embryonic stem cells or induction of pluripotent stem cells into epiblast-like cells is possible to increase primordial germ cell-like cells if they are being cultured in the media with BMP-4 (Hayashi, Ohta, Kurimoto, Aramaki, & Saitou, 2011). However, the technology of the iPSC differentiation into germ cell is well developed for the human clinical applications (Gassei & Orwig, 2016; Figure 1).

3.3 | In vitro differentiation of PGCS towards a spermatogenic cell fate

Some coordinated steps are needed for the early gametogenesis process, including specification of PGC, movement to and colonization of the gonadal ridges, followed by differentiation into more mature gametes (Linher, Dyce, & Li, 2009). Currently, researchers have demonstrated the differentiation capability of ESCs into gametes (Geijsen et al., 2004; Hubner et al., 2003; Kerkis et al., 2007; Lacham-Kaplan, Chy, & Trounson, 2006; Linher et al., 2009; Nayernia et al., 2006; Novak et al., 2006; Qing et al., 2007; Toyooka, Tsunekawa, Akasu, & Noce, 2003). Several studies have used murine ESCs differentiation into embryoid bodies (EBs), the ability of which to generate putative PGC-like cells was approved later (Geijsen et al., 2004; Toyooka et al., 2003; J. A. West, Park, Daley, & Geijsen, 2006).

The gametogenesis process from the PGCs formation to functional gametes has not been wholly recreated in vitro in any species of mammalian. Currently, the most possible methods to generate functional oocytes or sperm from PGCs are based on in vitro transplantation of tissues, which contain PGCs, from embryos or after PGCs being reaggregated in vitro with somatic cells of gonads into the gonads of prepuberal/adult hosts (Ge, Chen, De Felici, & Shen, 2015).

ESCs are involved in many fundamental studies on pluripotency and differentiation to germ cells. The differentiation capability of mESCs into PGC-like cells, which are able to be engrafted into testis and to form sperm, has been reported as well (Toyooka et al., 2003). PGCs and haploid cells have been reported to emerge from hESCs (Aflatoonian & Moore, 2006). While spontaneous differentiation of pluripotent stem cells was the basis of the mentioned studies, the gonadal microenvironment and signaling molecules can strongly affect germ cell differentiation (Li et al., 2014).

Recently, germ cells production from iPSCs has been demonstrated by several reports. For the first time, PGC-like cells production was shown by coculture with fetal gonads of human (Park et al., 2009). Supplementation of BMP4 enhances the differentiation of hiPSCs to VASA-GFP-positive PGC-like cells (Eguizabal et al., 2011).

4 | DIFFERENTIATION OF GERM CELLS USING IPSCS AND ESCS

Cocultures containing embryonic fibroblasts increase differentiation of pluripotent cells into PGCs (Park et al., 2009) and sertoli cells (Geens, Sermon, Van de Velde, & Tournaye, 2011; F. D. West et al., 2008), which in response increase the differentiation of pluripotent stem cells to PGCs. Although desired results may be obtained using these coculture systems, biologically or chemically defined factors

**FIGURE 1** To generate induced pluripotent stem cells (iPSCs), retroviruses encoding four factors of pluripotency (OCT4, c-MYC, KLF4, and SOX2) are used to transduce adult somatic cells. Fully reprogrammed iPSCs were selected and spread and can be used in human disease models, cell therapy, and drug screening. The own cells of patient are capable to be used to derive iPSCs, of which several kinds of somatic cells with the same genetic information as the patient can be differentiated [Color figure can be viewed at wileyonlinelibrary.com]
are preferred for induction due to their ability to improve the cells’ safety for clinical application and enhance the reproducibility of differentiation procedure. Therefore, application of defined media with growth factors is considered as a standard option for differentiation induction (Amini Mahabadi et al., 2018). In this respect, BMP8b and BMP4 promote the ESCs differentiation into PGC-like cells (Hiller, Liu, Blumenthal, Gearhart, & Kerr, 2010), and retinoic acid (RA) can be used for meiosis stimulation (Eguizabal et al., 2011). Furthermore, cytokines such as forskolin, leukemia inhibitory factor (LIF), and SCF increase germ line differentiation from pluripotent stem cells by enhancing in vitro self-renewal of SSCs named GDNF (Eguizabal et al., 2011; F. D. West et al., 2010), and adenylylate cyclase activator. In addition, manipulations may occur in gene expression to control lineage specification of differentiating pluripotent stem cells. In this respect, it could be stated that overexpression of VASA and DAZL promotes PGCs to be formed from hESCs, whereas the overexpression of BOULE and DAZ promotes the development of the haploid germ (Kee et al., 2009). When haploid cells are induced by culturing the iPSC-derived PGCs in bFGF, forskolin, LIF, and an inhibitor of CYP26 (a P450 enzyme), and all-trans RA can be inactivated (Eguizabal et al., 2011). The rate of meiotic cell formation was improved by Medrano et al. through employing plasmids to induce DAZ and VASA overexpression in hiPSCs (Medrano, Ramathal, Nguyen, Simon, & Reijo Pera, 2012).

Molecular mechanisms for the development of primate germline cells and functional assays in vivo which can be extrapolated for the differentiation of human germ cell will be revealed by further studies (Teramura & Frampton, 2013; Figure 2). It has been demonstrated that ncRNAs play the regulatory role in stem cells. Primary explorations have discovered small ncRNAs, such as miRNA, Piwi-interacting RNA (piRNA), and small interfering RNA (siRNAs) in stem cells, all of which have been reported to play a regulatory role in processes such as male germ cell development. Nonetheless, further research is required to determine the precise function of ncRNAs in the development of male germ cells (Lee, Xiao, & Rennert, 2012).

5 | NONCODING RNA DEFINITION

Non-encoding RNAs (noncoding RNAs) can be characterized into two groups of regulatory noncoding RNAs and housekeeping noncoding RNAs. With a regulatory role, RNA is mainly divided into two types based on size: long noncoding RNA (lncRNAs) and short noncoding RNAs (e.g., miRNAs, siRNAs, and piRNAs; Wei, Huang, Yang, & Kang, 2017).

Considered as major players in defining a cell’s identity, ncRNA molecules were previously thought to exert only passive roles. Alongside with the coding portion of the genome, the correlation of noncoding counterpart with the greater complexity of higher eukaryote is now clear (Mattick, 2011). Recently, ncRNAs have been characterized as new regulatory factors in gene expression profile of pluripotent cells. Among small noncoding RNAs with the size of less than 200 nucleotides, miRNAs are now recognized as the major regulators of metabolism, development, homeostasis, and differentiation in all multicellular organisms (Rosa & Brivanlou, 2013; Rottiers & Näär, 2012).

Currently, the involvement of miRNAs in developmental processes (e.g., development of human preimplantation development) has been confirmed. In addition, they have been recognized as considerable adjusting molecules for dedifferentiation and differentiation of cells (Kedde & Agami, 2008). In addition, it seems that dissemination and progression of various types of cancer (e.g., ovarian cancer) are greatly affected by the dysregulation of miRNA expression (e.g., modulation miRNAs).

**FIGURE 2** The sources of cells, from which spermatogonia stem cells can be derived. (a) The isolation of pluripotent inner mass cells are obtained from donated fertilization embryos which are at the blastocyst stage, followed by indefinite expansion in culture as human embryonic stem cells (hESCs). (b) As an alternative way, patient-specific induced pluripotent stem cells (iPSCs) are able to be reprogrammed from somatic cells, such as fibroblasts, by transduction using a cocktail of embryonic transcription factors (sex-determining region Y-box 2 [Sox2], octamer-binding transcription factor 4 [Oct4], c-Myc, and Kruppel-like factor 4 [Klf4]) [Color figure can be viewed at wileyonlinelibrary.com]
miRNAs, together with exosomes, have a great potential to be used for prognosis, therapy, and biomarkers of different diseases, including infertility. miRNAs may play an important role in modulating gene expression during human preimplantation development from primordial germ cells to the embryo (Virant-Klun, Stahlberg, Kubista, & Skutella, 2016).

Some of the previously recognized roles of miRNAs have been observed in impaired endometrial receptivity, altered embryo development, implantation failure after ART, and in ectopic pregnancy and pregnancies of unknown location (Galliano & Pellicer, 2014).

miRNAs play critical roles in reprogramming process, pluripotency maintenance, and differentiation of stem cells. Moreover, the significant function of miRNAs in the determination of stem cell fate indicates the way miRNAs regulate mammalian development in vivo (Li, Long, Han, Yuan, & Wang, 2017). During spermatogenesis, miRNAs are expressed in a cell-specific or stage-specific manner. Nonetheless, the underlying mechanisms and roles of most of the miRNAs in spermatogenesis are still not clear. In the end, particular miRNAs in seminal plasma or spermatozoa will be applied as possible biomarkers for infertility of men. Etiology of male infertility and sterility becomes more clear with the explanation of the miRNAs and the clarification of their adjusting mechanisms (Chen, Li, Guo, Zhang, & Zeng, 2017). As a result, particular miRNAs have been used as possible biomarkers for infertility of men (Yadav & Kotaja, 2014).

To perform hepatitis C-related human clinical trials and nonhuman primate preclinical trials, researchers have tested some of the most developed anti-miR therapies (e.g., micro-RNA modulation) to assess their efficiency (Beavers, Nelson, & Duvall, 2015). There are two possible categories of the miRNA therapy, including miRNA inhibition and replacement therapies with the ability of downregulating and upregulating the expression of the miRNA, respectively. However, this classification depends on the expression status of the target miRNA (Peng, Chen, & Leong, 2015).

5.1 | The role of miRNAs in promoting epigenetic modifications for reprogramming

Various small molecules and specific miRNAs and several different small molecules were used to improve the efficiency of reprogramming (Subramanyam et al., 2011). It has been suggested that the pathway of miRNA play a critical role in both germline stem cell maintenance and PGC specification in invertebrates (Gangaraju & Lin, 2009).

A number of miRNAs, including miR-291-3p, miR-294, and miR-295, belongs to the cluster of miR-290, which has been reported to be able to increase the colony number of iPSCs and ESCs (Judson et al., 2009). Another study has demonstrated the importance of miRNAs in reprogramming, in which fibroblasts that lack all mature miRNAs could not generate iPSCs (Kim et al., 2012). Therefore, miRNAs are characterized as necessary factors for not only proper differentiation but also dedifferentiation of fibroblasts (Luningschrör, Hauser, Kaltschmidt, & Kaltschmidt, 2013).

miRNAs have been demonstrated to regulate several genes implicated in pluripotency, which are required for specification of germ cells. miR-145, for example, is able to suppress OCT4 expression, and partially repress SOX2 expression in ESCs of human, resulting in promotion of their differentiation (Xu et al., 2009). In addition, miR-134, -296, and -470 play an important role in regulation of SOX2, NANOG, and OCT4 in ESCs. The pluripotency markers SOX2, NANOG, and OCT4 find promoters of miRNAs and bind directly to them. For example, OCT4 binds to promoter of miR-302 cluster, which is specific for ESCs. This collaboration between these molecules leads to the regulation of cell fate

**FIGURE 3** The role of microRNAs (miRs) in pluripotent stem cell differentiation [Color figure can be viewed at wileyonlinelibrary.com]
5.2 | Are hESCs and hiPSCs more similar in their noncoding RNA expression?

Most cell types are suggested to express a unique sequence of ncRNAs including miRNAs (Signal, Gloss, & Dinger, 2016). It has been reported that miRNAs suppress their homologous target RNAs from expression by associating in a complex known as RISC (RNA-induced silencing complex; Pelosi et al., 2011). The pattern of miRNA expression changes alongside with individual cells differentiation and tissue development (Yi et al., 2009). Vast differences in expression have been demonstrated by assessing miRNA expression profiles of undifferentiated hiPSCs, hESCs, and fibroblasts, including more than 100 miRNAs between these two fibroblasts and pluripotent populations (Table 1; Chin et al., 2009).

A few number of miRNAs is expressed in different profiles in hiPSCs and hESCs. In an independent study, it was concluded that most of these miRNAs were expressed differentially between independently derived hiPSCs and hESCs (Wilson et al., 2009). Because each of these miRNAs have been shown to have multiple targets, there is a possibility that even about 11 of them could reveal the reason behind the occurrence of late hiPSCs signature (Chin et al., 2009).

In addition, two sequences of miR-371/372/373 and miR-302 encoding the human homologs of the mouse 290–295 cluster have been shown to be the enhancers of the reprogramming process (Judson et al., 2009). Clearly, more investigations are required to clarify the role of these and other ncRNAs in the ESCs and iPSCs state maintenance and the reprogramming process.

5.3 | The effect of miRNA in male germ cell development

Use of miRNAs in the development of germ cells has been functionally demonstrated (Fernandez-Perez, Brieno-Enriquez, Isoler-Alcaraz, Larriba, & Del Mazo, 2018). The repression of the miRNA Let7 is considered the best characterized pathway in germ cells of mammalian, which is mediated by the RNA-binding protein Lin28 to send translation permission to Blimp1 during the first stages of determining mouse germ cells (J. A. West et al., 2009). Furthermore, the reciprocal regulation pathway of Let7 and Lin28 extends its role to spermatogenesis (later stages) as regulators of pluripotency are in conjugation with miR-9 and miR-125a (Zhong et al., 2010). Moreover, miR-125 plays a critical role in post transcriptional repression of Oct4 during the stages of male meiotic silencing (Medrano et al., 2013).

Altogether, miRNAs are mostly required to regulate the development of male germ cells, which occurs in rodents, includes mitosis, meiosis, and spermatogenesis (Saito et al., 2015) (Table 2). However, more experiments must be conducted to show which miRNAs are involved in spermatogenesis, in particular its three main stages in human, pachytene spermatocytes, spermatagonia and round spermatids (Liu et al., 2015). In addition, miRNAs have a significant effect on meiotic and post-meiotic cells. The expression of miR-34c is upregulated in spermatocytes, and apoptosis is triggered by round spermatids (Romero et al., 2011). One mechanism that can partially mediate this process is when transcription factor ATF-1 is targeted. Therefore, miR-34c is essential for the development of germ cells. Moreover, it has been shown that miR-469 targets protamine and transition protein 2 (TP2) miRNAs to be repressed in round spermatids and pachytene spermatocytes (Dai et al., 2011). In addition, the degradation of TP2 mRNA cleavage is controlled by miR-122a, and the miRNA of heat shock factor 2 can be directly targeted by miR-18 at the spermatogenesis stage (Chen et al., 2017). miR-221/222 are also required in the regulation of spermatogonia undifferentiated state (Q.-E Yang, Racicot, Kaucher, Oatley, & Oatley, 2013).

5.4 | The effect of miRNA in iPSCs and ESCs into male germ cell differentiation

High levels of miR-372 are expressed by iPS and hESCs-derived primordial germ cell-like cells (PGCLCs). Conversely, high levels of let-7 are expressed by somatic cells (Melton, Judson, & Blelloch, 2010). It has been demonstrated that when the levels of miRNA are manipulated by knockdown with miRNA sponges or introduction of miRNA mimics, let-7 antagonizes while miR-372 promotes differentiation. PGCLC production increases by knockdown of the individual miR-372 targets, including MECP2, SMARCC1, RBL2, CDKN1, TGFBR2, and RHOC, whereas differentiation of PGCLC is suppressed by knockdown of the let-7 targets, including NMYC.
and CMYC. These findings discovered that a miR-372/let-7 axis plays a critical role to regulate the specification of PGC (Tran et al., 2016).

It has also been reported that cluster of miR-290–295 plays a role in ESCs where it is a direct target of the Sox2, Nanog, and Oct4 regulatory network (Marson et al., 2008). During the development of germ cells, spermatogonia and PGCs highly express the cluster of miR-17–92, which is reported to increase cell cycling, and the ESCs-specific cluster of miR-290–295.

There is a correlation between the cluster of miR-290 and potency of development, the expression of miR-290–295 decreases following the differentiation of ESCs into germ cells. In addition, a few members of the miR-290 cluster have been reported to be able to 10 folds enhance the reprogramming efficiency by Sox2, Oct4, and Klf4 (Judson et al., 2009). Moreover, members of this cluster can promote the transition of G1-S and thereby the rapid ESCs proliferation characteristics (Wang et al., 2008). Furthermore, it has been shown that the miR-290 cluster is involved in indirect control of de novo DNA methylation in ESCs (Medeiros et al., 2011).

Although the first miRNAs upregulated in the embryo development are the cluster of miR-290, there is no need for this cluster in ESC pluripotency or preimplantation development. Instead, lack of miR-290–295 had a great impact between midgestation and implantation and during the development of germ cells. Approximately three-quarters of deficient embryos lacking of miR-290–295 were lost during the development of embryos (Medeiros et al., 2011).

## 5.5 The effect of miRNAs in iPSC and ESCs formation

Introduction of miR-290 has strongly improved the formation of iPSC, while no effect has been observed using other members of the

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Expression</th>
<th>References</th>
<th>Function</th>
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</thead>
<tbody>
<tr>
<td>miR-449</td>
<td>Localized to spermatids and spermatocytes</td>
<td>Buchold et al. (2010)</td>
<td>Prohibits proliferation of a germ cell line</td>
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<tr>
<td>miR-34b</td>
<td>Upregulation in testis</td>
<td>Vogt et al. (2011), Yu et al. (2014)</td>
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<td>miR-34a</td>
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<td>Saito et al. (2015)</td>
<td>Represses proliferation, promotes apoptosis</td>
</tr>
<tr>
<td>miR-34c</td>
<td>Highly expressed in pachytene spermatocytes and round spermatids</td>
<td>(Saito et al. (2015), Wu et al. (2011)</td>
<td>Cycle regulator mGSC apoptosis</td>
</tr>
<tr>
<td>miR-184</td>
<td>Localized in mouse testis germ cells</td>
<td>Liu et al. (2013), McIver et al. (2012)</td>
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<tr>
<td>miR-24</td>
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<td>Liu et al. (2013)</td>
<td>Meiosis</td>
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<tr>
<td>miR-214</td>
<td>Pachytene spermatocytes</td>
<td>Liu et al. (2013), Liu et al., 2015</td>
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<tr>
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<td>miR-469</td>
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<td>Regulates the chromatin remodeling</td>
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<td>Expression at high levels in spermatocytes</td>
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<td>Maturation of male germ cells</td>
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<tr>
<td>miR-122a</td>
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<td>Remodeling of chromatin</td>
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<td>miR-355</td>
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<td>Ito et al. (2010)</td>
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<td>miR-181b</td>
<td>Upregulation in adult testis</td>
<td>Ito et al. (2010)</td>
<td>Transcriptional regulation</td>
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<tr>
<td>miR-181c</td>
<td>Upregulation in adult testis</td>
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<td>Transcriptional regulation</td>
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<tr>
<td>miR-185</td>
<td>In pachytene spermatocytes</td>
<td>Liu et al. (2013)</td>
<td>Cell cycle regulator</td>
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<td>miR-191</td>
<td>In beta pachytene spermatocytes</td>
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<td>Required for normal sperm morphology</td>
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<td>miR-10a</td>
<td>Enriched in the spermatogonial cell population compared with somatic cells of Day 6 testis</td>
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<td>miR-21</td>
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<td>miR-509-3</td>
<td>Expressed in human testis</td>
<td>McIver et al. (2012)</td>
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Note. ESCs: embryonic stem cells; miR: microRNA; PGCs: primordial germ cells; SSC: spermatogonial stem cell.
same cluster, such as miR-293 and miR-292-3p. Interestingly, the promoter of these miRNAs is the location where c-Myc binds, suggesting that their operation is in downstream of c-Myc. c-Myc demonstrated to have a predominant role in suppression of miR-29a and miR-21 at the level of transcription. Both of these miRNAs have been shown to act as p53 expression inhibitor, the role of which in modulating iPSC reprogramming is well-known (Banito et al., 2009; Hong et al., 2009). The role of p53 pathway, which is known to suppress tumor development, as a roadblock for iPSCs generation, has been recently reported (Lin, Choi, Hicks, & He, 2012).

Although protein-coding genes are considered as the main targets of p53, several miRNAs have been reported vital in the pathway of p53, which has opened a new area for researchers to explore their role during cell programming. Three miRNAs of miR-199a, miR-145, and miR-145 are induced by p53, all of which have been demonstrated to inhibit the generation of iPSCs via different mechanism (Jain et al., 2012; Wang et al., 2012). Conversely, p53 is directly targeted by miR-138 and p21, a well-known component of the p53 pathway, which is targeted by several miRNAs, such as miR-290, miR-106a/b and miR-93, all of which are able to promote iPSC generation (Gingold et al., 2014; Luginbühl et al., 2017).

While the miR-290 cluster was chosen based on its expression during ESCs differentiation, other miRNAs have been candidates in another study based on their role in the upregulation of iPSC reprogramming, in its early stages in particular (Li, Yang, Nakashima, & Rana, 2011). The generation of iPSCs is greatly enhanced by the overexpression of miR-93 and miR-106b, two members of the miR-106a, while the efficiency of reprogramming decreases by the knockdown of the same miRNAs as well as miR-25, another member of the same cluster, using miR-inhibitors. Certainly, the formation of iPSCs is not improved necessarily by the factors essential for self-renewal, but some barriers might even be presented by these factors for cell reprogramming (Luginbühl et al., 2017).

It has been shown that the efficiency of iPSCs reprogramming is enhanced by several miRNAs when they are expressed along with a few Yamanaka factors (Subramanyam et al., 2011). For example, the cluster of miR-17–92 can regulate MYC, and there can be an increase in the levels of miR-92 by MYC overexpression (Jia et al., 2013; Wilson et al., 2010).

Regardless of the reprogrammed cells, pluripotent cells possess two distinct categories of miRNA patterns derived from embryonic or somatic cells (Neveu et al., 2010). Some families of miRNA, including miR-34, miR-29a and miR-21, interfere with the reprogramming process to enhance the iPSCs generation (C.-S. Yang, Li, & Rana, 2011). Inhibition of these miRNAs is associated with increased efficiency of reprogramming. For example, genetic ablation of miR-34a by MEFs has enhanced the efficiency of reprogramming, suggesting that miR-34a can interfere with reprogramming. The cluster of miR-34 consists of three miRNAs: miR-34a, miR-34b, and miR-34c. Reprogramming of somatic cells is promoted by knockout of miR-34a or miR-34a/b while it has been demonstrated that the ablation of miR-34a has a stronger effect on the generation of iPSCs, compared to miR-34b and miR-34c (Luningschror et al., 2013). The cluster of miR-34 seems to act as a repressor for the target genes of ESCs and iPSc fate depends on the activity of the specific cell cycle regulating (ESCC) miRNAs of ESC and iPSc, which are induced by the core pluripotency factors including Nanog, OCT4, KLF4, SOX2, LIN28, and MYC [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 4** Regulatory networks of microRNAs (miRs) able to control the differentiation and self-renewal of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Model for the various roles of several different miRNA families in the gene regulatory network which maintains differentiating and pluripotent cell states. Maintenance of ESCs and iPSc fate depends on the activity of the specific cell cycle regulating (ESCC) miRNAs of ESC and iPSc, which are induced by the core pluripotency factors including Nanog, OCT4, KLF4, SOX2, LIN28, and MYC [Color figure can be viewed at wileyonlinelibrary.com]
p53. Among these targets, N-Myc, Sox2, and Nanog were found to be post-transcriptionally regulated by the cluster of miR-34 during iPSCs induction (Choi et al., 2011; Figure 4).

6 CONCLUSION

The roles of miRNAs in self-renewal and differentiation of iPSCs and ESCs are increasingly evident. In addition, their function in stem cells is rapidly being discovered. Thanks to the introduction of new technologies, such as deep-sequencing and robust tools, for the isolation of stem cells, it is projected that further studies will reveal more miRNAs and their functions in diverse stem cell systems, as well as their differentiated progeny from various human tissues.

However, to discover the increasingly diverse characteristics of miRNA, more studies are required, including a deeper mechanistic exploration into structure, localization and interacting partners, to interpret their involvement in cell programming.

According to the results of the study, the ability of fine-tuning the levels of various factors in miRNA makes it capable of directing the fate of stem cells. Regenerative medicine includes a practical strategy known as manipulation of the miRNA level in stem cells ex vivo. To perform hepatitis C-related human clinical trials and nonhuman primate preclinical trials, researchers have tested some of the most developed anti-miR therapies (e.g., micro-RNA modulation) to assess their efficiency. There are two possible categories of the miRNA therapy, including miRNA inhibition and replacement therapies with the ability of downregulating and upregulating the expression of the miRNA, respectively. However, this classification depends on the expression status of the target miRNA. In regenerative medicine, some of the therapeutic impacts of miRNA include angiogenesis, wound healing, cardiac regeneration, neurogenesis, bone regeneration, and skeletal muscle.

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