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Determination and Regulation of 'Stemness' by MicroRNAs

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1. Introduction

Embryonic stem cells (ESCs) are pluripotent cells derived from the inner cell mass of pre-implantation blastocysts, and by definition they are the cells that can self-renew indefinitely and differentiate into diverse cell types found in three embryonic germ layers (Thomson et al., 1998). In addition to allowing investigation of early events of embryonic development, the remarkable attributes of ESCs raise the possibility of using them as potential source of raw material for cell-based therapies (Mantel et al., 2007; Yu and Thomson, 2008). Molecular mechanisms of self-renewal and differentiation have been intensively studied during last two decades, and significant progresses have been achieved with regards to several core issues. Transcriptional regulation is one area in which key findings have been made culminating in generation of induced pluripotent stem cells (iPS) (Lee et al., 2006). Still, it goes without saying that much remains unknown about the proliferation and differentiation essential nature of 'stemness' at the molecular level.

Recent years have witnessed the establishment of microRNAs (miRNAs) as important regulators of development, homeostasis and metabolism of plants and animals (Bartel, 2004). These noncoding small RNAs have been discovered in virtually all eukaryotic organisms, from the brown algae (Cock et al., 2010) to the human, and their list is growing rapidly, in excess of 5000 having been identified so far. Over 1,000 miRNAs have been experimentally validated in mammals, and evolutionary conservation seen amongst miRNAs and across species strongly indicates functional and mechanistic significance of these small molecules (Bentwich et al., 2005). The best known post-transcriptional role of miRNAs is inhibition of translation which occurs via annealing to their target sequence in 3'UTR often accompanied by degradation of target mRNAs. It has been proposed that up to 60% of human genes are regulated by miRNAs which implies that each miRNA can target several different mRNAs as confirmed by both computational and experimental studies (Friedman et al., 2009). The expression pattern of the miRNAs frequently shows a consistent and significant correlation with the phenotypic transitions of the cells, such as proliferation, differentiation and apoptosis. Accordingly, several recent studies have demonstrated critical involvement of miRNAs in early developmental processes such as neurogenesis (Makeyev et al., 2007), myogenesis (Rao et al., 2006), cardiogenesis (Zaho et al., 2005) and hematopoiesis (Chen et al., 2004). Mammalian cells thus appear to produce miRNAs in a cell-type-specific manner to regulate unique subsets of genes specifically expressed in the host cells.

Of importance is that the cell-type specificity rule of miRNA expression and function applies to ESCs as well. Several recent studies reported expression of unique clusters of miRNAs in ESCs. These include miRNAs of human and mouse miR-302 clusters, mouse miR-290 cluster and human miR-371 cluster (Houbaviy et al., 2003; Suh et al., 2004; Landgraf et al., 2008). Based on the ESC-specific expression of these miRNAs, it was hypothesized that some of the miRNAs from these clusters may function as determinants or regulators of 'stemness', a word embodying the salient characteristics of ESCs: self-renewal and pluripotency (Blakaj and Lin, 2008; Kim et al., 2009). As the latest reports on regulation and function indicate that the hypothesis is likely to be more than mere speculation (Marson et al., 2008; Lin et al., 2008; Lee et al., 2008; Card et al., 2008; Barroso-Deljesus et al., 2008; Lee et al., 2010), it has been inevitably envisioned that miRNAs may be useful tools for controlling the proliferation and the differentiation of ESCs. In addition, other lines of investigation showed that ES-like stem cells including iPS and multipotent spermatogonial stem cells (mSSCs) as well as embryonal carcinoma cells (ECCs) share to a varying extent the expression profile of miRNAs. This in turn seems to suggest that expression profiling of miRNAs can be a way of 'measuring' the 'stemness' of a given type of cells. In this chapter, we will introduce the recent discovery of miRNAs in ESC and ES-like stem cells, describe molecular and functional characterization of identified miRNAs and provide perspectives on issues and the research agenda of this flourishing field.

2. Identification of the ESC specific miRNAs

Houbaviy and coworkers reported in 2003 the isolation of 15 novel miRNAs from a mouse ESC cDNA library which included members of miR-290 cluster and predicted the existence of human homologues (Houbaviy et al., 2003). Using a similar approach, Suh et al. (2004) reported the cloning of 17 novel miRNAs belonging to miR-302 and miR-371 clusters from human ESCs. Remarkably, the miRNAs identified from the three clusters showed varying degrees of conservation between the two species which immediately led to a speculation about significant and specific functions in ESCs. In addition, murine miR-367 was isolated through a computational approach by Sewer et al. (2005), and Mineno et al. (2006) identified mouse miR-302b, miR-302d and miR-367 using a massive parallel signature sequencing technique. More recently, Landgraf et al. (2008) cloned and validated additional novel miRNAs from human and mouse ESCs (human miR-302d* and miR-367*; mouse miR-302b*, miR-302b, miR-302c*, miR-302c, miR-302a* and miR-302d; * denotes the minor miRNA species in case two excised miRNAs are generated, one from each arm) by a large-scale cloning. Altogether, the miRNA registry (miRBase 15; http://www.mirbase.org/) currently lists 10 miRNAs in the human miR-302 cluster and 8 miRNAs in the mouse miR-302 cluster (Table 1). ESC-specific expression of some of these miRNAs (miR-371, miR-372, miR-373 and miR-373*) has been confirmed by Northern blotting (Suh et al. 2004). Subsequently, Landgraf et al. (2008) reported identification of another member of the miR-371 cluster, miR-371-5p and corrected the sequence of miR-371 yielding miR-371-3p (Table 2). Currently, there are five miRNAs in the miR-371 cluster.

Of the 15 novel miRNAs reported by Houbaviy et al. (2003), 8 miRNAs belong to the miR-290 cluster (miR-290, miR-291-s, miR-291-as, miR-292-s, miR-292-as, miR-293, miR-294 and miR-295). Later, Sewer et al. (2005) isolated two additional miRNAs (miR-291b-5p and miR-291b-3p) in the same cluster, through a bioinformatics analysis. More recently, 4 more miRNAs (miR-290-3p, miR-293*, miR-294*, and miR-295*) have been added to the miR-290 cluster by direct cloning from mouse ESCs and ECCs (F19) (Landgraf et al., 2008). Currently, miRNA registry (miRBase 15) lists 14 miRNAs in the mouse miR-290 cluster (Table 3).

ID	Accession[a]		Sequences[a]	Genome Cont
	Stem-loop	Mature		
hsa-miR-302b	MI0000772	hsa-miR-302b* (MIMAT0000714)	acuuuaacauggaagugcuuuc	4: 113789090-113 [-] LARP7; intro
		hsa-miR-302b (MIMAT0000715)	uaagugcuuccauguuuuaguag	
hsa-miR-302c	MI0000773	hsa-miR-302c* (MIMAT0000716)	uuuaacaugggguaccugcug	4: 113788968-113 [-] LARP7; intro
		hsa-miR-302c (MIMAT0000717)	uaagugcuuccauguuucagugg	
hsa-miR-302a	MI0000738	hsa-miR-302a* (MIMAT0000683)	acuuaaacguggauguacuugcu	4: 113788788-113 [-] LARP7; intro
		hsa-miR-302a (MIMAT0000684)	uaagugcuuccauguuuugguga	
hsa-miR-302d	MI0000774	hsa-miR-302d* (MIMAT0004685)	acuuuaacauggaggcacuugc	4: 113788609-113 [-] LARP7; intro
		hsa-miR-302d (MIMAT0000718)	uaagugcuuccauguuugagugu	
hsa-miR-367	MI0000775	hsa-miR-367* (MIMAT0004686)	acuguugcuaauaugcaacucu	4: 113788479-113 [-] LARP7; intro
		hsa-miR-367 (MIMAT0000719)	aauugcacuuuagcaaugguga	
mmu-miR-302b	MI0003716	mmu-miR-302b* (MIMAT0003373)	acuuuaacaugggaaugcuuucu	3: 127248146-127 [+] Larp7; intron
		mmu-miR-302b (MIMAT0003374)	uaagugcuuccauguuuuaguag	
mmu-miR-302c	MI0003717	mmu-miR-302c* (MIMAT0003375)	gcuuuaacaugggguuaccugc	3: 127248281-127 [+] Larp7; intron
		mmu-miR-302c (MIMAT0003376)	aagugcuuccauguuucagugg	
mmu-miR-302a	MI0000402	mmu-miR-302a* (MIMAT0004579)	acuuaaacgugguuguacuugc	3: 127248414-127 [+] Larp7; intron
		mmu-miR-302a (MIMAT0000380)	uaagugcuuccauguuuugguga	
mmu-miR-302d	MI0003718	mmu-miR-302d (MIMAT0003377)	uaagugcuuccauguuugagugu	3: 127248542-127 [+] Larp7; intron
mmu-miR-367	MI0003531	mmu-miR-367 (MIMAT0003181)	aauugcacuuuagcaaugguga	3: 127248651-127 [+] Larp7; intron

[a] Accession number and sequences from the miRBase 15 (http://www.mirbase.org/)

[b] References, 1; Suh MR et al., (2004), 2; Landgraf P et al., (2007), 3; Houbaviy HB et al., (2003), 4; Voorhoeve P. M et al., (2006)

Table 1. Current miRBase 15 registered mature miRNA sequences of miR-302 cluster. Accession numbers for precursors andmature miRNAs, current mature miRNA sequences, genomic locations, expressed cell lines and references are listed.

ID	Accession[a]		Sequences[a]	Genome Conte
	Stem-loop	Mature		
hsa-miR-371	MI0000779	hsa-miR-371-5p (MIMAT0004687)	acucaaacuguggggcacu	19: 58982741-5898 [+] intergenic
		hsa-miR-371-3p (MIMAT0000723)	aagugccgccaucuuuugagugu	
hsa-miR-372	MI0000780	hsa-miR-372 (MIMAT0000724)	aaagugcugcgacauuugagcgu	19: 58982956-5898 [+] intergenic
hsa-mir-373	MI0000781	hsa-miR-373* (MIMAT0000725)	acucaaaaugggggcgcuuucc	19: 58983771-5898 [+] intergenic
		hsa-miR-373 (MIMAT0000726)	gaagugcuucgauuuuggggugu	

[a] Accession number and sequences from the miRBase 15 (http://www.mirbase.org/)

[b] References, 1; Suh MR et al., (2004), 2; Landgraf P et al., (2007), 3; Houbaviy HB et al., (2003), 4; Voorhoeve P. M et al., (2006)

Table 2. Current miRBase 15 registered mature miRNA sequences of miR-371 cluster. Accession numbers for precursors andmature miRNAs, current mature miRNA sequences, genomic locations, expressed cell lines and references are listed.

ID	Accession[a]		Sequences[a]	Genome Cont
	Stem-loop	Mature		
mmu-miR-290	MI0000388	mmu-miR-290-5p (MIMAT0000366)	acucaaacuaugggggcacuuu	7: 3218627-32187 intergenic
		mmu-miR-290-3p (MIMAT0004572)	aaagugccgccuaguuuuaagccc	
mmu-miR-291a	MI0000389	mmu-miR-291a-5p (MIMAT0000367)	caucaaaguggaggcccucucu	7: 3218920-32190 intergenic
		mmu-miR-291a-3p (MIMAT0000368)	aaagugcuuccacuuugugugc	
mmu-miR-292	MI0000390	mmu-miR-292-5p (MIMAT0000369)	acucaaacuggggcucuuuug	7: 3219190-32192 intergenic
		mmu-miR-292-3p (MIMAT0000370)	aaagugccgccagguuuugagugu	
mmu-miR-291b	MI0003539	mmu-miR-291b-5p (MIMAT0003189)	gaucaaaguggaggcccucucc	7: 3219483-32195 intergenic
		mmu-miR-291b-3p (MIMAT0003190)	aaagugcauccauuuuguuugu	
mmu-miR-293	MI0000391	mmu-miR-293* (MIMAT0004573)	acucaaacugugugacauuuug	7: 3220344-32204 intergenic
		mmu-miR-293 (MIMAT0000371)	agugccgcagaguuuguagugu	
mmu-miR-294	MI0000392	mmu-miR-294* (MIMAT0004574)	acucaaaauggaggcccuaucu	7: 3220642-32207 intergenic
		mmu-miR-294 (MIMAT0000372)	aaagugcuucccuuuugugugu	
mmu-miR-295	MI0000393	mmu-miR-295* (MIMAT0004575)	acucaaauguggggcacacuuc	7: 3220774-32208 intergenic
		mmu-miR-295 (MIMAT0000373)	aaagugcuacuacuuuugagucu	

[a] Accession number and sequences from the miRBase 15 (http://www.mirbase.org/)

[b] References, 1; Suh MR et al., (2004), 2; Landgraf P et al., (2007), 3; Houbaviy HB et al., (2003), 4; Voorhoeve P. M et al., (2006)

Table 3. Current miRBase 15 registered mature miRNA sequences of miR-290 cluster. Accession numbers for precursors andmature miRNAs, current mature miRNA sequences, genomic locations, expressed cell lines and references are listed.

3. Molecular characterization of the ESC specific miRNAs

3.1 miR-302 cluster members

Since the discovery in mouse and human, the existence of a conserved miR-302 cluster has been proposed in multiple species. For *Gallus gallus* (International Chicken Genome Sequencing Consortium, 2004), *Monodelphis domestica* (Devor & Samollow, 2008) , *Macaca mulatta* (Yue et al., 2008), *Pan troglodytes* (Baev et al., 2009), *Bos Taurus* (Artzi et al., 2008), *Canis familiaris* (Artzi et al., 2008), *Pongo pygmaeus* (Brameier, 2010), *Equus caballus* (Zhou et al., 2009), and *Ornithorhynchus anatinus* (Murchison et al., 2008), the miR-302 cluster has been identified by genomic sequence analysis, whereas in *Xenopus tropicalis*, the expression in early blastula has been experimentally validated (Watanabe et al., 2005).

Most of the detailed studies have been carried out with the members of human and mouse miR-302 clusters which show remarkably high degree of conservation. The precursor forms of orthologous miRNAs show the homology rates from 85% to 93%, while except for miR-302c and miR-367 all orthologous pairs show a perfect match in the mature form. Individual miRNAs also show strong homology to one another, consistent with the common origin, with the membership of miR-367 being somewhat controversial (Kim et al., 2009). According to miRBase 15, miR-367 belongs to a separate family, even though it is generated from the same polycistronic primary transcript as the rest of miRNAs of miR-302 cluster (Suh et al., 2004). Based on the weak homology, it is proposed that miR-302 family members and miR-367 target different sets of mRNAs and consequently perform different functions.

Human miR-302 cluster locus is located within a 700-bp region on chromosome 4, and the corresponding mouse cluster is found within a region of about 600 bp on chromosome 3. Interestingly, both human and mouse miR-302 loci are found within intron 8 of the La ribonucleoprotein domain family, member 7 (LARP7) gene, which is known to act through the 7SK ribonucleoprotein system to negatively regulate polymerase II-mediated transcription (Markert et al., 2008). It is not currently determined whether miRNAs of miR-302 cluster and LARP7 have regulatory interaction or functional coordination.

3.2 miR-290 and miR-371 cluster members

miRNAs of mouse miR-290 cluster appear to be expressed in ESC-specific manner, conserved across mammalian species and clustered within 2.2 kb of one another. Similarity of sequence to members of the mouse miR-290 cluster led to the prediction of the existence of miRNAs of human miR-371 cluster which was demonstrated experimentally. Human miR-371 cluster is located in an intergenic region, spanning approximately 1050 bp, on chromosome 19. The miR-371 cluster has also been identified in *Macaca mulatta* (Yue et al., 2008), *Pan troglodytes* (Baev et al., 2009), *Bos Taurus* (Artzi et al., 2008), *Canis familiaris* (Artzi et al., 2008), *Pongo pygmaeus* (Brameier, 2010), and *Equus caballus* (Zhou et al., 2009) by genomic sequence analysis while the homologous miR-290 cluster has been identified in *Rattus norvegicus* (Linsen et al., 2010).

Although miRNAs belonging to miR-371 cluster and miR-290 cluster are all classified as miR-290 family members at miRBase, homology rates among precursors and mature forms are relatively low compared to intra- and inter-species homology seen in miR-302 cluster members (Kim et al., 2009). Nevertheless, based on that seed region are much better conserved, it was hypothesized that at least some regulatory targets are shared. This awaits further examination as functional analyses miR-371 and miR-290 clusters reported thus far do not fully support this notion

3.3 Expression of miRNAs in ES-like pluripotent stem cells

An important insight into the significance of ESC-specific expression of the miRNAs in fact came from their expression in other pluripotent cell types. Specifically, expression of the miR-302 cluster has been found in ECCs derived from testicular and ovarian teratocarcinoma tissues (PA1) (Zeuthen et al., 1980), leading to the generalization that the miR-302 cluster is specific for cells capable of forming embryoid bodies (EBs) and differentiating into diverse cell types.

Somatic cells have been successfully reprogrammed by fusion with ES cells, and it has thus been suggested that some factors in ES cells are capable of reprogramming somatic cells into pluripotent stem cells (Cowan et al., 2005; Yu et al., 2006). This was dramatically demonstrated by Takahashi and Yamanaka in 2006 when they identified Oct-3/4, Sox2, c-Myc, and Klf4 as the pluripotency-inducing factors by introducing these genes into mouse embryonic or adult fibroblasts and generating iPS. Studies on miRNA expression pattern in various iPS and multipotent spermatogonial stem cells (mSSCs) are beginning to be reported. For example, Chin et al. (2009) examined if miRNAs are expressed in human iPS at the level and in a pattern as in hESCs. Expression profiling of all known miRNAs was performed on hESCs, iPS, and the fibroblasts from which iPS had been derived. Hierarchical clustering with the 105 miRNAs expressed showed that there is little difference in miRNA expression pattern between the pluripotent cells, i.e. iPS and hESCs. Conversely, all of the pluripotent cell lines had vastly different miRNA expression profiles from those of fibroblasts. These observations are overall highly suggestive of that a strong correlation exists between essential features of stem cells and the miRNA expression profiles. Still, the situation is likely to be much more complex. Specifically, it was noticed that a few miRNAs were consistently expressed differently between iPS and hESCs. This finding was also confirmed by another group using a different set of hESCs and iPS (Wilson et al., 2009), indicating that a distinct miRNA pattern is essentially reproducible among different reprogrammed cells and that iPS have a miRNA signature that distinguishes them from hESCs. Study by Wilson et al. (2009) in particular highlights the differences between the two pluripotent cell types with respect to the expression of the miR-371/372/373 cluster.

3.4 miRNA expression profile as an indicator of 'stemness'

Stadler et al. (2010) analyzed changes in the expression of miRNAs and mRNAs in nine different hESC lines during early commitment and also examined the expression of key ESC-enriched miRNAs in early developmental stages in several species. They demonstrated that several previously defined hESC-specific miRNA group members (belonging to the miR-302, -17, and - 515 families and the miR-371-373 cluster) and several other hESC-enriched miRNAs are down-regulated rapidly in response to differentiation. They further found that mRNAs upregulated upon differentiation are enriched in terms of harboring potential target sites for the down-regulated miRNAs. Interestingly, they also observed that the expression of ESC-enriched miRNAs bearing common seed sequences was modulated in a discordant manner while the cells transitioned through early embryonic states. In human and monkey ESCs, as well as human iPS, the miR-371-373 cluster was consistently upregulated while the miR-302 family was mildly downregulated when the cells were chemically treated to regress to an earlier developmental state. Similarly, miR-302b, but not miR-295, was expressed at higher levels in murine epiblast stem cells (mEpiSC) as compared to mouse ESCs which represents an earlier developmental state. These data for the first time raise the possibility that miRNAs bearing identical seed sequences could have distinct

functions from one another and during sub-stages of early embryonic development. Perhaps more importantly, these results suggest that the relative expression levels of related miRNAs may serve as indicators for defining the developmental state of ESCs and other stem cell lines, such as iPS.

Another series of reprogramming experiments are also worthy of close attention. The conversion of spermatogonial stem cells (SSCs) from neonatal hybrid (Kanatsu-Shinohara et al., 2004), neonatal inbred (Kim et al., 2010), adult inbred (Oh et al., 2009), and adult transgenic mice (Guan et al., 2006; Izadyar et al., 2008; Seandel et al., 2007) into ES-like cells (multipotent spermatogonial stem cells, mSSCs) has been reported. Interestingly, SSCs and mSSCs exhibit significant differences in gene expression and epigenetic properties even in the cases of identical genetic origin (Kanatsu-Shinohara et al., 2008). In particular, mSSCs have lost the expression of germ stem cell-specific genes and showed enhanced expression of pluripotent stem cell-specific genes. Importantly, Zovoislis et al. (2010) reported that miRNAs of miR-290 and 302 clusters are expressed in mSSCs and that the 290-miRNA family is functionally connected with Oct-4 in maintenance of the pluripotent state. It should be noted however that detection of miR-302 family members likely results from the presence of a proportion of spontaneously differentiating cells as members of the 302-miRNA family have been shown to be induced during first stages of in vitro differentiation in all pluripotent cell types tested. In sum, in addition to histone modification, promoter methylation, and transcription factor expression, cell type specific expression and dynamic modulation of miRNAs reflect a layer of regulatory control in ESCs and at the same time represent signature of 'stemness'.

3.5 Transcriptional regulation of expression by ESC-specific transcription factors

ESC-specific expression of the miRNAs of miR-302, miR-290, and miR-371 clusters has gained an added significance from a series of recent studies (Marson et al., 2008; Card et al., 2008; Barroso-Deljesus et al., 2008). Strikingly, transcription factors that have been shown to be critical determinants of 'stemness' of ESC were also demonstrated to be involved in the regulation of these miRNA cluster. Specifically, in the study by Marson et al. (2008), potential binding sites for Nanog, Oct3/4, Sox2 and Tcf3 have been shown to be present in the 5' region of the miR-302 clusters. Furthermore, down-regulation of Oct3/4 led to reduced expression of the miRNAs of this cluster. Card et al. (2008) also showed that Oct3/4, Sox2 and Nanog bind to the chromosomal region 5' to the miR-302 cluster and that Oct3/4 is required for the expression of the miR-302 transcript. Rex1, another ESC-specific transcription factor, was also predicted to bind to the same region (Barroso-Deljesus et al., 2008). Similar transcriptional regulation seems to be in operation for miR-290 and miR-371 clusters. Marson et al. (2008) showed that the two clusters have potential binding sites for Oct4, Sox2, Nanog and Tcf3 and that inhibition of Oct3/4 leads to down-regulation of miR-290 expression. Together, these studies strongly suggest that miRNAs expressed specifically in ESCs are in fact integral elements of the regulatory network, on one hand controlled by the ESC-specific transcriptional program and on the other likely controlling characteristics of ESC at the molecular level.

4. Role of the ESC-specific miRNAs

4.1 Studying the function of miRNAs in ESC

The current understanding of the molecular function of ESC-specific miRNAs is, simply but, limited. This in part stems from the technical demand in handling ESCs, especially those of

human origin. Ectopically expressing cDNAs, siRNAs, or miRNAs in human ESCs is difficult as these cells typically grow as aggregates. Delivery of foreign DNA or RNA can be much more easily achieved with mouse ESCs which are initially seeded as individual cells that can be grown into clones. How miRNAs determine or affect the self-renewal and pluripotency, the essential characteristics of stem cells, can be tested using mouse ESCs, and much can be inferred about the function of orthologous human miRNAs. It goes without saying that species difference must be kept in mind even in the case of unambiguous orthologues. There are additional difficulties even with mouse ESCs. For example miRNAs originating from the same cluster likely have overlapping target mRNAs which would make determining the function of individual miRNAs difficult. At any rate, an efficient delivery system and inducible vector constructs for small RNA molecules would be clearly useful. Recently developed advanced tools such as miRNA- or small interfering RNA (siRNA)-expressing lentiviral or retroviral systems with pseudotyping feature for a broad host cell range should be valuable tools (Chang et al., 2006). Additional features such as inducibility and cell type specificity can be incorporated in principle assisting precise functional analysis of miRNAs in murine and human ESCs (Chang et al., 2006). Identification of potential targets initially with bioinformatics approach and subsequently with 'wet-lab' validation also represents an important part of functional analyses. There are challenges here as well. Target prediction algorithms must be continually refined based on results of validation experiments, but exhaustive and systematic efforts are rather limited. Also, these prediction programs should ideally be linked with various gene-expression pattern databases, but again at this point a user-friendly in silico analysis program with such features does not exist.

4.2 Function of miR-302 cluster members

Functional conservation of the miR-302 cluster in ESCs of various species can be readily hypothesized based on the high degree of homology, conservation of genomic loci and cell type specific expression, as well as the regulation by ESC-specific transcription factors. In spite of the aforementioned difficulties, direct functional tests in ESC of miR-302 cluster are beginning to be reported. One notable study by Card et al., (2008) showed that ectopic expression of individual miR-302 members in both primary and transformed cell lines leads to increase in the proportion of cells in the S phase and decrease in the proportion of cells in the G1 phase. Consistently, bare expression led to the opposite phenotype. Cyclin D1, a G1 regulator, was predicted as a target of multiple members of miR-302 cluster, and miR-302 miRNA expression indeed led to inhibition of Cyclin D1 translation. These data indicate that one of the primary functions of miR-302 in ESC is cell cycle regulation. Subsequently, Lee et al., (2008) found that miR-302b indirectly regulates Oct4 and directly targets Cyclin D2, developmental regulators during gastrulation. Such observation suggests that at least miR-302b participates in maintaining the pluripotency of ECCs and likely of ESCs. A noteworthy study on the function and potential application of miR-302 has been reported by Lin et al. in 2008. They showed that expression of miR-302 induced reprogramming of human skin cancer cells into ESC-like cells (Lin et al., 2008). These miRNA-induced pluripotent stem cells shared 86% of their expressed genes with ESCs including the hallmark ESC marker genes such as Oct3/4, Sox2, SSEA-2 and SSEA-4. Furthermore, the miRNA-induced pluripotent cells were able to differentiate into multiple types of cells including neurons, chondrocytes, fibroblasts and spermatogonial cells in vitro. More recently, Scheel et al. ran a functional screen using a

large miRNA expression library and reported identification of the miR-302 cluster as a potent suppressor of p63 accumulation in various cell types. It was shown that miR-302 miRNAs reduce mRNA and proteins levels of p63 through two target sites within the 3' UTR. The role of miR-302 members was also confirmed in testicular cancer cells in which the endogenous miR-302 contributes to the suppression of p63. It was also proposed that miRNAs of the miR-302 cluster also contribute to the elimination of p63 mRNA in mature oocytes. Thus, miR-302 appears to be a part of the stringent regulatory mechanism for p63 in germ cells, reminiscent of the tight control for p53 levels in somatic cells.

4.3 Function of miR-290 and miR-371 clusters

The function of miRNAs of the miR-290 cluster has been studied in diverse contexts. First, a definitive loss-of-function analysis was carried out with gene-targeted mouse. Homozygous loss of miR-290–295 locus, which resulted in frequent embryonic lethality, led to infertility among female survivors. Notably they lacked germ cells, indicating that at least some of the members may be responsible for the maintenance of pluripotency (Giraldez et al., 2006; Marson et al., 2008). Benetti et al. (2008) showed that miRNAs of the miR-290 cluster silence Rbl2 which functions as a transcriptional repressor of the Dnmt3a and Dnmt3b enzymes and that these enzymes as well as miRNAs are down-regulated in Dicer1 -/- cells. In an independent study, Sinkkonen et al., (2008) demonstrated that de novo DNA methylation is defective in Dicer1-deficient ESCs consistent with indirect control of the expression of DNA methyltransferases by the miRNAs of miR-290 cluster. The mechanistic link between members of miR-290 cluster and de novo DNA methylation in ESCs indicates that these miRNAs are involved in the epigenetic control of gene expression. In addition, Hayashi et al., (2008) reported the requirement of miRNA biogenesis in primordial germ cell development and early spermatogenesis. miR-290 cluster members were among the most highly expressed in male germline cells suggesting a potential role in this particular developmental program. Wang et al. (2008) reported that members of the miR-290 family rescue cellular proliferation defect seen in *Dgcr8*-/- ESCs. Apparently, these miRNAs function by suppressing several key regulators of the G1/S transition which leads to release of cells from the arrest in G1 phase. Finally, Judson et al. (2009) demonstrated that introduction of miR-291-3p, miR-294 and miR-295 into fibroblasts enhances the production of mouse iPS cells by Oct4, Sox2 and Klf4.

Several recent reports describe the function of the miRNAs of miR-371 cluster. Most notably, Voorhoeve et al., (2006) demonstrated that miR-372/373 miRNAs promote cellular transformation in cooperation with oncogenes. It was also shown that the expression of the miR-372/373 cluster was seen in subsets of ECC lines such as Tera1, 2102Ep and 833KE but not in NT2 and NCCIT lines. Voorhoeve et al. noted a correlation between the expression of the miR-372/373 cluster and p53 status in these ECC lines. Specifically, whereas all three expresser cell lines contain high levels of wt-p53, NT2 expresses wt-p53 at a low-level and NCCIT has only single mutated allele and no wild type allele. Based on these findings, they proposed that miRNAs of the miR-372/373 cluster contribute to tumorigenesis from cells that retain wt-p53. Interestingly, Duale et al. (2007) showed that cisplatin represses the oncogenic properties of the miR-372/373 cluster, and Huang et al. (2008) demonstrated that miR-373 and miR-520 stimulated migration and invasion of cancer cells in vitro and in vivo. Related reports on the function of miR-372/373 members are also noteworthy. It was reported that E-cadherin and CSDC2 contain potential target sites highly complementary to miR-373 within their promoters. Transfection of miR-373 readily induced the expression of

the two genes, and induction was specifically dependent on the presence of both miR-373 and the proposed target promoter sites (Place et al., 2008).

5. Conclusion

Recently reported lines of evidence, some of which have been described thus far, all point to that miRNAs belonging to the three clusters, miR-302, miR-209, and miR-371, represent major regulators of pluripotent stem cells. They most likely participate in controlling all key aspects of 'stemness' including pluripotency, proliferation, and differentiation (Fig. 1). The latest series of reports indicate that ESC-specific miRNAs are regulated by ESC-specific transcription factors and that these miRNAs in turn control ESC-specific transcription factors. This fact alone should be sufficient to conclude that ESC-specific miRNAs are an integral part of the regulatory network of ESCs. More importantly, functional analyses based on the expression and inhibition of miRNAs in ESCs have begun to be reported, and preliminary data are consistent with that miRNAs is one of the determinants of the 'stemness'. As miRNAs are established as key regulators of numerous cellular events, ways of utilizing them as molecular tools are also being envisioned. This certainly also applies to ESCs: if miRNAs are regulators of 'stemness', we should be able to use them to control proliferation and differentiation of ESCs. Another use for miRNAs may be in reprogramming primary somatic cells into ESC-like cells with self-renewal and pluripotency. It is possible that miRNAs, organized in a single polycistronic transcript, may be a more convenient or efficient tool than a series of transcription factors. Furthermore, direct delivery of RNA into somatic cells would represent a safer alternative than transduction of retroviral vectors. It goes without saying that through the target analysis of miRNAs, we should also be able to gain insight into the gene regulatory networks and signaling pathways involved in particular characteristics or behaviors of ESCs, which is the fundamental prerequisite for applying ESCs for the regenerative therapy.

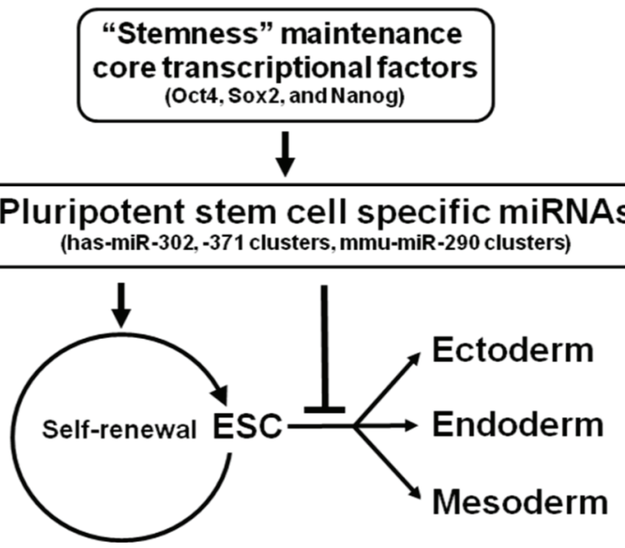

Fig. 1. Determination and regulation of "stemness" by miRNAs. miRNAs are regulated by core transcription factors of ESCs and in turn promote self-renewal and inhibit differentiation of ESCs .

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7. References

Ambros V & Chen X. (2007). The regulation of genes and genomes by small RNAs. *Development,* 134, 1635-1641.

Artzi S, Kiezun A & Shomron N. (2008). miRNAminer: a tool for homologous microRNA gene search. *BMC Bioinformatics*, 23,9-39.

Baev V, Daskalova E & Minkov I. (2009). Computational identification of novel microRNA homologs in the chimpanzee genome. *Comput Biol Chem,* 33(1), 62-70.

Barroso-Deljesus A, Romero-Lopez C, Lucena-Aguilar G, Melen GJ, Sanchez L, Ligero G, Berzal-Herranz A & Menendez P. (2008). Embryonic stem cell specific miR302-367 cluster: Human Gene Structure and Functional Characterization of its Core Promoter. *Mol Cell Biol,* 28, 6609-6619.

Bartel DP. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell,* 116, 281-297.

Benetti R, Gonzalo S, Jaco I, Munoz P, Gonzalez S, Schoeftner S, Murchison E, Andl T, Chen T, Klatt P, Li E, Serrano M, Millar S, Hannon G & Blasco MA. (2008). A mammalian microRNA cluster controls DNA methylation and telomere recombination via Rbl2-dependent regulation of DNA methyltransferases. *Nat Struct Mol Biol,* 15, 268-279.

Bentwich, I., Avniel, A., Karov, Y., Aharonov, R., Gilad, S., Barad, O., Barzilai, A., Einat, P., Einav, U. & Meiri, E. (2005). Identification of hundreds of conserved and nonconserved human microRNAs. *Nat Genet,* 37, 766-770.

Blakaj A & Lin H. (2008). Piecing together the mosaic of early mammalian development through microRNAs. *J Biol Chem,* 283, 9505-9508.

Brameier M. (2010). Genome-wide comparative analysis of microRNAs in three non-human primates. *BMC Res Notes,* 9, 3-64.

Card DA, Hebbar PB, Li L, Trotter KW, Komatsu Y, Mishina Y & Archer TK. (2008). Oct4/Sox2-regulated miR-302 targets cyclin D1 in human embryonic stem cells. *Mol Cell Biol*, 28, 6426-6438.

Chang K, Elledge SJ & Hannon GJ. (2006). Lessons from Nature: microRNA-based shRNA libraries. *Nat Methods,* 3, 707-714.

Chen CZ, Li L, Lodish HF & Bartel DP. (2004). MicroRNAs modulate hematopoietic lineage differentiation. *Science,* 303, 83-86.

Chin MH, Mason MJ, Xie W, Volinia S, Singer M, Peterson C, Ambartsumyan G, Aimiuwu O, Richter L, Zhang J, Khvorostov I, Ott V, Grunstein M, Lavon N, Benvenisty N, Croce CM, Clark AT, Baxter T, Pyle AD, Teitell MA, Pelegrini M, Plath K & Lowry WE. (2009). Induced Pluripotent Stem Cells and Embryonic Stem Cells Are Distinguishedby Gene Expression Signatures. *Cell Stem Cell*, 5, 111–123.

Cock JM, Sterck L, Rouzé P, Scornet D, Allen AE, Amoutzias G, Anthouard V, Artiguenave F, Aury JM, Badger JH, Beszteri B, Billiau K, Bonnet E, Bothwell JH, Bowler C, Boyen C, Brownlee C, Carrano CJ, Charrier B, Cho GY, Coelho SM, Collén J, Corre E, Da Silva C, Delage L, Delaroque N, Dittami SM, Doulbeau S, Elias M, Farnham G, Gachon CM, Gschloessl B, Heesch S, Jabbari K, Jubin C, Kawai H, Kimura K, Kloareg B, Küpper FC, Lang D, Le Bail A, Leblanc C, Lerouge P, Lohr M, Lopez PJ,

Martens C, Maumus F, Michel G, Miranda-Saavedra D, Morales J, Moreau H, Motomura T, Nagasato C, Napoli CA, Nelson DR, Nyvall-Collén P, Peters AF, Pommier C, Potin P, Poulain J, Quesneville H, Read B, Rensing SA, Ritter A, Rousvoal S, Samanta M, Samson G, Schroeder DC, Ségurens B, Strittmatter M, Tonon T, Tregear JW, Valentin K, von Dassow P, Yamagishi T, Van de Peer Y, & Wincker P. (2010). The Ectocarpus genome and the independent evolution of multicellularity in brown algae. *Nature*, 465 (7298), 617–621.

Cowan CA, Atienza J, Melton DA & Eggan K. (2005). Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science,* 309, 1369-1373.

Devor EJ & Samollow PB. (2008). In vitro and in silico annotation of conserved and nonconserved microRNAs in the genome of the marsupial Monodelphis domestica. *J Hered,* 99, 66-72.

Duale N, Lindeman B, Komada M, Olsen AK, Andreassen A, Soderlund EJ & Brunborg G. (2007). Molecular portrait of cisplatin induced response in human testis cancer cell lines based on gene expression profiles. *Mol Cancer,* 6, 53.

Friedländer MR, Chen W, Adamidi C, Maaskola J, Einspanier R, Knespel S & Rajewsky N. (2008). Discovering microRNAs from deep sequencing data using miRDeep. *Nat Biotechnol*, 26(4), 407-15.

Friedman, R. C., Farh, K. K., Burge, C. B & Bartel, D. P. (2009). Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res,* 19, 92-105.

Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM, Baskerville S, Hammond SM, Bartel DP & Schier AF. (2005). MicroRNAs regulate brain morphogenesis in zebrafish. *Science,* 308, 833-838.

Giraldez AJ, Mishima Y, Rihel J, Grocock RJ, Van Dongen S, Inoue K, Enright AJ & Schier AF. (2006). Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science,* 312, 75-79.

Guan K, Nayernia K, Maier LS, Wagner S, Dressel R, Lee JH, Nolte J, Wolf F, Li M, Engel W & Hasenfuss G. (2006). Pluripotency of spermatogonial stem cells from adult mouse testis. *Nature*, 440, 1199-1203.

Hayashi K, Chuva de Sousa Lopes SM, Kaneda M, Tang F, Hajkova P, Lao K, O'Carroll D, Das PP, Tarakhovsky A, Miska EA & Surani MA. (2008). MicroRNA biogenesis is required for mouse primordial germ cell development and spermatogenesis. *PLoS ONE,* 3, e1738.

Houbaviy HB, Murray MF & Sharp PA. (2003). Embryonic stem cell-specific MicroRNAs. *Dev Cell,* 5, 351-358.

Huang Q, Gumireddy K, Schrier M, le Sage C, Nagel R, Nair S, Egan DA, Li A, Huang G, Klein-Szanto AJ, Gimotty PA, Katsaros D, Coukos G, Zhang L, Pure E & Agami R. (2008). The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. *Nat Cell Biol,* 10, 202-210.

International Chicken Genome Sequencing Consortium, Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. (2004). *Nature,* 432, 695-716.

Izadyar F, Pau F, Marh J, Slepko N, Wang T, Gonzalez R, Ramos T, Howerton K, Sayre C & Silva F. (2008). Generation of multipotent cell lines from a distinct population of male germ line stem cells. *Reproduction*, 135, 771-784.

Judson RL, Babiarz JE, Venere M & Blelloch R. (2009). Embryonic stem cell–specific microRNAs promote induced pluripotency. *Nat Biotechnol*, 27, 459-461.

Kanatsu-Shinohara M, Inoue K, Lee J, Yoshimoto M, Ogonuki N, Miki H, Baba S, Kato T, Kazuki Y, Toyokuni S, Toyoshima M, Niwa O, Oshimura M, Heike T, Nakahata T,

Ishino F, Ogura A & Shinohara T. (2004). Generation of pluripotent stem cells from neonatal mouse testis. *Cell*, 119, 1001-1012.

Kanatsu-Shinohara M, Lee J, Inoue K, Ogonuki N, Miki H, Toyokuni S, Ikawa M, Nakamura T, Ogura A & Shinohara T. (2008). Pluripotency of a single spermatogonial stem cell in mice. *Biol Reprod*, 78, 681-687.

Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, Livingston DM & Rajewsky K. (2005). Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. *Genes Dev*, 19, 489-501.

Kim HJ, Lee HJ, Lim JJ, Kwak KH, Kim JS, Kim JH, Han YM, Kim KS & Lee DR. (2010). Identification of intermediate state as spermatogonial stem cells reprogram to multipotent cells. *Mol Cells*, 29, 519-526.

Kim KS, Kim JS, Lee MR, Jeong HS & Kim J. (2009) A Study of microRNAs *in silico* and *in vivo*: Emerging Regulators of Embryonic Stem Cells. *FEBS Journal*, 276, 2140-2149.

Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M, Lin C, Socci ND, Hermida L, Fulci V, Chiaretti S, Foa R, Schliwka J, Fuchs U, Novosel A, Muller RU, Schermer B, Bissels U, Inman J, Phan Q, Chien M, Weir DB, Choksi R, De Vita G, Frezzetti D, Trompeter HI, Hornung V, Teng G, Hartmann G, Palkovits M, Di Lauro R, Wernet P, Macino G, Rogler CE, Nagle JW, Ju J, Papavasiliou FN, Benzing T, Lichter P, Tam W, Brownstein MJ, Bosio A, Borkhardt A, Russo JJ, Sander C,

Lee MR, Kim JS & Kim KS. (2010). miR-124a is Important for Migratory Cell Fate Transition During Gastrulation of Human Embryonic Stem Cells. Stem Cells, 28, 1550-1559.

Lee NS, Kim JS, Cho WJ, Lee MR, Steiner R, Gompers A, Ling D, Zhang J, Strom P, Behlke M, Moon SH, Salvaterra PM, Jove R & Kim KS. (2008). miR-302b maintains "stemness" of human embryonal carcinoma cells by post-transcriptional regulation of Cyclin D2 expression. *Biochem Biophys Res Commun*, 377, 434-440.

Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, Chevalier B, Johnstone SE, Cole MF, Isono K, Koseki H, Fuchikami T, Abe K, Murray HL, Zucker JP, Yuan B, Bell GW, Herbolsheimer E, Hannett NM, Sun K, Odom DT, Otte AP, Volkert TL, Bartel DP, Melton DA, Gifford DK, Jaenisch R & Young RA. (2006). Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell*, 125, 301-313.

Lewis BP, Burge CB & Bartel DP. (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*, 120, 15-20.

Linsen SE, de Wit E, de Bruijn E & Cuppen E. (2010). Small RNA expression and strain specificity in the rat, *BMC Genomics*, 11, 249.

Lin SL, Chang DC, Chang-Lin S, Lin CH, Wu DT, Chen DT & Ying SY. (2008). Mir-302 reprograms human skin cancer cells into a pluripotent ES-cell-like state. *RNA*, 14, 115-2124.

Makeyev EV, Zhang J, Carrasco MA & Maniatis T. (2007). The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. *Mol Cell*, 27, 435-448.

Markert A, Grimm M, Martinez J, Wiesner J, Meyerhans A, Meyuhas O, Sickmann A & Fischer U. (2008). The La-related protein LARP7 is a component of the 7SK ribonucleoprotein and affects transcription of cellular and viral polymerase II genes. *EMBO Rep*, 9, 569-575.

Mantel C, Guo Y, Lee MR, Kim MK, Han MK, Shibayama H, Fukuda S, Yoder MC, Pelus LM, Kim KS & Broxmeyer HE. (2007). Checkpoint-apoptosis uncoupling in human

and mouse embryonic stem cells: a source of karyotpic instability. *Blood*, 109, 4518-4527.

Marson A, Levine SS, Cole MF, Frampton GM, Brambrink T, Johnstone S, Guenther MG, Johnston WK, Wernig M, Newman J, Calabrese JM, Dennis LM, Volkert TL, Gupta S, Love J, Hannett N, Sharp PA, Bartel DP, Jaenisch R & Young RA. (2008). Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells. *Cell,* 134, 521-533.

Mineno J, Okamoto S, Ando T, Sato M, Chono H, Izu H, Takayama M, Asada K, Mirochnitchenko O, Inouye M & Kato I. (2006). The expression profile of microRNAs in mouse embryos. *Nucleic Acids Res,* 34, 1765-1771.

Murchison EP, Kheradpour P, Sachidanandam R, Smith C, Hodges E, Xuan Z, Kellis M, Grützner F, Stark A & Hannon GJ. (2008). Conservation of small RNA pathways in platypus. *Genome Res*, 18(6), 995-1004.

Obernosterer G, Leuschner PJ, Alenius M & Martinez J. (2006). Post-transcriptional regulation of microRNA expression. *RNA,* 12, 1161-1167.

Oh SH, Jung YH, Gupta MK, Uhm SJ & Lee HT. (2009). H19 gene is epigenetically stable in mouse multipotent germline stem cells. *Mol Cells*, 27, 635-640.

Palmieri A, Pezzetti F, Brunelli G, Zollino I, Scapoli L, Martinelli M, Arlotti M & Carinci F. (2007). Differences in osteoblast miRNA induced by cell binding domain of collagen and silicate-based synthetic bone. *J Biomed Sci,* 14, 777-782.

Place RF, Li LC, Pookot D, Noonan EJ & Dahiya R. (2008). MicroRNA-373 induces expression of genes with complementary promoter sequences. *Proc Natl Acad Sci U S A,* 105, 1608-1613.

Rao PK, Kumar RM, Farkhondeh M, Baskerville S & Lodish HF. (2006). Myogenic factors that regulate expression of muscle-specific microRNAs. *Proc Natl Acad Sci U S A,* 103, 8721-8726.

Seandel M, James D, Shmelkov SV, Falciatori I, Kim J, Chavala S, Scherr DS, Zhang F, Torres R, Gale NW, Yancopoulos GD, Murphy A, Valenzuela DM, Hobbs RM, Pandolfi PP & Rafii S. (2007). Generation of functional multipotent adult stem cells from GPR125+ germline progenitors. *Nature*, 449, 346-350.

Sewer A, Paul N, Landgraf P, Aravin A, Pfeffer S, Brownstein MJ, Tuschl T, van Nimwegen E & Zavolan M. (2005). Identification of clustered microRNAs using an ab initio prediction method. *BMC Bioinformatics,* 6, 267.

Sinkkonen L, Hugenschmidt T, Berninger P, Gaidatzis D, Mohn F, Artus-Revel CG, Zavolan M, Svoboda P & Filipowicz W. (2008). MicroRNAs control de novo DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells. *Nat Struct Mol Biol,* 15, 259-267.

Suh MR, Lee Y, Kim JY, Kim SK, Moon SH, Lee JY, Cha KY, Chung HM, Yoon HS, Moon SY, Kim VN & Kim KS. (2004). Human embryonic stem cells express a unique set of microRNAs. *Dev Biol,* 270, 488-498.

Stadler B, Ivanovska I, Mehta K, Song S, Nelson A, Tan Y, Mathieu J, Darby C, Blau CA, Ware C, Peters G, Miller DG, Shen L, Cleary MA & Ruohola-Baker H. (2010). Characterization of microRNAs Involved in Embryonic Stem Cell States. *Stem Cells Dev*,19,935-950.

Takahashi K, & Yamanaka S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, 126, 663-676.

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS & Jones JM. (1998). Embryonic stem cell lines derived from human blastocysts. *Science,* 282, 1145-1147.

Ventura A, Young AG, Winslow MM, Lintault L, Meissner A, Erkeland SJ, Newman J, Bronson RT, Crowley D, Stone JR, Jaenisch R, Sharp PA & Jacks T. (2008). Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miRNA clusters. *Cell,* 132, 875-886.

Voorhoeve PM, le Sage C, Schrier M, Gillis AJ, Stoop H, Nagel R, Liu YP, van Duijse J, Drost J, Griekspoor A, Zlotorynski E, Yabuta N, De Vita G, Nojima H, Looijenga LH & Agami R. (2006). A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. *Cell,* 124, 1169-1181.

Wagner W, Horn P, Castoldi M, Diehlmann A, Bork S, Saffrich R, Benes V, Blake J, Pfister S, Eckstein V & Ho AD. (2008). Replicative senescence of mesenchymal stem cells: a continuous and organized process. *PLoS ONE,* 3, e2213.

Wang Y, Baskerville S, Shenoy A, Babiarz JE, Baehner L & Blelloch R. (2008). Embryonic Stem Cell Specific MicroRNAs Regulate the G1/S Transition and Promote Rapid Proliferation. *Nat Genet*, 40,1478–1483.

Watanabe T, Takeda A, Mise K, Okuno T, Suzuki T, Minami N & Imai H. (2005). Stage-specific expression of microRNAs during Xenopus development. *FEBS Lett,* 579, 318-324.

Wilson KD, Venkatasubrahmanyam S, Jia F, Sun N, Butte AJ & Wu JC. (2009). MicroRNA Profiling of Human-Induced Pluripotent Stem Cells. *Stem cell Dev*, 18, 749-757.

Yeom KH, Lee Y, Han J, Suh MR & Kim VN. (2006). Characterization of DGCR8/Pasha, the essential cofactor for Drosha in primary miRNA processing. *Nucleic Acids Res,* 34, 4622-4629.

Yu J, Vodyanik MA, He P, Slukvin II & Thomson JA. (2006). Human embryonic stem cells reprogram myeloid precursors following cell-cell fusion. *Stem Cells*, 24, 168-176.

Yu J & Thomson JA. (2008). Pluripotent stem cell lines. *Genes Dev,* 22, 1987-1997.

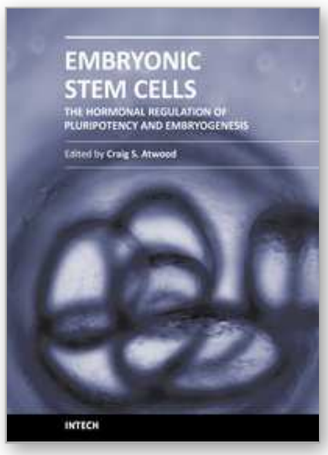
Yue J, Sheng Y & Orwig KE. (2008). Identification of novel homologous microRNA genes in the rhesus macaque genome. *BMC Genomics,* 9, 8.

Zeuthen J, Norgaard JO, Avner P, Fellous M, Wartiovaara J, Vaheri A, Rosen A & Giovanella BC. (1980). Characterization of a human ovarian teratocarcinoma-derived cell line. *Int J Cancer,* 25, 19-32.

Zhao Y, Samal E & Srivastava D. (2005). Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. *Nature,* 436, 214-220.

Zhou M, Wang Q, Sun J, Li X, Xu L, Yang H, Shi H, Ning S, Chen L, Li Y, He T & Zheng Y. (2009). In silico detection and characteristics of novel microRNA genes in the Equus caballus genome using an integrated ab initio and comparative genomic approach. *Genomics,* 94(2), 125-31.

Zovoilis A, Pantazi A, Smorag L, Opitz L, Salinas Riester G, Wolf M, Zechner U, Holubowska A, Stewart CL & Engel W. (2010). Embryonic stem cell related miRNAs are involved in differentiation of pluripotent cells originating from the germ line. *Mol Hum Reprod*, doi: 10.1093/molehr/gaq053.



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Pluripotency is a prerequisite for the subsequent coordinated differentiation of embryonic stem cells into all tissues of the body. This book describes recent advances in our understanding of pluripotency and the hormonal regulation of embryonic stem cell differentiation into tissue types derived from the ectoderm, mesoderm and endoderm.

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