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Review

MicroRNAs in pluripotency, reprogramming and cell fate induction

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ABSTRACT

Pluripotent stem cells display a unique expression pattern of microRNAs (miRNAs). These ~22 nucleotide non-coding RNAs have established a crucial role in controlling gene expression of pluripotent stem cells at the post-transcriptional level. Recent studies made important advances in identifying miRNA regulated processes like de novo DNA methylation, progression of the cell cycle and regulation of cell fate decision. miRNAs have also the ability to reprogram somatic cells to pluripotent stem cells and on the other hand, to induce differentiation of pluripotent stem cells into distinct somatic lineages. Previously it was published that miRNAs can direct reprogramming on its own. Here we provide evidence and critically discuss that the effect of miRNA depends on co-expression of the classical reprogramming factors. During transition between these different cell fates distinct miRNAs adjust the levels of specific transcriptional programs and confer robustness to differentiation processes. This results in a complex network between miRNAs and their targets. The fact that miRNAs itself can also be regulated by its targets establishes complex regulatory loops. Based on bioinformatical predictions, each miRNA theoretically has hundreds of target genes making it even more challenging to understand the complete network between miRNAs and their targets.

Contents

1. Introduction

Pluripotent cells are capable to differentiate into all cell types within the body including the germ cells. These characteristics are displayed by cells of the inner cell mass (ICM) from early embryos enabling them to form the complete adult organism. The pluripotent state of cells from the ICM can be captured in vitro by placing blastocysts in culture, leading to the generation of pluripotent embryonic stem cells (ESCs) [1].

The derivation of complete animals by transplantation of somatic nuclei into eggs was the first demonstration that cell fates are neither restricted nor irreversible [2]. Subsequent work demonstrated that the determined fate of differentiated cells can be reversed by nuclear transfer, cell fusion or iPS (induced pluripotent stem cell) technology [2–6]. These approaches offer the opportunity to generate pluripotent embryonic-like cells from adult, somatic cells of the same individual, opening the field of personalized regenerative medicine. Furthermore, the developments in this field have been honored by the Nobel Prize for Physiology or Medicine in 2012. The Nobel Prize was awarded to Sir John Gurdon, who cloned a frog by nuclear transfer in 1962 [7] and to Shinya Yamanaka, who invented iPS technology [6].

In contrast to nuclear transfer and cell fusion, induced pluripotent stem cells (iPSCs) are generated by overexpression of the transcription

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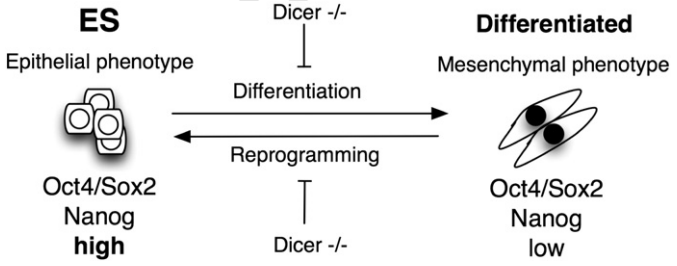
factors Oct4, Sox2, Klf4 and c-Myc and do not rely on the usage of unfertilized eggs making them ethically less controversial. On the other hand, iPSC generation is less efficient, appears to be slower and it still remains questionable if iPSCs are in full terms equivalent to ESCs or pluripotent cells derived by nuclear transfer [8,9]. Several studies indicate that subtle differences in gene expression and chromatin modifications lead to partially reprogrammed iPSCs, which retain an epigenetic memory [10–12]. This manifests in the tendency of tissue-specific iPSCs to more efficiently differentiate to somatic cells of their tissue of origin. Reduced pluripotency has also been associated with loss of imprinting at the *Dlk1–Dio3* locus [13]. iPSC lines that lost imprinting at this specific locus poorly contributed to chimeric mice and were not able to generate "all-iPSC mice" in tetraploid complementation assays [13]. This study also demonstrates the power of tetraploid complementation assays to investigate full developmental potential of iPSCs. Subsequent investigations suggest that imprinting at the *Dlk1–Dio3* locus is not an absolute marker for pluripotency. Expression and stoichiometry of reprogramming factors as well as other parameters rather seem to influence the grade of pluripotency [14]. So far it remains unclear whether such subtle biological differences between ES cells and fully reprogrammed iPSCs have any functional consequences, which might challenge the use of iPSCs in disease research [11,15]. Nevertheless, some promising reports carried out with ESCs demonstrated that pluripotent cells are capable to differentiate into specific cell types in vitro which are capable to integrate and participate in tissue repair when transplanted into animal disease models [16,17].

The regulation of pluripotency is controlled by a complex regulatory network including several transcription factors and chromatin modifying enzymes. The core transcriptional network consists of Oct4, Nanog and Sox2. All three transcription factors are highly expressed in the ICM, epiblast and undifferentiated ESCs. Disruption of each of these genes in mice results in early embryonic lethality due to loss of pluripotent cells within the ICM of preimplantation embryos [18,19]. While, c-Myc and Klf4 are used in some reprogramming approaches starting with fibroblasts those seem not to be part of the core transcriptional network in ESCs. In fact even Oct4 alone is able to reprogram neuroectodermal cells from mice and men. The transcriptional regulation of pluripotency and the unique chromatin status of pluripotent cells as well as the different states of pluripotency are extensively reviewed elsewhere [20–24].

Another important role in the regulation of pluripotency and lineage specification has recently emerged for non-coding microRNAs that are associated with the main regulatory circuitry by modulating gene expression at the post-transcriptional level (Fig. 1) [25]. This review focuses on the emerging role of miRNAs in cell fate regulation of pluripotent stem cells. Within the next chapters miRNAs mediated regulation of pluripotency and reprogramming is introduced and miRNA mediated cell fate specification will be discussed.



Q2 **Fig. 1.** Differentiation of embryonic stem cells (ESCs). Embryonic stem cells are characterized by a transcriptional network involving the transcription factors Oct4, Sox2 and Nanog, which are downregulated in many cell types during differentiation. The inverse process (reprogramming) can be initiated by forced expression of some of these factors in combination with others such as c-Myc and Klf4. Surprisingly, both processes seem to rely on miRNAs, since knockout of Dicer inhibits both processes.

2. Biogenesis of miRNAs in mice and men

MicroRNAs (miRNAs) were initially discovered in *Caenorhabditis elegans* during a genetic screen to uncover molecules involved in the regulation of nematode development [26]. Since then, a fundamental role has been established for these small ~22 nucleotide non-coding RNAs in controlling gene expression at the post-transcriptional level. Hundreds of miRNAs have been discovered in most eukaryotic species [27], located in intergenic regions as well as in exons or introns of other genes.

Most miRNAs are transcribed by Polymerase II as long primary, capped and polyadenylated transcripts (pri-miRNAs) [28], which are processed into their mature form in a complex multistep process.

Briefly, within the nucleus, the pri-miRNA is converted by a microprocessor-complex to the precursor miRNA (pre-miRNA). The microprocessor-complex consists of the RNase type III endonuclease Drosha, which is associated to the Di George syndrome critical region gene 8 (DGCR8) and additional co-factors. This complex recognizes the hairpin secondary structures embedded in the pri-miRNA [29–32]. The resulting ~70 nucleotide hairpin pre-miRNA is excised from the pri-miRNA and recognized by the Exportin-5/Ran-GTP complex, actively exporting the pre-miRNA out of the nucleus [33–35]. A small subclass of miRNAs, located in small introns, the so called mirtrons, can bypass the Drosha-mediated processing, which is crucial for the vast majority of the other miRNAs [36]. After entering the cytoplasm, the pre-miRNA is further processed by another RNase-type III enzyme, the so called Dicer, to its mature ~22 nucleotide miRNA–miRNA* duplex. Dicer contains a PAZ-domain (Pie/Argonaut/Zwille) recognizing the 3′-overhangs of the pre-miRNAs generated by the microprocessor [37,38]. The dicer-enzyme cuts the pre-miRNAs within the stem loop and creates thereby the ~22–24 nucleotide miRNA-duplex, which remains bound to the dicer.

In most cases only one strand of this duplex, the designated "major" strand, gets incorporated into the multi-protein miRNA ribonucleoprotein complex (miRNP or miRISC complex). The "minor" strand, referred to as miRNA*, gets degraded after exclusion from the miRISC complex. Some large scale studies provided strong evidence, that the miRNA* strand also has a biological function, since a larger number of miRNA* sequences could be detected among the total miRNA population than expected.

The miRNA loaded miRISC complex is used to guide it to its target sites. Previous studies focused on the target sites within the 3′UTR (untranslated region) whereby miRNA targeting within the 5′UTR and cds (coding sequence) has been overlooked. Thus, recent sequencing data using anti-Ago immunoprecipitation and cross-linking have reported target sites e.g. in cultivated HEK 293 cells from 5′UTR to cds and 3′UTR, targeting 4647 transcripts (21% of 22,466 unique HEK293 cell transcripts) [39]. Ago1–4 seemed to bind to the same target sequences: 84% of the miRNA target sites were located in exons, whereas 4% bound to 5′UTR, 50% to cds and 46% to 3′UTR. According to data by Hafner and coworkers miRNA sites in cds seem to result in small significant mRNA destabilization in HEK293 cells.

Target interaction of miRNA and mRNA seems to involve a seed-pairing interaction with a match of approximately 7 nt near the 5′end of the 22nt miRNA (e.g. positions 2–7) with sites in the target region of mRNA [40]. Targeting-sites that cannot be explained by the canonical seed-match model seem to involve non-Watson–Crick base pairing with G-bulges at positions 5–6 and suggest an alternative mode of microRNA target recognition, making target gene identification even more challenging [41].

Initial studies suggested that animal miRNAs exclusively mediate target gene silencing by translational repression. Recently, there is growing evidence that animal miRNAs can induce mRNA degradation as well as translational repression [42]. Translational repression has been proposed to occur at four different stages: initiation of translation, elongation of translation, protein degradation during translation

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