The Role of RNA Interference in Stem Cell Biology: Beyond the Mutant Phenotypes

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Abstract

Complex gene regulation systems ensure the maintenance of cellular identity during early development in mammals. Eukaryotic small RNAs have emerged as critical players in RNA interference (RNAi) by mediating gene silencing during embryonic stem cell self-renewal. Most of the proteins involved in the biogenesis of small RNAs are essential for proliferation and differentiation into the three germ layers of mouse embryonic stem cells. In the last decade, new functions for some RNAi proteins, independent of their roles in RNAi pathways, have been demonstrated in different biological systems. In parallel, new concepts in stem cell biology have emerged. Here, we review and integrate the current understanding of how RNAi proteins regulate stem cell identity with the new advances in the stem cell field and the recent non-canonical functions of the RNAi proteins. Finally, we propose a reevaluation of all RNAi mutant phenotypes, as non-canonical (small non-coding RNA independent) functions may contribute to the molecular mechanisms governing mouse embryonic stem cells commitment.

Introduction

The first report of RNA interference (RNAi) in 1998 and the discovery of its effector molecules, the small interfering RNAs (siRNAs) [1,2], have revolutionized our understanding of gene regulation. Since then, the biological importance of these silencing small RNAs (sRNAs) and other classes of non-coding sRNAs has been evidenced through a staggering array of publications implicating these molecules in virtually all aspects of cell biology. MicroRNAs (miRNAs), in particular, influence many biological processes including immune responses, hormonal regulation, phase transitioning and patterning, and embryonic and post-embryonic development [3]. Tight and stringent regulation of the sRNA pathways is a cellular necessity, as perturbations in the expression and/or activity of these small molecules have considerable deleterious effects including genetic instability, sterility, and loss of cellular identity. Recent research indicates that miRNAs have important roles in regulating stem cell self-renewal and differentiation by repressing the translation of selected mRNAs in stem cells and differentiating daughter cells [4]. More importantly, mouse embryonic stem cells (mESCs) deficient of *Dicer* or *Dgcr8*, two essential genes involved in miRNA biogenesis, fail to produce miRNAs and manifest defects in proliferation and differentiation, which confirms the essential role for the RNAi genes in early mouse development [5–7]. Unfortunately, the *Dicer* and *Dgcr8* mutant mESCs reported by different laboratories have a distinct background, making their comparison difficult. Nevertheless, the aforementioned *Dicer*- and *Dgcr8*-deficient mESCs present differences

in their phenotype (e.g., proliferation and cell cycle defects, impaired differentiation) [6–8], suggesting that the role of DICER in the siRNA pathway could have a specific function in mESCs homeostasis [7] or that DICER and/or DGCR8 proteins might have non-canonical functions other than their specific role in the miRNA pathway. Moreover, recent breakthroughs in stem cell biology now allow to distinguish between the exit from pluripotency (an event necessary for mESC commitment) and the differentiation of mESCs (their capacity to activate differentiation programs).

In this perspective, we present previous and emerging concepts in RNAi and stem cell biology fields. Finally, we propose that these new concepts should call for a reinvestigation of the RNAi mutant phenotypes in the light of their putative non-canonical functions in stem cell biology.

State-Of-The-Art and New Advances in Stem Cell Biology

In 1981, two groups successfully isolated cells directly from mouse blastocyst for the first time [9,10]. Derived from the whole delayed blastocyst [9] or from the inner cell mass (ICM) isolated by immunosurgery [10], early embryonic cells exhibited the capacity to generate well-differentiated teratomas and presented a normal karyotype [9–11]. The ICM-isolated cells were named mESCs [10]. The first studies implemented the culture protocols of mESCs using feeder cells and serum-supplemented medium [9]. The establishment of these culture conditions highlighted either the presence (in the serum and in the conditioned medium) or the production (by the feeder cells) of factors favoring the survival of mESCs [9,10,12,13].

Despite these mESC isolation breakthroughs [9,10], the original empirically established protocols and culture conditions did not allow isolation of ESCs from other mammals. At the beginning, ESCs could be derived only from mouse embryos of inbred 129 strain and more rarely from the C57BL/6 strain [14]. Deconvolution of the mixture of the factors present in the serum and of the interactions between mESCs and the feeder cells allowed researchers to identify a key differentiation-inhibiting protein: the cytokine leukemia inhibitory factor (LIF) [15,16]. Addition of LIF to the serum allowed derivation and long-term culture of mESCs in the absence of feeder cells [15,16]. However, this improvement was still limited to the 129 mouse strains. Briefly, LIF promotes self-renewal by repressing the differentiation programs via the stimulation of the signal transducer and activator of transcription 3 [17–20]. Moreover, LIF alone was not sufficient to sustain mESC culture, as serum withdraw led to the differentiation of the cells [21]. Later, the bone morphogenetic factor-4 (BMP4) was found to also support mESC propagation [22]. In BMP4 plus LIF condition, mESCs from the 129 background were successfully derived and cultured in a feeder-free and serum-free environment [22]. Remarkably, LIF has also been shown to promote differentiation by stimulating the mitogen-activated protein kinase/Extracellular Signal-Regulated Kinase (ERK pathway) [23]. Indeed, suppression of the ERK pathway improved mESC self-renewal [24]. Finally, it was reported that mESCs produce significant amount of another potent activator of the ERK pathway: the fibroblast growth factor-4 [24,25]. Therefore, new approaches using ERK pathway repressors started to be employed. In conclusion, a new culture condition combining LIF, BMP4, and ERK pathway inhibitors allowed the reproducible derivation of mESCs from C57BL/6 and CBA strains in a feeder-free and serum-free environment [26].

In the early 2000s, studies revealed a new antagonist of mESC self-renewal, the glycogen synthase kinase-3 (GSK3), and further investigations showed that its inhibition enhanced mESC propagation [27–30]. GSK3 negatively regulates a range of cellular and intracellular pathways, including the canonical Wnt/ß-catenin pathway [31,32]. The big breakthrough happened in 2008, when Ying and colleagues defined a new medium combining three inhibitors: an FGF receptor inhibitor (SU5042), an ERK pathway inhibitor (PD184352), and a GSK3 inhibitor (CHIR99021) [33]. Mouse ESCs cultured in this new medium named 3i (for three inhibitors) displayed a stronger self-renewal compared to mESCs cultured in serum plus LIF or LIF and BMP4 conditions and even propagated in the absence of LIF or serum [33]. Afterwards, a single high potency and selectively MEK stands for MAP (Mitogen-Activated Protein) Kinase/ERK (Extracellular Signal-Regulated Kinase) Kinase (MEK) inhibitor (PD0325901) and a GSK3 inhibitor were shown to be sufficient to support the long-term culture of mESCs [34]; therefore, this new culture condition was coined 2i. Thus, it is now acknowledged that in 2i medium, mESCs reach a ground-state naïve pluripotency, resembling to the epiblast of the mature mouse blastocyst [35].

In addition, the 2i culture condition overcame the non-permissive strain barrier and enabled the derivation of ESCs from other mouse backgrounds [33,36–38] and from other rodent species [39,40]. More recently, the 2i media has permitted the derivation of haploid mESCs [41]. These cells are a tremendous valuable tool because they allow the usage of genetic screens, which will extend genome exploration into developmentally and medically relevant pathways [42].

Impact of these New Discoveries on the Pluripotency Network in mESCs

Several transcription factors are critical for the maintenance of the naïve pluripotent state of mESCs including OCT4, SOX2, and NANOG (OSN). Their