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Review

THE ROLE OF MICRORNAS IN RABBIT PREIMPLANTATION EMBRYOS AND PLURIPOTENT STEM CELLS

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ABSTRACT

Although several groups are able to generate rabbit embryonic stem cells (ESCs) and induced pluripotency cells (iPS) cell lines using different derivation methods, it is still difficult to establish it, and the pluripotent lines, therefore, remain poorly characterized. Since ESCs are derived from early blastocysts, they can reflect the potential characteristics of their embryonic founder population. Therefore, it is important to compare the expression pattern of both miRNAs and proteins known to play regulatory roles during early lineage specification. Our group aimed to explore ESC-specific miRNA expression pattern from early embryonic stages to rabbit embryonic stem-like (ES-like) cell for the first time to get more insight into their potential regulatory mechanism during embryonic development and in stem cell properties.

Key words: rabbit; embryo; epiblast; hypoblast; stem cell; microRNA

INTRODUCTION

Overview of rabbit embryonic stem cell derivation

Human ESCs are potential source for regenerative medicine, tissue engineering, cell-based transplantation, disease pathology and drug discovery (Thomson *et al*., 1998). In spite of their great medical potential, human ESCs generation requires the destruction of developing human embryos which restrict their intended application ethically. By generation of iPSCs from human somatic cells, it was expected to overcome the ethical controversy associated with human ESCs research (Takahashi *et al.*, 2007; Park *et al*., 2008). To overcome the limitations associated with human ESCs, many efforts have been put to generate the stable animal ESC lines in order to use in regenerative medicine. The rabbit ESCs represent an alternative animal model to study human diseases since rabbit ESCs resemble primate ESCs more closely than mouse ESCs (Wang *et al*., 2007). The establishment of rabbit ESCs has been reported by several groups (Honda *et al.*, 2009), (Catunda *et al.*, 2008; Intawicha *et al*., 2009). Although there are reports indicating that rabbit ESCs might be passaged for longer period over 20 passages (Intawicha *et al*., 2013), but it needs to be maintained manually and pick the only colonies which still keep the typical ESC morphology and stayed undifferentiated (Honda, 2013; Osteil *et al*., 2013;

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Tancos *et al.*, 2012). Rabbit pluripotent stem cells were defined as EpiSC-type or primed state stem cells; these are rarely competent to contribute to blastocyst chimeras and are, therefore, developmentally and functionally distinct from naive state mouse ES cells (Osteil *et al*., 2013), (Intawicha *et al.*, 2013). The first live born ES-derived rabbit chimera was reported in 2011 (Zakhartchenko *et al.*, 2011), but there are no reports about the germ line transmission.

Regulatory function of miRNA during early development

MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate multiple biological processes. They post-transcriptionally regulate gene expression mostly through binding to complementary regions within 3‘UTRs of their target mRNAs leading to translational repression or cleavage. Increasing experimental evidence implies an important regulatory role of miRNAs during early embryonic development and in embryonic stem cell biology.

Regulation of gene expression in developmental processes is an important aspect of miRNA function. Many experiments have been performed to dissect the miRNA pathway in various organisms demonstrating that miRNAs are essential for proper embryonic development. Mouse pre-implantation embryos express developmental stage-specific miRNAs (Mineno *et al.*, 2006; Viswanathan *et al.*, 2009). Interestingly, several stem cell specific miRNAs, such as miR-290 cluster, were shown to be the first de novo expressed miRNAs in mouse embryos at 2-4-cell stage, with an increasing expression through the blastocyst stage (Zeng and Schultz, 2005), (Viswanathan *et al*., 2009), (Kutter and Svoboda, 2008). Moreover, miR-290 cluster deficiency in mouse embryos causes penetrant embryonic lethality and germ cell defects implying the important role of this cluster in embryonic development (Medeiros *et al*., 2011).

Regulatory Function of miRNA in ESCs

MicroRNAs have also been identified as important regulators of embryonic stem cell properties (Cao *et al*., 2008), (Marson *et al.*, 2008). The mmu-miR-290 cluster has been reported as an ESC-specific miRNA which expressed highly in undifferentiated ESCs (Marson *et al.*, 2008) and inhibited the ESC differentiation (Zovoilis *et al*., 2009). This cluster has an important regulatory function in undifferentiated ESCs through direct control of de novo DNA methylation (Benetti *et al.*, 2008; Sinkkonen *et al.*, 2008), cell cycle regulation by suppressing the G1-S transition and targeting cell cycle regulators (Lichner *et al.*, 2011; Wang *et al.*, 2008). In addition, the promoter of this cluster is a direct target of key ESC transcription factors, such as OCT4, SOX2 and NANOG (Marson *et al.*, 2008). The human homolog of mmu-miR-290 cluster, miR-371 cluster was also identified to be expressed in human ESCs and does not reappear in any somatic lineage. This cluster shares the chromosomal region with C19MC cluster (chromosome 19 miRNA Cluster) which is a highly repetitive region containing 46 members of the super-family and shows high sequence similarity to both hsa-miR-371 and mmu-miR-290 clusters (Bentwich *et al.*, 2005; Lichner *et al.*, 2011). The miR-302 cluster is also highly expressed in both human and mouse ESCs and down-regulated upon differentiation (Chen *et al.*, 2007). Similar to miR-290 family this cluster is a cell cycle regulator; cyclin D1 and Cdk4 are post-transcriptionally regulated by miR-302 cluster in human ESCs resulting in positive regulation of ESC self-renewal (Card *et al.*, 2008). Furthermore, this cluster might positively regulate the Nodal/Activin pathway, therefore contributing to the maintenance of pluripotency (Barroso-delJesus *et al.*, 2008).

A microRNA expression profile in rabbit embryos and stem cells

By applying SOLID deep sequencing technique, we reported the miRNA expression profile during early rabbit embryonic development and in ES-like cells (Maraghechi *et al.*, 2013). We could identify a total of 1693 expressing rabbit miRNAs based on comparison of obtained sequence reads to the known human, mouse and bovine miRNA databases (miRBase). We detected and characterized ESC-specific miRNAs of early steps of rabbit ESC line establishment and their embryonic founder cell population of attached ICM clumps and embryonic disc. We described and analyzed the expression profile of pluripotency-associated miRNAs in rabbit embryos and embryonic stem-like (ES-like) cells. The rabbit specific ocu-miR-302, ocu-miR-290 clusters and three homologs of human C19MC cluster (ocu-miR-512, ocu-miR-520e and ocu-miR-498) were identified in rabbit preimplantation embryos and ES-like cells. The ocu-miR-302 cluster was highly similar to its human homolog, while ocu-miR-290 revealed a low level of evolutionary conservation with its mouse homologous cluster. The expression of ocu-miR-302 cluster members began at 3.5 days post coitum - early blastocyst stage and they stayed highly expressed in rabbit ES-like cells. In contrast, high expression level of ocu-miR-290

cluster members was detected during preimplantation embryonic development, but low level of expression was found in rabbit ES-like cells.

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