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Validation of rabbit microRNA sequences identified by SOLiD™ sequencing in rabbit preimplantation embryos and embryonic stem-like cells

*G Elen¹, N Kinga¹, M Pouneh¹, B Babett¹ & B Zsuzsanna¹

¹Agricultural Biotechnology Center, Animal Biotechnology, Godollo, Hungary

MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate multiple biological processes. Increasing experimental evidence implies an important regulatory role of miRNAs during early embryonic development and in embryonic stem cell biology.

More than 1000 human (*Homo sapiens*) and more than 800 mouse (*Mus musculus*) microRNAs are known, but not any yet in rabbit (*Oryctolagus cuniculus*). We aimed to explore rabbit pluripotent stem cell (rabPSC) specific miRNA expression pattern using SOLiD™ System (Applied Biosystems, CA, USA) Small RNA analysis of samples isolated from early embryonic stages of rabbit embryos and embryonic stem-like cells, to get more insight into their potential regulatory mechanism in pluripotent stem cells. In this study we compared the data of SOLiD sequencing with the results of real-time PCR analysis. As a result of comparison, it could be established that the read numbers of SOLiD sequencing cannot be directly compared with the relative expression levels of real-time PCR analysis, although in some cases we could estimate the order of magnitude.

Next, we described and analyzed the expression profile of pluripotency-associated miRNAs in rabbit embryos and embryonic stem-like (ES-like) cells for the first time. 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 dpc early blastocyst stage and they highly expressed in rabbit ES-like cells. In contrast, high expression levels of ocu-miR-290 cluster members were detected during preimplantation embryonic development, but a low level of expression was detected in rabbit ES-like cells. Differential expression of the ocu-miR-302 cluster and ocu-miR-512 miRNA was detected in rabbit trophoblast and embryoblast. The predominant expression of ocu-miR-512 in rabbit trophoblast may propose its trophoblast specificity.

We suggest that the expression of ocu-miR-302 cluster is more characteristic for the rabbit ES-like cell specific, while the ocu-miR-290 cluster may play a crucial role during early embryonic development. This study represents the first identification of pluripotency associated miRNAs in rabbit early embryos during embryonic development and ES-like cells, and compared the miRNA expression profile of SOLiD sequencing and real time PCR analysis.

Financial & competing interests disclosure

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