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188 MicroRNA characterization in equine induced pluripotent stem cells

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Abstract

Cell reprogramming has been well described in mouse and human cells. The expression of specific microRNAs has demonstrated to be essential for pluripotent maintenance and cell differentiation, but not much information is available in domestic species. A single microRNA can regulate the expression of hundreds of mRNA targets, a property given by a short sequence (called “seed”) in positions 2 to 8 from the 5' end that is complementary to the 3' untranslated region (UTR) tail of specific mRNAs. We aimed to generate horse induced pluripotent stem cells (iPSC), characterise them, and evaluate the expression of different microRNAs (miR-302a, b, c, d, miR-205, miR-145, miR-9, miR-96, miR-125b, and miR-296) in pluripotency and differentiation. Both cell states were evaluated (pluripotency and differentiation) in order to understand more deeply the complex network of transcriptional regulation in different contexts but with the same genomic background. Two equine iPSC lines (named L2 and L3) were characterised after the reprogramming of equine fibroblasts with the 4 human Yamanaka factors (*OCT-4*, *SOX-2*, *c-MYC*, *KLF4*). The pluripotency of both lines was assessed by phosphatase alkaline activity, expression of *OCT-4*, *NANOG*, and *REX1* by RT-PCR, and by immunofluorescence of OCT-4, SOX-2, and c-MYC. *In vitro* differentiation to embryo bodies (EB) showed the capacity of the iPSC to differentiate into ectodermal, endodermal, and mesodermal phenotypes. MicroRNA expression was analysed by quantitative RT-PCR and resulted in higher expression of the miR-302 family, miR-9, and miR-96 in L2 and L3 *v.* fibroblasts ($P \leq 0.05$), as previously shown in human pluripotent cells. Moreover, down-regulation of miR-145 and miR-205 was observed. After differentiation to EB, greater expression of miR-96 was observed in the EB compared with iPSC, and the expression of miR-205 was induced but only in the EB-L2. In addition, we performed *in silico* analysis of horse and human microRNAs. First, we compared the horse-miR-302/367 cluster with the human-miR-302/367 cluster and determined a 75% homology between them. Moreover, the seed region of the horse-miR-302 family resulted complementary to the 3' UTR of horse cell cycle regulator genes *CDK2, CYCLIN D1*, and *E2F1*, and to the 3' UTR of the *RHOC* gene, which is involved in the epithelial-mesenchymal transition. The miR-145 seed sequence was complementary to the 3' UTR region of the *OCT-4* and *KLF-4* horse genes. With respect to miR-9 and miR-96, the seed sequence of these genes were complementary to the *HES1* and *PAX-6* genes. In all cases, the same gene targets were previously demonstrated in humans. In conclusion, we report the generation and characterization of equine iPSC and determined for the first time the expression of microRNAs in equine pluripotent cells. Moreover, several results led us to think that the horse microRNAs evaluated herein are highly conserved in sequence and function with respect to the human species. It will now be necessary to generate directed differentiations to derivatives of the 3 germ layers in order to strengthen our results. This is the first report to evaluate the expression and possible targets of microRNAs in pluripotent cells from domestic animals.

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