Comparative generation of induced pluripotent cells (IPS) derived from human and animal models

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Abstract

Re-acquisition of cellular pluripotency is routinely accomplished in animal models after nuclear transfer (NT) of somatic cells, however, isolation and in vitro maintenance of pluripotent embryonic stem cells is challenging in animals due to the lack of knowledge on specific pluripotency mechanisms. The advent of iPS cells generation may represent a new possibility of using pluripotent cells derived from large animals for either enhancing animal production trough NT or for its use as suitable models in translational medicine. This study aimed to generate bovine and equine induced pluripotent stem cells (biPS and eiPS) using murine and human pluripotency induction protocols (miPS and hiPS) as controls. Bovine, equine, human and mouse iPS cells were produced trough lentiviral transduction of mesenchymal or fibroblasts cells with a polycistronic excisable vector containing human or mouse OCT4, SOX2, C-MYC and KLF4 pluripotency-related transcription factors (h or m OSMK). Cells were transduced overnight, cultured in vitro for 5 days and transferred to MEFs in iPS culture media (KO DMEM/F12 supplemented with 20% KSR, 1% glutamine, 1% neaa, 1% antibiotics and 10ng/ml bFGF with LIF and/or 2i when needed) for at least 21 days. Clonal iPS lineages were produced and a minimum of 3 different clonal lines for each species was characterized regarding morphology, protein expression of pluripotency factors by immunofluorescence, alkaline phosphatase detection, embryoid body formation, in vitro differentiation, in vivo teratoma formation and required supplementation. Bovine cells could be reprogrammed with mouse factors but not with human OSMK; however, equine cells could be better reprogrammed with human OSMK. Human and equine colonies were formed more rapidly when compared to bovine and murine iPS colonies and presented a flat shaped morphology, whereas biPS and miPS presented dome-shaped colony morphology. All lineages studied were positive for alkaline phosphatase, OCT4, SOX2 and NANOG protein detection and they formed embryioid bodies; however, teratomas were observed only when biPS or miPS were injected in nude balb/c mice. hiPS could not be enzymatic dissociated for passaging. LIF was used for mIPS culture, and 2i was not essential for iPS generation in any species. In summary,

iPS cells from bovine, equine, human and mouse models were generated in similar conditions, however, biPS resembled miPS whereas eiPS resembled hiPS cells. A better understanding of nuclei reprogramming mechanisms in different species should enhance the efficiency of reproductive biotechnologies and allow the use of pluripotent cells in cellular therapies.

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