Acta Scientiae Veterinariae, 2011. 39(Suppl1): s81.

ISSN 1679-9216 (Online)

Veterinary applications in regenerative medicine; development of induced pluripotential stem cells (iPSC) in dogs

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ABSTRACT

***Background*:** Pluripotent stem cells such as embryonic stem cells can give rise to derivatives of all three germ layers and thus have great potential for clinical applications related to regenerative medicine. By analyzing totipotent or pluripotent gene expression signatures from eggs, embryonic blastomeres and embryonic stem cells, Takahashi & Yamanaka (2006) developed a method to directly rewire the circuitry of adult somatic cells to pluripotency by transfection with a range of transcription factors. Ectopic expression of four transcription factors *Oct4, Klf4, Sox2* and *c-Myc* (OKSM) was capable of resetting the adult somatic cell to pluripotency. This approach has now been applied to a range of mammalian species including mice, humans, horses and pigs. Our interest is the application of this technology to the dog both for clinical application in veterinary medicine and as a way to understanding some of the limitations and strengths of this technology when applied to humans.
***Review*:** Here we report the derivation of iPS cells from adult canine fibroblast by retroviral OSKM transduction. The isolated canine iPS cells were expanded in three different iPS culture media (FGF2, LIF and FGF2 plus LIF) and only the cells cultured in FGF2 plus LIF showed strong AP activity and to express pluripotency markers, *POU5F1 (OCT4), SOX2, NANOG* and *LIN28* as well as ES cells-specific genes (*PODXL, DPPA5, FGF5, REX1* and *LAMP1*). To determine the ability of the cell to differentiate into derivatives of all tree germ layers; endoderm, mesoderm and ectoderm, we utilized both embryoid body formation and directed differentiation using chemical inducers. *In vitro* differentiation by formation of embryoid bodies (EBs) and directed differentiation showed cell derivatives of all three germ layers as confirmed by expression for AFP, *CXCR4* and SOX17 (endoderm), desmin (DES), vimentin (VIM), *MSX1* and *BMP2* (mesoderm) and glial fibrillary acidic proten (GFAP), TUJ1, *NCAM* and *bIII-tubulin* (*TUBB*, ectoderm). *In vivo* differentiation was tested by development of teratomas after injection into immunodeficient mice. Results indicated that the putative canine iPS cells were capable of creating solid tumors that expressed markers for all three germ layers.
***Conclusion*:** Embryonic stem cells have tremendous potential in the area of regenerative medicine but the difficulties in their isolation, and the inability to rapidly and efficiently develop lines that can be genetically matched to the recipient, reduces their clinical usefulness. In contrast, pluripotential stem cells (iPS) developed through direct reprogramming with transcription factors allow the derivation of patient-derived stem cells. This will allow the development of cell lines that can be used in the area of regenerative medicine in both human and veterinary medicine. However, there are still some issues of stability and culture requirements that must be elucidated before this technology can be fully applied in the clinics.
Keywords: induced pluripotent stem cells, stem cells, dogs, regenerative medicine.

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