A249 Cloning, Transgenesis and Stem Cells

Integration-free induction of cell pluripotency in the porcine model as a translational model

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The generation of pluripotent stem cells (induced pluripotent stem cells, iPSCs) through forced expression of known transcription factors *in vitro* (Takahashi and Yamanaka 2006) opened a new era for translational and regenerative medicine. The swine is a very adequate species to model several conditions and syndromes due to its well-known production, maintenance and physiological similarities to humans. Initial methods of pluripotency induction relied on integration of viral vectors into host genome that, although efficient, may lead to undesired mutations and residual transgene expression possibly resulting in tumorigenicity and differentiation. Alternative methods are desired aiming the safety for future clinical use of iPSCs. Herein, we aimed to generate porcine iPSCs by use of episomal vectors due to its non-viral and non-integrative properties, that can replicate extrachromosomally and it is gradually and spontaneously eliminated from the cells. Porcine fibroblasts were nucleotransfected (Nucleofactor 2b kit, cat.#VPI-1002, Lonza) with either Addgene´s human episomal vectors containing pluripotency-related transcription factors, pCXLE-hOCT3/4-shp53-F (hOCT4, shRNA against p53, cat.#27077), pCXLE-hSK (hSOX2, hKLF4, cat.#27078) and pCXL.E-hUL (hL-MYC, hLIN28. cat.#27080) or mouse episomal vectors pEB-C5 (mOCT4, mSOX2, mKLF4, mc-MYC, mLIN28, cat.#282229) and pEB-Tg (SV 40 Large T antigen, cat.#28213). After 2 days of the nucleofection, the media was changed every other day and supplemented with 0.05mM sodium butyrate (cat.#303410, Sigma) until D12. On D6, 2x 10^4 cells were transferred into a new 10cm^2 well previously covered with Geltrex (cat. #A14133-02, Gibco) and cultured from that moment on with E8 (cat.#A15169-01, Gibco). Cells were cultured for more than 40 days in 38.5ºC and 5% CO_2. So far, morphology and presence of Alkaline Phosphatase activity with the Leukocyte Alkaline Phosphatase kit (cat.# 86R, Sigma) were analyzed. Flat, compact and clear edged colonies formed by round cells with high nucleus-cytoplasm ratio were observed on the human vector group as early as D20, at D27 they were tested and positive for AP activity. Those results suggest a successful reprogramming of the porcine cells into iPSCs by episomal vectors with the human based gene sequences. No colonies were formed on the mouse vector group until D40. It was observed a change in cellular morphology where the cells gained characteristics similar of the other species´ iPS cells. Future steps include a more complete characterization regarding gene expression and protein detection. Also, optimization of procedures in order to achieve a higher efficiency of colony formation is still need to be performed. Such results may greatly contribute to the understanding of the role of these pluripotency factors and the generation of a safe and reproducible model of study for further cellular therapies used for regenerative and translational medicine.

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