A207 Cloning, transgenesis and stem cells

Induced pluripotent stem cells (iPSCs) derived from urine progenitor cells in the swine: a novel non-invasive method for regenerative medicine

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The swine model is of special interest as a biomedical model due to its immunological and physiological similarity with the human model, and non-invasive collection of cells for iPSCs generation would facilitate its use. Herein we aimed to derive urine progenitor cells (UPCs) *in vitro* cultures from urine samples, still unpublished for species other than human, and to reprogram them *in vitro* into pluripotency. For that, urine samples (approximately 250ml) were collected from three females. Isolation and culture were performed following human UPCs protocol (Steichen et al., 2017). Briefly, the urine was centrifuged at 300 x g, the pellet washed in DPBS (Sigma), resuspended and cultured in 45% DMEM high glucose (Life Technologies), 5% FBS, 50% REBM media (renal epithelial basal media, Lonza) supplemented with 1% glutamine, 1% MEM neaa, 1% penicillin/streptomycin (all Life Technologies) and REGM supplements : hEGF, Insulin, Hydrocortisone, GA-1000, FBS, Transferrin, Triiodothyronine, Epinephrine (Lonza) and 10ng/mL bFGF (Peprotech). After approximately one week, epithelial-like cells were observed in colonies. The cells from one female were submitted to transduction of murine OSKM (OCT4, SOX2, KLF4 and C-MYC - STEMCCA lentiviral vector, Millipore). After 4-5 days, cells were plated onto MEFs and cultured in KnockOut DMEM/F12, 20% KnockOut Serum Replacement, MEM neaa, L-Glutamine, 2-Mercaptoethanol and penicillin/streptomycin (all Life Technologies) supplemented with 10ng/ml bFGF (Peprotech). At approximately 12 days after transduction, colonies presenting typical pluripotent morphology were observed and evaluated regarding efficiency of colony formation and alkaline phosphatase detection. Three clonal lineages (C1, C4 and C6) were further maintained *in vitro* and characterized regarding pluripotency markers for more than 20 passages. The overall reprogramming efficiency observed was 8,455% (percentage of colonies observed in relation to the number of transduced cells plated). All three colonies were positive for alkaline phosphatase in passages 22, 21 and 21, respectively. Immunocytochemistry analysis revealed that C6 was positive for the pluripotency markers OCT4 (1: 100, cat # SC8628), SOX2 (1: 500, cat # ab97959; Abcam), SSEA1 (1: 50, cat # SC21702, Santa Cruz), TRA1- 81, and NANOG (1: 100 # catab77095, Abcam), meanwhile the colonies C1 and C4 were positive only for OCT4 and SOX2. In conclusion, it was possible herein to reprogram cells derived from urine samples into iPSCs that were maintained in culture *in vitro* for at least 20 passages. Further analysis is still needed to prove the complete reprogramming of these cells; however, these results already open a new possibility to generate models of *in vitro* diseases from a non-invasive source in an unprecedented way.

Financial Support: FAPESP (2019/02811-2, 2015/26818-5), CNPq 433133/2018-0 and CAPES.