A205 Cloning, transgenesis and stem cells

Successful generation of induced pluripotent stem cells (eiPS) derived from skin fibroblasts of an aged equine

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Cellular aging is a limitation in cellular reprogramming since it is associated with cell senescence. As the cell ages, an upregulation of pathways such as p53, $p16^{INK4A}$, and $p21^{CIPI}$ occurs leading to cell cycle arrest along with alterations in cell morphology and metabolism. Considering the difficulty on reprogramming of aged cells, the objective of the work was to achieve reprogramming into pluripotency of a more than 20 years old horse. Therefore, a skin fragment was collected from the dorsal lateral metacarpophalangeal region, taken to the lab and fibroblasts were recovered after a 3 hours digestion period with Collagenase IV (#C2674 Sigma Aldrich). The fibroblasts were then seeded in a 6 well plate ($2x10^4$ cells per well) and the lentiviral vector STEMCCA containing the human sequences of OCT4, SOX2, KLF4, and c-MYC was used for transduction. Six days after transduction cells were seeded in mouse embryonic fibroblast (MEF) layer ($4,75x10^4$ cells per well). The reprogramming efficiency was calculated by dividing the number of formed colonies by the number of seeded cells. The eiPS colonies were evaluated regarding their morphology and detection of alkaline phosphatase, immunocytochemistry for Oct4 (#sc8628, Santa Cruz), Sox2 (#ab97958, Abcam), Nanog (#ab21624, Abcam), SSEA-1 (MAB 4301, Millipore), TRA-1-60 (Mab 4360, Millipore) and TRA-1-81 (Mab 4381, Millipore). The transcript levels were determined by RT-qPCR, for pluripotency genes OCT4, REX-1, NANOG, and SOX2. Therefore, the cycle threshold (Ct) values of the target genes were normalized by the average of Ct values of the housekeeping genes (HPRT1 and PPIA) and the fold changes were then calculated using the $2^{(-\Delta CT)}$ equation. After 16 days of the transduction, colonies were visualized, being primarily identified by their typical morphology: tightly packed cells with a high nuclear/cytoplasm ratio. The efficiency of the reprogramming process was 0,059% (28 colonies from $4,75x10^4$ seeded cells). Colonies were positive for alkaline phosphatase at passages 4 and 12. Immunocytochemistry revealed that cells were found to be positive for OCT4, NANOG, SSEA-1, and TRA-1-81. Cells showed endogenous expression of the pluripotency genes OCT4 (0,3670 ± 0,1032, n=3), REX-1 (0,0391 ± 0,0005, n=3), NANOG (0,1421 ± 0,2903, n=3) and SOX2 (0,0034 ± 0,0020, n=3), being the Ct values all minor than 31,8, using specific equine primers. Herein we conclude that although age is considered as a great barrier to the reprogramming of somatic cells, it was possible to achieve successful reprogramming in an animal in advanced age in our conditions. Financial Support: FAPESP (2018/04009-6; 2015/26818-5) and CAPES.