Failure in the induction of pluripotency in rabbit adipose stem cells: a high proliferation problem?

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Abstract

By the pluripotency induction, adult somatic cells acquire very similar behavior to embryonic stem cells, eliminating ethical issues related to the use of such research. However, the mechanisms involved in induced pluripotent stem (iPS) cells are not yet fully elucidated. The aim of this study was to induce pluripotency in rabbit adipose stem (ADS) cells. Rabbit ADS cells were collected and characterized by their morphology, dynamic growth, differentiation potential, viability under cryopreservation and phenotypic profile. The ADS cells were transduced with a lentiviral vector containing four human or murine pluripotency factors (OCT4, KLF4, SOX2 and c-MYC). Cells were maintained in IMDM medium, supplemented with 10% bovine fetal serum for six days, when they were trypsinized and 5x103 cells were platted on mitomycin-treated murine embryonic fibroblasts (MEFs) feeder layers with iPS medium. Several protocols were tested to acquire the pluripotent state, including the addition of MEK and GSK inhibitors or human LIF, but just partial reprogramming was detected. In order to verify the integration efficiency of pluripotent factors into the rabbit ADS cells, the cells were transduced with only one lentiviral vector containing an individual factor (OCT4 or SOX2), or with a combination of the two factors; those were conjugated with fluorescent reporters, vexGFP (OCT4) and mCitrine (SOX2). The integration efficiency was analyzed and sorted by flow cytometer, and analyzed by confocal microscopy. Rabbit ADS cells were viable after cryopreservation and demonstrated a mesenchymal stem cell phenotype as fibroblastic morphology, differentiation potential in adipocytes, osteocytes and chondrocytes and a positive expression of CD73 and CD90 and negative expression of CD34 and CD45. An exceptional high proliferation potential was observed. The pluripotency induction resulted in only partial reprogramming morphology in all protocols tested. By the integration efficiency assay, OCT4 factor was detected into the cells, but not the SOX2. Rabbit ADS are easy and fast to collect, isolate and proliferate in vitro and share the same characteristics of other mesenchymal stem cells, with an exceptional high proliferation rate. The pluripotent state could not be reached using several strategies, and just partial reprogramming cells were detected. By the integration assay, we verified that the OCT4 factor was properly transduced into the ADS cell, but the SOX2 factor could not be detected. The failure in the pluripotency induction and in the integration of SOX2 into the cells remains unclear, and further studies are necessary to better explain the mechanisms involved in the reprogramming. A possible link between proliferation and integration of SOX2 should be further

investigated. We expect that managing the proliferation rate of rabbit ADS cells, the iPS cells can be generated. Sponsored by FAPESP.

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