Talk

Generation of induced pluripotent stem cell (iPSC) lines from urine samples.

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Keywords: Induced Pluripotent Stem Cells; Viral Transfection; Cell Therapy; Urine Cells.

ABSTRACT

Motivation:

Induced pluripotent stem cell (iPSC) lines arise from the need of seeking alternative sources of stem cells for cellular and genetic therapies to avoid ethical aspects related to the use of embryonic stem cells. Urine represents a rich source of epitelial cell that can undergo a dedifferentiation process to generate iPSCs (Zhou et al, 2011). Interestingly, the method to obtain iPSC from urine derived cells (UDC) requiers a non-invasive technique that avoids biopsies and other surgical techniques.

In others reports, iPSC derived from UDC are been used to produce cardiomyocites (Feng et al , 2018), retinal cells (Clegga et Foltza, 2019) and beta cells (Maehr, 2011) , for the treatment of such as myocardial infarctions, degenerative retinal diseases and type 1 diabetes, respectively, among other diseases.

Methods:

For isolation and cultivation of UDC, we used the protocol described by Zhou et al. Specific culture media were used for the selection of UDC colonies suitable for transfection and for iPSC culture maintenance. The most remarkable tecnique used was a commercial ,non-integrative, viral system, named Sendai, for the transfection of UDC with transcription factors (hKlf4, hOct3/4, hSox2, cMyc).

Once iPSC line was stabilized, it was characterized by techniques such as alkaline phosphatase assay, PCR, immunofluorescence, flow cytometry for nuclear (OCT-4, SOX-2, NANOG, TELO) and surface (TRA-1-60, TRA-1-81 and SSEA4) pluripotency markers. Differentiation potential of iPSCs into the three germ layers was performed using STEMdiff Trilineage Differentiation Kit protocol (STEMCELL Technologies)

Results:

Cultured urine epithelial cells were successfully transfected and formed colonies iPSC-like. Characterization of these colonies confirmed expression of pluripotency markers and alkaline phosphatase. The absence of exogenous pluripotency vector expression was verified by PCR.

Conclusions:

So far, we can accept that it has been possible to isolate, cultivate and transform UDCs into iPSCs. Now, we need to further characterize our iPSC line, by studying its karyotype and testing its ability to form teratomas in vivo. To apply this procedure in cell therapy, we should repeat all culture processes under Good Manufacturing Practices (GMP). The generation of iPSC lines represents a great advance for cell therapy and tissues bioengineering.

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