Somatic cell reprogramming using NF- κ B inhibitory strategies

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Method Article

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Abstract

This protocol describes somatic cell reprogramming methodologies using NF- κ B inhibitors step-by-step. Thus, we have used NF- κ B pharmacological and genetic inhibitory strategies for the reprogramming of human fibroblasts from normal and accelerated ageing. These strategies can be used for implementing reprogramming methodologies in ageing conditions.

Reagents

- HEK-293T (ATCC).
- Human primary fibroblasts (Coriell cell repository).
- IKK-VII inhibitor (Selleckchem).
- Sodium salicylate (Sigma).
- pMX-OCT4, SOX2, KLF4 and c-MYC plasmids (Addgene).
- pBAGE-I κ B α -SR and IKK2-KI plasmids (Addgene).
- pCL-Ampho retroviral packaging plasmid (Addgene).
- HEK-293T medium: DMEM medium supplemented with 10% of fetal bovine serum (FBS).
- Human fibroblasts medium: DMEM medium supplemented with 15% FBS, nonessential amino acids, 2-mercaptoethanol and sodium pyruvate.
- Human iPSC medium: DMEM-F12 medium supplemented with 20% knockout serum replacement (KSR), nonessential amino acids, 2-mercaptoethanol, penicillin/streptomycin, GlutaMax, and bFGF (4 ng/mL).

Procedure

****Cell reprogramming with NF- κ B pharmacological inhibitors****

- 1) Retroviral vectors expressing human OCT4, SOX2, KLF4 and c-MYC were transfected in HEK-293T cells (5×10^6 cells per 10 cm plate each) along with packaging plasmids to produce retrovirus.
- 2) Human primary fibroblasts were seeded at a density of 1×10^5 cells per well in 6-well plates.
- 3) Twenty four h after plating, cells were infected with retroviral supernatants and the same procedure was repeated every 12 h for 2 days (a total of four additions) (Figure 1).
- 4) The day after last infection (day 3), medium was replaced with fibroblasts medium, and kept for three more days. NF- κ B inhibitors were added at this point and maintained along the reprogramming process (IKK-VII, 0.5 μ M and sodium salicylate, 0.2 mM) (Note 1).
- 5) At day 7, cells were trypsinized and reseeded on feeder plates (Note 2).
- 6) On day 8, medium was changed to human iPSC medium.
- 7) Cultures were maintained with daily medium changes for 4 weeks. At that point, colonies were isolated and expanded on a feeder layer or Tra-1-60 stained for evaluating reprogramming efficiency.

****Cell reprogramming with NF- κ B genetic inhibitors****

- 1) Retroviral vectors expressing human OCT4, SOX2, KLF4, c-MYC, I κ B α -SR or IKK2-KI were transfected in HEK-293T cells (5×10^6 cells per 10 cm plate each) along with packaging plasmids to produce retrovirus.
- 2) Human primary fibroblasts were seeded at a density of 1×10^5 cells per well in 6-well plates.
- 3) Twenty four h after plating, cells were infected with retroviral supernatants and the same procedure was repeated every 12 h for 2 days (a total of four additions).
- 4) Cells were trypsinized at day 7 and reseeded on feeder plates. On day 8, medium was changed to human iPSC medium and cultures were maintained with daily medium changes for 4 weeks.

Troubleshooting

Notes 1) We have observed that NF-κB-inhibition during the first two weeks is sufficient to achieve the complete effect on reprogramming efficiency. Thus, NF-κB inhibitors could be removed from iPSC medium with no effect on reprogramming efficiency after that time point. 2) Human iPSCs were cultured on mitomycin-inactivated mouse embryonic fibroblasts \(\MEFs).

Figures

Figure 1

Scheme of somatic cell reprogramming process with NF-κB inhibitors