¹Xiaoli Shen, ²Feng Chen, ¹Lili Han, ¹Haiyan Tang, ¹Saimei Lin, ¹Xiaoqing Liu, ¹Lifang Lin, ¹Leng Pan, ¹Xiaodong Pu, ¹Xiaoli Shen. ¹Fujian Provincial Key Laboratory of Cardiovascular Disease, Affiliated Fujian Provincial Hospital, Fujian Medical University; ²Emergency Center of Fujian Province, Affiliated Fujian Provincial Hospital, Fujian Medical University

α.

Objectives To construct and pack Lentivirus vector which includes the four genes as Oct4, Sox2, c-Myc, Klf4, and infect primary cultured human foreskin fibroblasts (HFF). Mainly to illustrate construction and packing of Lentivirus vectors and investigate the optimum condition of target gene over-expression lentivirus particle infecting human foreskin fibroblasts cells.

Methods

1. To acquire objective genes from the plasmid which includes Oct4, Sox2, c-Myc, and Klf4. Enzyme linearised the Lentivirus vector and then directional connected them with objective genes, the production transformed into competent bacteria. Comparison analyzed and sequenced the positive clones, if the result was correct, which was successfully constructed objective plasmid, and then ultra-purified extract endotoxin from the objective plasmid. Use Western Blot to test objective gene expression. Use three plasmid system to transfect 293T cells for packing Lentivirus and test the titer of Lentivirus;
2. Use collagenase I digestion and tissue culture technique to separate and culture HFF, and then use trypsin digestion to separate and culture mouse embryonic fibroblasts (MEF), separately observed the two kinds cells grow, and appraised them with immunocytochemistry SABC method, Mitomycin C processed MEF to set up the feeder which was needed to culture cells;
3. Packed empty lentivirus vector with GFP and infected HFF; fished out the best MOI data; Forth: Randomly divided the third generation HFF in good condition into three group: objective infected gene, empty lentivirus vector, and contrastive group. Polyinfected HFF with lentivirus vector which includes the four genes and consecutive infected for twice. After 4 days, given it condition of stem cells culture, observe the morphological changes to get the best laboratory condition.

Results

1. Successfully constructed and packed Lentivirus vector which includes Oct4, Sox2, c-Myc, and Klf4, the sequence of gene was consistent with objective sequence, titre was 1×10^9 TU/ml;
2. 0.02% collagenase I digestion and attachment explants culture technique can found stable HFF lines in vitro, cells began to migrate after 4 days of cells culture, showed shuttle shape and had arms, after 6 days, major free cells began to form and binding, after 10 days, cells overspread the bottom, tryptic finished digestion and passage. Maximum production and high-activity MEF could be got through 0.0625% trypsin graded digestion method, cells shuttle and polygon shape, and completely attached wall within 24 h. immunocytochemical test prove positive;
3. Treated MEF confluent the 25 cm² culture flask with 1 ml 15 µg/ml mitomycin C for 1.5 H, could set up the feeder which was needed to culture cells; Forth: fished out the best MOI was 30; compared with contrastive group, there were no obvious multipotential stem cells in objective infected gene, but the cells form were changed from shuttle into ellipSe or semi-ellipSe; there were no obvious cells form change of empty lentivirus vector HFF, the cells kept shuttle shape.

Conclusions Successfully primary cultured HFF and MEF; Successfully constructed and packed Lentivirus vectors which includes Oct4, Sox2, c-Myc, and Klf4 with high titre, HFF can be

GW23-e1762

THE STUDY OF CONSTRUCTION AND PACKING OF LENTIVIRAL VECTOR-OCT4, SOX2, C-MYC, KLF4 AND INFECTING HUMAN SKIN FIBROBLASTS

doi:10.1136/heartjnl-2012-302920b.23

infected by target gene over-expression lentivirus particle which start to change in morphology.