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THE BASIC STUDY OF REPROGRAMMING HUMAN POSTNATAL FORESKIN FIBROBLASTS INTO INDUCED PLURIPOTENT STEM CELLS IN VITRO

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Objectives To establish the process of culturing primary human postnatal foreskin fibroblasts (hPFF) line and the hPFF were identified by Mitomycin C as feeder cells. Lentivirus vector which included the four genes as Oct4, Sox2, c-Myc, Klf4 infected hPFF, to investigate the optimum condition of target gene over-expression lentivirus particle infecting hPFF which were reprogrammed into induced pluripotent stem cell in vitro.

Methods First: Using collagenase I digestion and tissue culture technique to separate and culture hPFF, Observing cells morphology under the inverted microscope, measuring cells viability by trypan blue assay, identify cells by immunocytochemical staining of Strept actividin-biotin complex (SABC). The Optional planting density of cell was determined according to the cellular growth curve detected by MTT colorimetric assay. The hPFF were identified by Mitomycin C as feeder cells. The optional concentration of mitomycin C and the time treating with mitomycin on MEFs were determined by MTT colorimetric assay;

Second: Using lentivirus particle expressing GFP infect hPFF observe fluorescence efficiency under the inverted microscope to get the best infecting condition and multiplicity of infection (MOI); Third: Randomly dividing hPFF in good condition into three groups: control group, lentivirus particle expressing GFP group, target gene infected group, lentivirus vectors consecutively infect hPFF. After four days, giving hPFF condition of stem cells culture, observing the morphological changes and Raman spectroscopy analyse three group cells spectral configuration to get the best laboratory condition.

Results First: 0.025% collagenase I digestion and tissue culture technique can found stable hPFF lines in vitro. cells began to migrate from tissue after five days, showed shuttle shape and had arms, after seven days, major free cells began to form and binding, after ten days, cells overspread the bottom, 0.25% tryptic finished digestion and passaged. The identified result was right by immunocytochemical staining of Strept actividin-biotin complex. hPFF proliferation could be efficiently repressed after being treated with mitomycin C10 μg/ml for 3.5 h or 15 μg/ml for 2.5 h and the quantity of hPFF could maintain at least for 1 week; Second: fishing out the best infecting multiplicity of infection was 40 in our laboratory condition. Third: in the same infection condition, compared with control group, morphology in lentivirus particle expressing GFP group were no obvious observed change under the inverted microscope, but cells in target gene infected group were different, changing from shuttle to spherical in morphology. spectral configuration was no obvious discrepancy between control group and lentivirus particle expressing GFP group by Raman spectroscopy, however cell spectral configuration of target gene infected group was different.

Conclusions The study successfully primary cultured hPFF, established hPFF lines in vitro and attained highly effective feeder layer cells. hPFF can be infected by target gene over-expression lentivirus particle and four target gene over- expression lentivirus particle were mainly factors that leaded cells change in morphology.

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