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THE STUDY OF TARGET GENE OVER-EXPRESSION LENTIVIRUS PARTICLE INFECTING HUMAN CARDIAC FIBROBLASTS IN VITRO

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

Objectives To investigate the optimum condition of target gene over-expression lentivirus particle infecting human cardiac fibroblasts by making lentivirus including the gene of KLF4, OCT4, SOX2, C-MYC.

Methods Take the right atrial appendage form patients of coronary heart disease and make human cardiac fibroblasts by the direct adherent culture, take Kunming pregnant mouses in 13.5–14.5 days and make embryonic fibroblasts by the trypsin digestion method. Observe cell under the inverted microscope, measure cell viability by trypan blue assay, identify cell by immunocytochemical staining. Construct and package gene over-expression lentivirus particle of KLF4, OCT4, SOX2, C-MYCand green fluorescent protein. Use lentivirus particle expressing GFP to infect human cardiac fibroblasts, observe fluorescence efficiency under the inverted microscope to get the best infecting condition and multiplicity of infection. Use target gene over-expression lentivirus particle of KLF4, OCT4, SOX2, C-MYC to infect human cardiac fibroblasts, observe the morphological changes to get the best laboratory condition.

Results By the direct adherent culture we get enough human cardiac fibroblasts, which contain little mixed-cell and cannot beat spontaneously. By 0.0625% trypsin digestion several times we get stable, sufficient and high-dynamic embryonic fibrolasts, we can get more pure MEFs by spreading to the third generation, then we can make feeder cell with MMC. After contracting and packaging lentivirus successfully, we use them to infect human cardiac fibroblasts, and finally we discover that cells start to change in morphology.

Conclusions The study demonstrate that human cardiac fibroblasts can be infected by target gene over-expression lentivirus particle of KLF4, OCT4, SOX2, C-MYC, which start to change in morphology.