The fusion of somatic cells and human embryonic stem cells by electrofusion method

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Generating pluripotent stem cells directly from somatic cells obtained from patients is one of the major methods to avoid tissues rejection in regenerative medicine. Beside somatic cell nuclear transfer, recent studies have shown that human embryonic stem cells (hESCs) have the ability to reprogram somatic cells after fusion by PEG. But the low efficiency of PEG induced fusion in the study might be one of the obstacles for the technology to be used practically. Electrofusion is a biomedically safe method that has been broadly used in nuclear transfer. This study is to investigate whether electrofusion would improve the fusion efficiency of hESCs with human embryonic fibroblasts (HEFs). A normal hESC line with 46, XX karyotype and a HEF line with 46, XY karyotype were used in this study. Before electrofusion, both of the cells were dissociated into single cells and were separately labeled by DIO and DID. 1×10^6 hESCs and 1×10^6 HEFs were fused using the Electro Cell Manipulator ECM 2001 in the optimized electrical parameters (AC:20v, 30s, DC:700v, 10 μ s, $\times 2$ times). The fusion efficiency was determined by fluorescence activated cell sorting (FACS) after fusion. Fused cell were seeded onto FBS-coated six-well plates in N2B27 serum free hESCs culture medium as described by others supplemented with 40 ng/mL bFGF. When hESC-like colonies appeared, the DIO and DID double positive colonies were mechanically picked up, passaged and analyzed by FISH for chromosome X and Y. The colonies contained the Y chromosome were considered as hybrid colonies. After fusion, FACS analysis showed that DID and DIO double positive cells was $21.5 \pm 2.0\%$ ($n = 2$) which is superior to the results described before by Cowan *et al.* using PEG for fusion ($0.95 \pm 0.03\%$, double positive cells with PEG), and this could be increased to 60.0% ($n = 1$) after FACS sorting. Approximately 2×10^5 cells after fusion were plated in each well of six-well plate. Six days after plating, small colonies started to appear in culture. In the ninth day, there were 70-80 colonies in each well. 4 of 30 colonies analyzed by FISH contained Y chromosome which indicated that these colonies were hybrid of HES and HEF. The reprogramming characters of this reprogrammed ES-like colonies are in further analysis.

Keywords: hybrid cell, human embryonic stem cells, reprogramming, electrofusion

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