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Functional Cardiomyocytes Formation Derived from Parthenogenetic Mouse Embryonic Stem Cells

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This study was to establish the use of parthenogenetic mouse ES (P-mES02) cells as a reproducible differentiation system for mouse cardiomyocytes. To induce differentiation, P -mES02 cells were dispersed by dissociation and the formation of ES cell aggregates in differentiation medium. After 7 days in differentiation culture, the embryoid bodies (EBs) were plated onto gelatin-coated dish. Cultures were observed daily using an inverted light microscope to determine the day of contraction onset and total duration of continuous contractile activity for each contracting focus. The effects of dimethyl sulfoxide (DMSO), a known stimulant, on differentiation into cardiomyocytic lineage were assessed by adding at a concentration of 0.75%. Contracting areas were mechanically dissected and then enzymatically dispersed using trypsin-EDTA. Cells were plated on glass coverslips, incubated for 48 h, fixed using 4% paraformaldehyde and treated with primary antibodies for 1 h at 37 ℃. Staining of sarcomeric α-actinin was performed using anti-sarcomeric α-actinin mAb's at a dilution of 1:800. After three washes with PBS, cells were incubated with FITC-conjugated anti-mouse IgG antibodies for α-actinin. Preparations were examined using fluorescence microscopy. Rhythmically contracting areas appeared at 15～17 days after plating. Spontaneously contracting areas appeared in 69.38% (DMSO) of the EBs. Cells from the spontaneously contracting areas within EBs were stained positively with anti-α-actinin. This study showed that the P-mES02 cell-derived cardiomyocytes displayed structural properties of cardiomyocytes and that the DMSO enhanced development of cardiomyocytes.

Key words) ***Parthenogenetic mouse embryonic stem cell, Cardiomyocyte, DMSO, Spontaneous contraction***