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Formation of Functional Cardiomyocytes Derived from Mouse Embryonic Stem Cells

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Pluripotent embryonic stem cells can differentiate into beating cardiomyocytes with proper culture conditions and stimulants via embryo-like aggregates. We describe here the use of mouse embryonic stem (mES03) cells as a reproducible differentiation system for cardiomyocyte. mES03 cells growing in colonies were dissociated and allowed to re-aggregated in suspension [embryoid body (EB) formation] . To induce cardiomyocytic differentiation, cells were exposed to 0.75% dimethyl sulfoxide (DMSO) during EB formation for 4 days and then another 4 days without DMSO (4+/4-). Thus treated EB was plated onto gelatin-coated dishes for differentiation. Spontaneously contracting colonies which appeared in approximately 4~5 days upon differentiation were mechanically dissected, enzymatically dispersed, plated onto coverslips, and then incubated for another 48~72 hrs. By RT-PCR, robust expression of cardiac myosin heavy chain α, cardiac muscle heavy polypeptide 7 β(β-MHC), cardiac transcription factor GATA4, and skeletal muscle-specific α_1-subunit of the L-type calcium channel ($\alpha_1 CaCh_{sm}$) were detected as early as 8 days after EB formation, but message of cardiac muscle-specific α_1-subunit of the L-type calcium channel ($\alpha_1 CaCh$) were reveled at a low level. In contrast, expression of myosin light chain (MLC-2V) and atrial natriuretic factor (ANF) were not detected during EB formation for 8 days. However, a strong expression of the atrial-specific ANF gene was expressed from day 8 onward, which were remained constant in EB. (cardiac specialization and terminal differentiation stage). Electrophysiological examination of spontaneously contracting cells showed ventricle-like action potential 17 days after the EB formation. This study indicates that mES03 cell-derived cardiomyocytes via 4+/4- protocol displayed biochemical and electrophysiological properties of subpopulation of cardiomyocytes.

Key words) ***Mouse embryonic stem cell, Cardiomyocyte, Gene expression, Electrophysiology***