

## Functional Cardiomyocytes Formation Derived from Mouse Embryonic Stem Cells

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Pluripotent embryonic stem (ES) cells differentiate spontaneously into beating cardiomyocytes via embryo-like aggregates. We describe the use of mouse embryonic stem (mES03) cells as a reproducible differentiation system for cardiomyocyte. To induce cardiomyocytic differentiation, mES03 cells were dissociated and allowed to aggregate (EB formation) at the presence of 0.75% dimethyl sulfoxide (DMSO) for 4 days and then another 4 days without DMSO (4+/4-). Thus treated EBs were plated onto gelatin-coated dish for differentiation. Spontaneously contracting colonies which appeared in approximately 4-5 days upon differentiation. Expression of cardiac-specific genes were determined by RT-PCR. Robust expression of myosin light chain (MLC-2V), cardiac myosin heavy chain  $\alpha$ , cardiac muscle heavy polypeptide 7  $\beta$  ( $\beta$ -MHC), cardiac transcription factor GATA4 and skeletal muscle-specific  $\alpha_1$ -subunit of the L-type calcium channel ( $\alpha_1$ CaCh<sub>sm</sub>) were detected as early as 8 days after EB formation, but message of cardiac muscle-specific  $\alpha_1$ -subunit of the L-type calcium channel ( $\alpha_1$ CaCh) were revealed at a low level. Strikingly, the expression of atrial natriuretic factor (ANF) was not detected. When spontaneous contracting cell masses were examined their electrophysiological features by patch-clamp technique, it showed ventricle-like action potential 17 days after the EB formation. This study indicates that mES03 cell-derived cardiomyocytes displayed biochemical and electrophysiological properties of cardiomyocytes and DMSO enhanced development of cardiomyocytes in 4+/4- method.

Key words) *mES cell, Cardiomyocyte, DMSO, Cardiac-specific gene, Electrophysiology*