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Embryonic Stem Cell Lines Derived from 8-cell Stage Mouse Single Blastomeres

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The current method for deriving embryonic stem (ES) cell lines from blastocyst requires embryo destruction. The aim of this study is to investigate the potential of 8-cell stage blastomere for establishment of ES cell lines and to improve the efficiency of establishment in mouse. Zona pellucida of Mouse (BDF1) embryos at 8-cell stage were removed with acid Tyrode and separated in Ca^{2+}-free medium by pipetting. Each blastomere was aggregated with a small clump of GFP-positive (G4-2) mouse embryonic stem (mES) cells in a small dimple created by pressing a needle into the bottom of a plastic tissue culture plate. After incubation for 48 h in mES cell growth medium supplemented with mouse leukaemia inhibitory factor (LIF) and MEK1 inhibitor, a growing bud of GFP-negative cells was observed on the sides of the majority of GFP-mES clusters. The aggregates were separated from GFP-positive mES cells mechanically with a microcapillary under a fluorescence microscope or with trypsin, which then were plated onto mitomycin C-treated mouse embryonic fibroblasts (MEFs) and cultured in mES cell growth medium until GFP-negative clumps became large enough for dispersion. Blastomere outgrowths that morphologically resembled ES cells were further cultured in the mES cell medium and produced mES cells that were maintained under these conditions and passaged.

By mechanical method mES like-cell line was established in 2.33 % of cultured blastomere and by trypsin-treatment was 2.59%. Both putative mES cell line derived from single blastomere expressed mES cells specific markers such as Naong and alkaline phosphatase. This study suggests that mES cells could be established from single blas-

tomere. This animal model could be applied to establishment of auto-logous human ES cells from biopsied blastomeres of preimplantation embryos in human IVF-ET program.

Key word) ***Mouse ES cell, Isolated blastomere, Animal model***