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Establishment of Mouse Pluripotent Stem Cells Generated from Primordial Germ Cells

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Pluripotent stem cells have been generated from two embryonic sources. ES cells are generated from ICM of blastocyst stage embryos, and embryonic germ (EG) cells are generated from primordial germ cells (PGCs). Both ES and EG cells are pluripotent and present important characteristics such as high levels of alkaline phosphatase (AP) activity, multi-cellular colony formation, normal and stable karyotypes, continuously passaging ability, and the capability of differentiation into all three embryonic germ layers. This study was performed to establish the culture system of mouse EG cells from mouse PGCs. PGCs collected from genital ridge of around embryonic day 11.5 and 12.5 mouse embryos (C57BL/6 × DBA/2) were cultured and subsequently passaged on a mitotically inactivated STO feeder cell layer. Cells were grown in DMEM supplemented with 15% FBS, 0.1mM nonessential amino acids, 0.1mM 2-mercaptoethanol, 2mM glutamine, 100 unit/ml of penicillin, 100ug/ml of streptomycin, 1,000 unit/ml of LIF, 6ng/ml of SCF, and 10ng/ml of bFGF. Cultures were grown in 5% CO_2, 95% humidity, 37℃ incubator and were routinely passaged every 3~4 days after primary culture. Over a period of 7~10 days in primary culture, PGCs proliferate to give rise to small multi-cellular clumps and these were form densely packed colonies, AP-positive cells resembling undifferentiated ES cells in morphology. Also these cells were confirmed mRNA expression of transcription factor Oct-4 and Nanog by RT-PCR. The cultured cells have been maintained on feeder layer for at least 10 passages and found to be shown normal karyotype. These results suggest that these cell lines derived from mouse primordial germ cells are presumably EG cell lines and these cell lines are thought to be very useful for transgenic animal production and ES cell study.

Key words: ***Primordial germ cell, Embryonic germ cell, Alkaline phosphatase, Oct-4, Nanog***