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Isolation and Culture of Embryonic Germ-like Cells from Porcine Mesonephros

Hye-Young Son[1,2], Min-Goo Kim[1,2], Jung-Im Yun[1,2], Jung-Eun Kim[1,2], Hye-Seon Kim[1,2], Eugene Lee[1,2] and Chang-Kye Lee[1,2]

[1]School of Agricultural Biotechnology, College of Agriculture and Life Sciences, [2]Xenotransplantation Research Center, and [3]College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea

Many attempts to establish embryonic stem (ES) cells from prei-mplantation stage embryos in pigs have come to a failure. An alternate source of pluripotent stem cells are embryonic germ (EG) cells derived from primordial germ cells (PGCs) of the genital ridge, which is deve-loped from mesonephros. Mesonephros is a vestige, transient renal organ that functions only during embryonic development. It is believed to be a source of multiple stem cells including somatic cells in the gonad, vascular endothelial cells and hematopoietic stem cells. Therefore, we tried to obtain putative stem cells from cells isolated from porcine mesonephros with the culture condition to establish EG cells.

Porcine fetuses from crossbred gilts were collected by hyste-rectomy between Days 25 and 30 of pregnancy (estrus = Day 0). Me-sonephros and genital ridges were separated each other and cells from mesonephros and PGCs from genital ridges were isolated by a physical method. Isolated cells were cultured in PES medium [50:50 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F10 medium supplemented with 15% fetal bovine serum (FBS), L-glutamine (1.7 mM), β-mercaptoethanol (0.1 mM), 1% MEM non-essential amino acids and 1% antibiotic-antimycotic] containing the cytokines, soluble recom-binant human basic fibroblast growth factor (bFGF;20 ng/mL), and hu-man leukemia inhibitory factor (hLIF;10ng/mL). Isolated cellswere cul-tured on fresh primary murine embryonic fibroblast feeder cells (PMEF) in a humidified environment of 5% CO_2 in air at 38℃. The colonies with

EG-like morphology were dissociated with 0.25% trypsin/1 mM EDTA for 10 min, and passed to fresh feeders.

After 5~8 days, colonies started to grow with typical EG-like morphology. More colonies were obtained from the culture of cells from mesonephros than PGCs. Porcine EG-like cells from mesonephros (pMN-EG-like cells) were passed to fresh feeder every 6~8 days and have been cultured upto 9th passages maintaining typical EG-like morphology. pMN-EG-like cells were stained for alkaline phosphatase throughout the culture. Futhermore, these cells were reacted with antibodies against Oct-4 and SSEA-1 by immunocytochemstry, indicating that these cells have characteristics of pluripotential stem cells. In order to characterize the pMN-EG-like cells with respect to their potential for differentiation, embryoid body (EB) formation was induced. EBs were started to form in 4 days and cystic structures in 2 weeks. EBs were then attached to the dishand cultured without cytokines. Spontaneously, EBs from pMN-EG-like cellscould give rise to differentiated cell types such as neuronal-like, epithelial-like, and fibroblast-like cells. Further studies to characterize differentiated cells from pMN-EG-like cells by immunocytochemstry and teratoma formation by injecting pMN-EG-like cells into SCID mice will be performed. In conclusion, EG-like cells could be obtained from culture of mesonephric cells from porcine fetus and further characterization of these cells is required.

We studied to determine the minimal combination of growth factors that maintain the proliferation and pluripotency of undifferentiated pMN-EG-like cells, and the result was that 20 ng/mL FGF2 sustained PMSCs with an effectiveness comparable to other growth factor combinations, SCF and hrLIF.