

Derivation and Application of Pluripotent Embryonic Germ Cells in Chicken

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Embryonic germ (EG) cell line derived from primordial germ cells (PGCs) is an undifferentiated and pluripotent stem cell. To date, EG cells with proven germline transmission have been completely established only in the mouse with embryonic stem (ES) cells. In this study, PGCs from 5.5-day-old (stage 28) chicken embryonic gonads were isolated and established a chicken EG cell line with EG culture medium supplemented with stem cell factor (SCF), leukemia inhibitory factor (LIF), basic fibroblast growth factor (bFGF), interleukin-11 (IL-11), and insulin-like growth factor-I (IGF-I). gPGCs on chicken gonadal stroma cells began to colonize 7 days after culture following significant ($p < 0.0001$) increase in cell population. For EG cell establishment, fully colonized gPGCs 10 to 15 days after primary culture were subcultured with chicken embryonic fibroblast monolayer. These cells grew continuously for 10 passages (4 months) on a feeder layer of mitotically active chicken embryonic fibroblasts. After several passages, these cells were characterized by screening with the Periodic acid-Schiff's reaction, anti-SSEA-1 antibody, and a proliferation assay. The chicken EG cells maintained characteristics of both gonadal PGCs and undifferentiated stem cells. When cultured in suspension, the chicken EG cells successfully formed an embryoid body and differentiated into a variety of cell types. The chicken EG cells were injected into stage X blastodermal layer and produced chimeric chickens with various differentiated tissues derived from the EG cells.

This study reports not only the somatic chimera but also chicken germline chimera production by established embryonic germ (EG) cell transplantation into recipient embryos. EG cells were derived from the culture of White Leghorn (WL, *I/I* gene) gonadal primordial germ cells (gPGCs) at the stage 28. Transplantation was conducted with Korean Ogot chicken embryos (*i/i* gene) at the stage 17. In subsequent testcross analysis, EG cells or gPGCs transplantation yielded total 17 germline chimeras; 2 out of 15 matured progenies in EG cell (13.3%) and 15 of 176 in gPGC cultured for 2 days or fully colonized (10 days) (8.5%). The efficiency of germline transmission was 1.5 to 14.6% in EG cells, while 0.2 to 27.6% in gPGCs. Chicken EG cells will be useful for the production of transgenic chickens and for studies of germ cell differentiation and genomic imprinting.