EFFICIENT DERIVATION OF PRIMORDIAL GERM CELLS FOR GENETIC PRESERVATION OF INDIGENOUS CHICKEN

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Introduction

Primordial germ cells (PGCs) are promising tools for producing transgenic chickens or preserving genetic resources in endangered birds or domestic avian species. Procuring PGCs from embryonic tissues, as described in the literature, is inefficient, unstable, and time-consuming. Moreover, germline transmission of PGCs tends to be decreasing with extended culture in vitro. Therefore, we set up to develop a robust method for isolation and rapid propagation of PGCs derived from embryonic gonads.

Materials and Methods

Chicken PGCs used in culture system optimization were labeled with EGFP. A total of 2,000 PGCs were seeded in different culture systems mKO (modified knockout DMEM), mKO-Feeder, FACs and cKO-F. Cell proliferation were examined in day 1, 3, 6, using a BioTek Cytation5 system.

In primary culture, PGCs were isolated from the Donglan chickens, a black chicken breed native to Guangxi Province, China. PGCs were examined every two days in the first 20days. Immunocytochemistry and RT-PCR were performed to confirm the expression of germ cell specific biomarker in the established cell lines. PGCs labeled with GFP were injected into recipient White Leghorns chicken embryos to evaluate the migration potential. Germline chimera were bred to wide-type chicken and donor-derived chicken is examined by the presence of black feather or skin in F1 chicken.

Results and Discussions

PGCs were more proliferative in mKO and mKO-Feeder than in the other two culture system. But while mKO-F system was used in the primary PGCs culture, cells ceased to grow 6 days. Then we used a hanging-insert system to obviate the direct contact of PGC and feeder cells in primary culture. Surprisingly, PGCs derived in mKO-FI (mKO-feeder-inserts) presented a robust proliferation and grew to 10^6 in 14 days. Proliferative cell lines could be established in over 90% of the isolations, much higher than those in FACs and cKO-F ($P<0.01$). Germ cell specific biomarkers, such as Nanog, pouV, Cvh, Dazl, Cdh, were detected by RT-PCR in the derived PGCs. And immunocytochemistry also showed that SSEA1 and DAZL were positive in these cells. In addition, these cells maintained gonad migration ability and up to 64% of germline-transmission efficiency was seed in F1 chicks. The mKO-FI culture system optimized in our research would not only allow efficient genetic banking of chicken species, but also facilitate production of chicken bearing a desired phenotype via genomic editing.