The Production of Germline Chimeras by Transfer of Gonadal Primordial Germ Cells Separated with Magnetic Cell Sorter System in Quail

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Abstract

Collection of large number of gonadal primordial germ cells(gPGCs) is a prerequisite factor for improving germline transmission efficacy in the aves. In this study, a magnetic-activated cell sorter(MACS) was applied for improving retrieval efficacy of quail gPGCs and the migration capacity of MACS-separated gPGCs was further examined after being transplanted to recipient embryos. We also induced germline chimeras by transfer of MACS-separated quail gPGCs at the efficiency of 17.4% on average.

(Key words : quail, MACS, gonadal PGCs, purification)

Introduction

The ave is only a species occurring germ cell migration through the cardiovascular system, thus this nature makes it possible to transplant germ cells into the blood vessel of early embryos for inducing germline transmission. Development of avian gonadal primordial germ cells(gPGCs) transplantation system into recipient embryos for germline chimera production greatly contributes to establishing an efficient bioreactor system using transgenic technology, which overcomes a lot of difficulties being found in the production of transgenic mammals. Furthermore, this technique will increase industrial applicability of avian gPGCs, since direct retrieval from the embryonic gonads and the efficient separation system can collect large number of PGCs. In this study, a magnetic activated cell sorter(MACS) was applied for improving retrieval efficacy of gPGCs and the migration capacity of MACS-separated gPGCs was further examined after being transplanted to recipient embryos.

Materials and Methods

① **Retrieval of Gonadal Primordial Germ Cells** : Primordial germ cells(PGCs) were retrieved from the gonads of Japanese wild-type quail(Coturnix japonica) embryos at 5.0 days of incubation according to the methods of Park and Han(2000). Gonadal tissues were then dissociated by gentle pipetting in 0.05%(v/v) trypsin solution supplemented with 0.53 mM EDTA.

② **Separation of gPGCs with MACS** : Gonadal cells of $1x10^6$ were incubated with QCR I antibodies for 20 minutes at the room temperature of 20 to 25℃. After washing with 1 ml buffer(PBS supplement with 0.5% BSA and 2 mM EDTA), the supernatant

was completely removed and then mixed with rat anti-mouse IgM microbeads for 15 minutes at 4℃. Cells were carefully washed by addition of 500uL buffer and proceeded to magnetic separation according to the manufacturers recommendations (Miltenyi Biotech, Germany).

③ **Staining of gPGCs with Specific Antibody** : Quail gPGCs were fixed with 1%(v/v) glutaraldehyde for 5 minutes and rinsed with 1×PBS twice. The QCR I antibody diluted 1:200 in PBS was added and subsequent steps were carried out using DAKO universal LSAB®kit, Peroxidase(DAKO, USA) according to the manufacturers instruction.

④ **Transplantation of gPGCs into the Recipient Embryos** : For the transplantation, a small window was made on the sharp end of recipient black quail egg and approximately 2uL of cell suspension containing 300 or 600 with or without MACS separation was injected into the dorsal aorta of recipient embryo using a micropipette. The egg window of the recipient embryo was sealed twice with paraffin film and then laid down with the pointed end at the bottom until next treatment.

Results

The population ratio of gPGCs significantly($P<0.0001$) increased after MACS separation : approximately 16.7 folds(45.1% vs. 2.7%). In the next series of experiment, MACS-separated gPGCs were injected into the dorsal aorta of recipient embryos at 2 days of incubation and monitored gPGC migration on 3 days after incubation. PHK26-labelled and transplanted quail gPGCs could be detected in the embryonic gonads of recipient embryos at 5 days. Furthermore, the manipulated wild quails, after sexual maturity, were mated with black quails for identifying germline chimeras. The efficiency of germline chimeras produced by transfer of MACS-separated quail gPGCs was on average 17.4%. The results of this study demonstrated that non-invasive separation method for quail gPGCs could be established with the use of MACS system and separated gPGCs could migrate into the embryonic gonads after transplantation into recipient embryos.

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