연제 6

Examination Of The Migratory Ability Of Primordial Germ Cells From Embryonic Gonads At Different Developmental Stages In Quail

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Abstract

Retaining migratory activity is a prerequisite for the manipulation and use of PGCs. This study was conducted to examine whether migratory activity is retained in the primordial germ cells (PGCs) from gonads at the later embryonic developmental stage. In the present study, gonads were dissected from 5-, 6- and 10-day-old quail embryos and treated with trypsin-EDTA for the degradation of gonadal tissue. Gonadal PGCs (gPGCs) were purified by Ficoll density gradient centrifugation and labeled with PKH26 fluorescent dye. The PKH26-labeled gPGCs were microinjected into the blood vessels of recipient quail embryo. After further incubation of 3 days, the manipulated recipients were embedded in paraffin and sectioned. The gPGCs were detected by their fluorescence under the fluorescent microscopy and the confocal laser microscopy. As a result, 10-day-old quail gPGCs as well as 5- and 6-day-old gPGCs, could migrate to recipient embryonic gonads and settle down. These results suggest that the 10-day-old gPGCs have the properties of circulating PGCs at early stage. Therefore, the PGCs from 10-day old embryonic gonads can be used for the tools of genetic manipulation.

Introduction

Avian PGCs enter the developing blood vessels at stage 10 to 12 (Hamburger and Hamilton, 1951), circulate in the blood stream and migrate into the

developing gonadal
anlage in which differentiate into germ cells. Generally, it is known that the PGCs differentiate after 6 days of incubation. The chick germ cells divide mitotically between 3 and 7 days of incubation (Erickson, 1974). It has examined by Maeda and his colleagues (1994) that 2C9-reactive PGCs were increased in number beyond the migrating stage. In the female gonads, the reactivity disappeared at 8 days of incubation, but the reactivity of male PGCs was gradually decreased and disappeared until 14 days of incubation. To date, the attempt on the production of germline chimeric birds using PGCs has been focused on the PGCs from early embryonic stage. However, the PGCs arrived in gonad proliferate into numerous cells through mitosis. Therefore, if these cells in gonads maintain the characteristics as circulating PGCs at later stage, a large number of PGCs can be easily isolated for the production of germline chimera. Although the characteristics of PGC have been extensively described in the chicken, little is known about in other avian species including Japanese quail (*Coturnix coturnix japonica*). In the present study, we characterized the PGCs from gonads at the later embryonic developmental stage and microinjected PGCs from different stages into recipient embryos to confirm the migratory activity to the gonadal ridges.

Materials and Methods

Embryonic gonads were dissected from 5-, 6- and 10-day-old quail embryos and single cell suspension was prepared by treatment with trypsin-EDTA. Gonadal primordial germ cells (gPGCs) were purified by Ficoll density gradient centrifugation and labeled with PKH26 fluorescent dye. The PKH26-labeled gPGCs were injected into the blood vessel of recipient quail embryo at 2.5 days. The manipulated recipients were incubated for further 3 days and embedded in paraffin for detection of foreign PGCs under the fluorescent microscopy and confocal laser microscopy.

Results and Discussion

Quail gPGCs were concentrated by Ficoll density gradient centrifugation. The purity of gPGCs after Ficoll density gradient centrifugation is about 73.1~82.5% which is independent the embryonic developmental stages. The gPGCs

were labeled with PKH26 fluorescent dye before those were injected into the blood vessel of the recipient. Injected gPGCs in the gonadal region of recipient embryo were identified as fluorescent spots. Gonadal PGCs of 5-, 6- and 10-day-old embryos could migrate into the recipient gonadal region. These results suggest that the PGCs settled in the gonad still retain the migratory activity into the gonads through the blood stream of recipient embryo. It was also shown that 10-day-old quail gPGCs could migrate and settle in the gonads, suggesting that the 10-day-old gPGCs hold the properties of circulating PGCs. Therefore, the PGCs from up to 10-day-old embryonic gonads can be used for the tools of genetic manipulation.

(Key words : quail, gonadal PGCs, PKH26)

References

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