연제 11

The capabilities of migration and differentiation of female primordial germ cells after transferring to male embryos

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Abstract

Comparing to mammals, male bird has the homozygote ZZ and female has the heterozygote ZW. Therefore, the sex of fertilized eggs is defined by female chromosome constitution. Although this cytological observation had been established, the molecular and cellular mechanism of germ cell differentiation are essentially unknown in aves. Especially, the differentiation of germ cells in mixed-sex chimeras has not yet been clearly elucidated. Primordial germ cells, which are the progenitors of sperm or egg after sexual maturity, firstly arise in the epiblast and migrate to embryonic gonads through the blood vessel. During the embryo development, these PGCs differentiate in the pathway of male or female, respectively and develop the sperm or egg cells after sexual maturity. In this paper, we confirmed that the female PGCs could migrate into the recipient male gonads after transferring and differentiate into germ cells in the embryonic stages. The primordial germ cells were isolated from the female embryonic gonads of 5.5-day-old incubation and re-injected into the male recipient embryos of 2-day-old incubation, which produced mixed-sex chimera in the germline. The finding in this study demonstrated the ability of migration and differentiation of gonadal primordial germ cells in mixed-sex chicken.

(**Key words** : primordial germ cell, migration, differentiation, mixed-sex)

Introduction

Unlike mammals, the avian embryos with the homozygote ZZ are males, whereas those with the heterozygote ZW are females. Although this cytological observation has been established, the molecular and cellular mechanism of germ cell differentiation are essentially unknown. Especially, the differentiation of germ cells in mixed-sex chimeras has not been elucidated as yet. The germ cells are originated from primordial germ cells (PGCs), which first arise in the epiblast and migrate to embryonic gonads through the blood vessel. During the embryo development, the PGCs differentiate in the pathway of male or female and develop the sperm or egg cells after sexual maturity, respectively.

Naito *et al.* (1999) demonstrated W-chromosome bearing sperm by using PCR method but the migration and differentiation of injected female PGCs in male embryonic gonads

have not been known. Thus, this study was conducted to confirm that the female PGCs could migrate into the recipient male gonads and differentiate into germ cells in the embryonic stages.

Materials and Methods

Experimental stocks

White Leghorns were used for donor of gonadal primordial germ cells (gPGCs) and Korean Ogol chickens for recipient embryos.

Direct-sexing of embryos without DNA extraction

To determine sex of a recipient embryo, blood was removed from 5-day-old embryo and the sample was boiled at 97℃ and 55℃ three times for 5 minutes each and subsequently subjected to PCR reaction. The primer sequences were as follows; 5′-ACC TGT CTC CCA AAA ATT CTG C-3′ and 5′-TGG GGT GAA ATG GGG TTG- 3′.

Isolation of gonadal primordial germ cells

Gonadal PGCs (gPGCs) were prepared by isolating embryonic gonads at stage 28 (5.5 days of incubation) and by dissociating the gonad tissue in trypsin-EDTA by gentle pipetting. After inactivation of trypsin-EDTA with DMEM containing 10% FBS, the cells were harvested by centrifugation.

Fluorescent PKH-26 labeling

gPGCs were labeled with PKH-26 according to the supplier s specification (ZYNAXIS Cell Science INC.) with a minor modification.

Microinjection into recipient embryo

gPGCs of White Leghorn were injected into the blood vessel of Korean Ogol chicken embryos at Stage 14-15 (53 hrs of incubation). A small window was made on the sharp end of egg and approximately 2-3 $\mu\ell$ of cell suspension was injected into the blood vessel of the 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 down until next experiments or hatching.

Detection of PKH-26 in recipient embryonic gonads

To identify PKH-26 labeled donor gPGCs, the whole gonads of recipient embryo were dissected and observed under the fluorescent microscope (IX70, Olympus).

Results

In this study, we demonstrated that, first, the female gPGCs could migrate into male recipient embryonic gonads when transferred and, second, the female gPGCs could be detected in gonads of male recipients at 5, 10, 14 days of incubation. This result indicated that female gPGCs could migrate into male recipient embryonic gonads and differentiate into germ cells in mixed-sex gonads during embryonic development.

적 요

조류의 경우에는 포유류와 달리 수정란의 성별이 암컷에 의하여 결정된다. 수컷은 동일접합체로 ZZ 염색체를, 암컷의 경우에는 이형접합체로 ZW 염색체를 갖기 때문이다. 현재까지 조류에 있어서 염색체 분석 등에 의한 암·수의 세포 유전학적인 특성은 많은 연구가 되어 있으나, 배발달 초기의 원시생식세포 등에 대해서는 많은 연구가 진행되어 있지 않다. 따라서 본 연구는 암컷의 원시생식세포를 분리하여 숫컷의 초기 배자에 주입함으로써 수용체 배자의 원시생식기내로 이동이 가능한지를 검증하였으며, 또한 수컷의 원시생식기내로의 이동 후 정상적으로 분열 및 분화가 가능한지를 초기 배발달 과정에서 확인하였다. 본 연구 결과, 암컷의 원시생식세포는 수컷의 수용체 배자에 재주입시 정상적인 원시생식기내로의 이동 능력을 보여주었으며, 분열·분화함을 알 수있었다.

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