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Introduction of Exogenous Gene into Chicken PGCs via Blastoderm

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Abstract: It was proved that an exogenous gene was successfully transferred to chicken embryo and Primordial Germ Cells (PGCs) by the injection of the blastoderm. In this experiment, an exogenous gene, Lac Z constructs encoding *Escherichia coli* beta-galactosidase was introduced into chicken blastoderm (stage X) just ovoposition using lipofection reagent. After injection of gene the eggs were incubated in routine manner until developmental stage 12-15. Survival rate of the treated embryo was 35.6%. Lac Z expression was mosaic manner and low efficiency. However, it was obtained Lac Z specific band from chicken PGCs by PCR method.

Key words: Chicken embryo, gene transfer, blastoderm, PGCs, exogenous gene, Lac Z

INTRODUCTION

Recently, the production of transgenic animals has been succeeded in animal biology. Transgenic mice have been shown to be useful in the study of regulation and progress of mammalian development. Transgenic mice are produced routinely by microinjection of DNA and chimeric animal using ES cell introduced exogenous gene.

In the poultry, transgenic chickens have been proved to be the excellent system for studying developmental biology and could be of great benefit to the breeding industry. However, the production of transgenic chickens may present a set of problems that such experimental manipulation is impractical because of the unique reproductive anatomy and physiology. Gene introduction for chicken have been examined using early chick embryos and Primordial Germ Cells (PGCs) (Inada *et al*., 1997; Furuta *et al*., 2000; Furuta and Fujihara, 2000). Avian PGCs have been said to originate from epiblast (Eyal-Giladi *et al*., 1981) appear in hypoblast of germinal crescent region, circulate while developing in the blood vasucular system at stage 12-16 and finally migrate into the germinal ridge (Kuwana, 1993). After the miglation, differentiation of the PGCs into ova or spermatozoa occurs at the site germinal ridge.

The utilization of PGCs are employed as a useful tool for introduction of exogenous gene. The gonad of germline chimeric chicken have also been investigated to effect by the transfer PGCs (Yamaguchi *et al*., 2000; Furuta *et al*., 2007, 2008). The techniques for introducing exogenous gene to cell have so far been studied using various methods. The viral vector method for introduction of exogenous gene has been found superior to other methods (Vick *et al*., 1993; Allioli *et al*., 1994). This method however, requires the handling of hazardous retroviruses and limits the size of insert DNA.

The present experiments were carried out to introduce marker gene, the Lac Z in chicken blastoderm of freshly oviposited eggs with lipofection by simple method. Special attention was paid to question whether the exogenous gene injected into blastoderm appears in PGCs.

MATERIALS AND METHODS

Recipient eggs: Fertilized eggs were obtained from white leghorn juria line hens (GHEN Corporation, Gifu Japan) were employed.

Introduction of marker gene solution: The lac Z : pSV-β-Galactosidase Control Vector (Promega, WI, USA) was used as a marker gene. The circular form lac Z (0.1 μg) was mixed with 1 μL of lipofectamine 2000 (Invitrogen, CA, USA) and volume was made up to 10 μL with OPTI-MEMI (Gibco, MD, USA). Sharp edge of the fertilized eggs were set to keep upward in an egg tray to observe a blastoderm easily. A window of approximately 10 mm in diameter was opened in sharp edge of egg. 2 μL Lac Z solution was injected into a central area of the blastoderm by the glass pipette (G-1, Narishige, Tokyo, Japan) through the window.

Control: A window of approximately 10 mm in diameter was opened in sharp edge of egg however, DNA solution was not injected into blastoderm.

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Incubation of the eggs: The treated eggs were incubated at 37.8°C and 60-70% of relative humidity for 3 days when developmental stage would be at 12-15 (Hamburger and Hamilton, 1951). At stage, the PGCs migrate to germinal ridges via blood stream.

Detection of Lac Z: The incubation eggs were cracked to check mortality. Blood samples were collected from embryonic vein by micro glass at stage 12-15. PGCs were isolated from blood (Yamamoto *et al*., 2007). The embryos were removed from yolk immersed in PBS and fixed with 1% glutaraldehyde in PBS. After washing three times with PBS, the samples were stained for 1 h with X-gal solution (0.05% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (Sigma, MO, USA), 1 mM $MgCl_2$, 0.1% Triton X-100, 3 mM potassium ferrocyanide (III) and 3 mM potassium hexacyanoferrate (II) trihydrate in PBS).

The total DNA was extracted from PGCs by the genomic DNA purification kit (Promega, WI, USA). The Polymerase Chain Reaction (PCR) analysis was carried out DNA samples in order to detect the presence of the Lac Z.

RESULTS AND DISCUSSION

The number of embryos surviving at 3 days (stage 12-15) incubation was 31 out of 87 manipulated (35.6%) and 28 out of 48 of windowed eggs (58.3%). The number of embryos surviving in the control was significantly greater ($p<0.05$) than in manipulated and windowed eggs. The number of abnormal development embryos was 4 out of 31 manipulated (12.9%) and 2 out of 28 of windowed eggs (7.1%) as shown in Table 1.

The expression of the Lac Z as revealed by β-galactosidase activity was detected in 3.7% of survival embryos (Table 2). The rate of detected specific Lac Z band from PGCs by PCR was 58.3% (Table 3).

Table 1: Number of survival and abnormity of embryos after stage 12-15 of incubation following transfer of exogenous gene

Groups	No. of embryos manipulatid	No. of surviving embryos (%)	No. of abnormal embryos (%)
Control	48	28 (58.3)	2(7.1)
Trail	87	31 (35.6)	4 (12.9)

Table 2: Expression of Lac Z gene in embryos by X-gal

Groups	No. of embryos observed	No. of Lac Z expresised embryos (%)
Control	22	0 (0)
Trail	27	1 (3.7)

Table 3: Detect of Lac Z gene from PGCs by PCR (%)

Groups	No. of detected Lac Z
Control	0/12 (0)
Trail	7/12 (58.3)

The chick embryo is a useful model which possible to study molecular approach on embryology. Several methods have been developed to introduce exogenous sgene into embryos (Momose *et al*., 1999; Ishii and Mikawa, 2005). These methods available for somatic transgenesis are very useful for the embryo into the most attractive model to examine function of genes.

A production of transgenic mouse by transfer of exogenous gene to germline is routine. In avian, however it is difficult to obtain single fertilized ova and be matured embryos by artificial hatch. The PGCs are widely going to clarify the mechanism of gene targeting and gene transfer which may provide some of benefits to the case of germ cells from mammalian. Germline chimeric chickens have already been produced by the transfer PGCs for restore genetic resources from endangered domestic chicken or some of the wild bird and produce transgenic chicken (Furuta *et al*., 2001; Van De Lavoir *et al*., 2006). The exogenous gene could be introduced into the PGCs for production of a transgenic chicken (Inada *et al*., 1997; Furuta *et al*., 2000; Furuta and Fujihara, 2000). It was focused to integrate exogenous gene into PGCs, which lead to germline incorporation of gene. However, efficiency of introducing exogenous gene into PGCs was very low. A germline transfer approaches is still challenging.

CONCLUSION

In this study, it was detected exogenous gene from PGCs that migrate to germinal ridges in blood vessel by introduce exogenous gene into the central area of the blastoderm which is an origin of PGCs. However, few positive cell of Lac Z staining have been obtained on the embryos. There is much scope for future studies on this subject.

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