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Selective decrease of chick embryonic primordial germ cells in vivo and in vitro by soft X-ray irradiation

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Abstract

The feasibility of soft (low-energy) X-ray irradiation as a means of depleting the endogenous primordial germ cell(s) (PGC) of chicken embryos, to improve the efficiency of germ cell-mediated transgenesis, was investigated. Eggs were subjected to a non-irradiated control treatment and embryos were exposed for 40 s to soft X-ray at 15, 16.5, or 18 kV (∼1.5, 1.65, and 1.8 Gy, respectively). Exposure of stage X embryos to each dose of X-ray resulted in a reduction of ∼50% in the number of PGC apparent at stage 28, whereas the total number of gonadal cells was unaffected. Irradiation (16.5 kV) of embryos at stage 9 or 14 also resulted in similar decreases in the number of PGC with no effect on the total number of gonadal cells. Irradiation did not affect embryo hatchability, compared with the non-irradiated control treatment, although the hatch rate increased with the age of embryos at the time of irradiation. Exposure of gonadal cells isolated from stage 28 embryos to X-ray (16.5 kV, ∼0.8 Gy) prevented the increase in PGC number during subsequent culture for 10 days; the increase in the total number of gonadal cells was not affected. In conclusion, exposure of chicken embryos to a low dose of soft X-rays is effective for depleting the endogenous PGC population without affecting embryo hatchability or somatic cell viability.

Keywords: Chicken; Primordial germ cell; Gonadal cell; X-irradiation; Hatchability; Cell viability

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1. Introduction

Development of an effective technology for germ cell-mediated transgenesis in the chicken will require improvement in the efficiency of germline transmission. Such improvement might be achieved by increasing the number of donor germ cells transplanted. To date, the number of transplanted germ cells has been increased either by prior extended culture to allow proliferation (Chang et al., 1997; Karagenç and Petitte, 2000; Park et al., 2003) or with the use of magnetic cell sorting (Kim et al., 2004). An alternative approach is to deplete endogenous germ cells of recipient embryos. Such depletion of primordial germ cell(s) (PGC) has been attempted by exposure of the host gonads to busulfan sulfate (Aige-Gil and Simkiss, 1991a) or by irradiation of the embryos with ultraviolet light (Aige-Gil and Simkiss, 1991b). However, both of these treatments were lethal and had limiting practical application. A simple method based on irradiation with soft (low-energy) X-rays (wavelength of 0.1–10 nm) has been applied to reduce the number of PGC in Japanese quail embryos (Li et al., 2001). The utility of this approach for preparation of recipient chicken embryos for PGC transfer was evaluated with the present study.

2. Materials and methods

2.1. Experimental animals

White Leghorn (WL) chickens were maintained at the University Animal Farm of Seoul National University, and mature females were artificially inseminated for collection of fertilized eggs. All procedures for animal management, reproduction, and surgery were performed in accordance with the guidelines of Seoul National University, and the study protocol was approved by the Institutional Review Board of the Department of Animal Science and Technology, Seoul National University in December 2002.

2.2. Experimental design

Developmental stages of chick embryos were determined on the basis of the normal tables of Eyal-Giladi and Kochav (1976) or Hamburger and Hamilton (1951) for stages before incubation (expressed in Roman numerals) or after incubation (expressed in Arabic numerals), respectively. Two series of experiments were performed for evaluation of the effects of X-ray irradiation.

In the first series of experiments, embryos at stages X, 9, and 14 were exposed to soft X-rays for 40 s and were then incubated until they developed into stage 28 (total of 5.5 days). The PGC were detected by staining with periodic acid-Schiff (PAS) solution and antibody to stage-specific embryonic antigen-1 (SSEA-1) as described previously (Jung et al., 2005). The numbers of gonadal cells and PGC were counted by our standard procedure (Park et al., 2003). Briefly, the gonadal cells seeded onto a 96-well plate were fixed with 1% (v:v) glutaraldehyde for 5 min and rinsed with 1× PBS twice. The cells were then treated with anti-SSEA-1 ascites fluid diluted 1:1000 in PBS and counting the cell number was proceeded according to the manufacturer's instruction of DAKO universal LSAB® kit, Peroxidase (DAKO, Carpinteria, CA). The viability of embryos on day 14 of incubation and the hatchability of irradiated and non-irradiated control embryos were also determined.

In the second series of experiments, gonadal cells retrieved from stage 28 embryos were either exposed to soft X-rays or not and were subsequently cultured in vitro for 10 days. The numbers