# Derivation of primordial germ cells from chicken blastodermal cells by BMP-2 and BMP-4 signaling

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## ABSTRACT

Primordial germ cells (PGCs) are the progenitors of the sperms or eggs of adult. Evidence suggests that the specification of primordial germ cells (PGCs) in the mammalian embryo does not depend on maternal determinants. Recent previous studies in the mouse has shown that several bone morphogenetic proteins (BMPs) are required for the formation of PGCs.

However, there is no study about the effect of BMPs on avian PGCs. Here, we studied the effects of recombinant human BMP-2 (rhBMP-2) and recombinant human BMP-4 (rhBMP-4) on chicken blastodermal cells in culture.

As a results, the addition of rhBMP-2 and rhBMP-4 increased the number of SSEA-1 positive cells in dose-dependent manner. However, there is no synergic effect by using both rhBMP-2 and rhBMP-4. **(Key words : PGC, BMP-2, BMP-4)**

## INTRODUCTION

In avian embryos, primordial germ cells (PGCs) originate from the epiblast and are identified at the germinal crescent area during the early primitive streak stage, then circulate in the blood stream and migrate to the developing gonadal anlage where they differentiate into germ cells.

Recent studies indicate that several BMPs may play a direct role for the formation of the germline in mammals. BMPs are members of TGF-β superfamily of growth factors that function as homodimers or heterodimers to signal through heteromeric receptor complexes and downstream SMAD proteins.

Lawson et al. (1999) showed that BMP-4 is required for PGC generation in the mouse and its activity is dose-dependent. In addition to Bmp4, Bmp8b and Bmp2 likely cooperate with Bmp4 in affecting the formation of PGCs. Bmp8b is expressed in the extraembryonic ectoderm in pregastrula and gastrula stage mouse embryos and is required for PGC generation (Ying et al, 2000). Ying et al (2001) showed that Bmp2 also plays a role in PGC induction and is primarily expressed in the endoderm of mouse pregastrula and gastrula embryos. Using a genetic approach, they showed that Bmp2 and Bmp4 had an additive effect on PGC generation.

In this paper, we have studied the effects of recombinant human BMP-2 (rhBMP-2) and recombinant human BMP-4 (rhBMP-4) on chicken blastodermal cells in culture.

## MATERIALS AND METHODS

Embryos were obtained from White Leghorn. The area pellucidae from stage X (Eyal-Giladi and Kochav, 1976) embryo was dissociated enzymatically using 0.25% trypsin/ 0.05% EDTA. Trypsin activity was neutralized by adding DMEM supplemented with 10% FBS, and a single-cell suspension was obtained by pipetting. Cells were seeded into the well of a 96-well plates containing onto mitomycin C-treated STO cell as a feeder. The seeding cell number was 5,000 per each well. Recombinant human BMP-2 (R&D systems) and recombinant human BMP-4 (R&D systems) were added into the culture medium at different concentrations ranging from 0 to 100 ng/㎖ and 0 to 20 ng/㎖, respectively. After 2 day in culture, immunohistochemical staining was performed for PGC identification using anti-SSEA-1 (MC-480) antibody. The following staining step was carried out using a labelled streptavidin biotin method (DAKO LSAB Kit, DAKO CORPORATION, USA) according to the manufacturer's instructions. The red colored cells were counted under the microscope.

## RESULTS & DISCUSSION

To examine the functions of BMPs in chicken PGCs, we added rhBMP-2 or rhBMP-4 into the culture of blastodermal cells. rhBMP-2 and rhBMP-4 increased the number of SSEA-1 positive cells in dose-dependent manner. Notably, dose of 75 ng/㎖ of rhBMP-2 and 10 ng/㎖ of rhBMP-4 showed a significant effect, respectively. The synergic effect by addition of rhBMP-2 and rhBMP-4 at a time didn't appear.

As a results, it is likely that signalling by BMP-2 and BMP-4 stimulates PGC derivation and growth. Enhancement of PGC number by BMP-2 and BMP-4 in culture is consistent with this supposition. The results show that BMP-2 and BMP-4 are functional for the derivation of PGCs from chicken blastodermal cells in culture. However, there is no additive effect on PGC generation by using BMP-2 and BMP-4 at the same time.

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