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Research Article

BMP4 can generate primordial germ cells from bone-marrow-derived pluripotent stem cells

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Abstract

Evidence of germ cell derivation from embryonic and somatic stem cells provides an in vitro model for the study of germ cell development, associated epigenetic modification and mammalian gametogenesis. More importantly, in vitro derived gametes also represent a potential strategy for treating infertility. In mammals, male and female gametes, oocyte and sperm, are derived from a specific cell population, PGCs (primordial germ cells) that segregate early in embryogenesis. We have isolated pluripotent SSEA-1$^+$ (stage-specific embryonic antigen-1$^+$) cells from mice bone marrow using a MACS (magnetic-activated cell sorting) system. SSEA-1$^+$ cells were directly separated from the suspension of MMCs (murine mononuclear cells) harvested from bone marrow of 2–4-week-old mice. Flow-cytometry assay immediately after sorting and culturing under undifferentiated condition showed 55 ± 7% and 87 ± 4% purity respectively. RT–PCR (reverse transcription–PCR) analysis after differentiation of SSEA-1$^+$ cells into derivations of three germ layers showed the pluripotency properties of isolated cells. SSEA-1$^+$ cells were induced to differentiate along germ cell lineage by adding BMP4 (bone morphogenic factor-4) to the medium. Regarding the expression of germ cell markers (PGCs, male and female germ cell lineage), it was found that adding exogenous BMP4 to culture medium could differentiate pluripotent SSEA-1$^+$ cells isolated from an adult tissue into gamete precursors, PGCs. Differentiated cells expressed specific molecular markers of PGCs, including Oct4, fragilis, Stella and Mvh (mouse vasa homologue). Therefore BMP4 is insufficient to induce SSEA-1$^+$ cells derived from PGCs to develop further into late germ cells in vitro.

Keywords: bone morphogenic protein 4 (BMP4); bone marrow; infertility; magnetic-activated cell sorting (MACS); primordial germ cell (PGC); SSEA-1$^+$ cells

1. Introduction

Infertility affects approximately 1 in 10 couples of reproductive age; epidemiological studies suggest an increasing incidence of inability to conceive children in developing countries (Iammarrone et al., 2003). Infertility is not just only on the female side, since the male is the main cause in approximately half the cases (Cousineau and Domar, 2007). Advances in assisted reproductive medicine and presentation of new effective treatment methods would be valuable in reproductive medicine (Cousineau and Domar, 2007). Successful derivation of gametes (sperm and oocyte) from embryonic and adult stem cells has been achieved (Hubner et al., 2003; Toyooka et al., 2003; Nayernia et al., 2004, 2006a, 2006b; Dyce et al., 2006; Kerkis et al., 2007; Lue et al., 2007; Aflatoonian et al., 2009; Hua et al., 2009; Hayashi et al., 2011). PGCs (primordial germ cells) can be derived from ESCs (embryonic stem cells) in vitro, and derivation of germ cells from stem cells represents a desirable model and potential strategy for treating infertility (Park et al., 2009). Stem-cell-derived germ cells also provide an in vitro model for the study of germ cell development, associated epigenetic modification and mammalian gametogenesis (Nayernia et al., 2006b; Aflatoonian et al., 2009).

Germ-line cells are the biological route for genetic transmission and reproducing totipotency from generation to generation. Murine embryonic precursors of gametes, PGCs, originate from founder cells of the proximal epiblast located at the base of the allantois in response to inductive signals emanating from the adjacent extra-embryonic ectoderm, including BMPs (bone morphogenic proteins), particularly BMP2, BMP4 and BMP8 (Eguizabal et al., 2009; Marques-Mari et al., 2009). PGCs migrate in the developing embryo through the dorsal mesentery towards the fetal gonads, gonadal ridge, where they proliferate and differentiate into gonocytes, the primitive germ cells (Donovan et al., 1986). They start to express germ-cell-specific genes, such as Mvh (mouse vasa homologue) and Gcna-1 (germ cell nuclear antigen 1) (Enders and May, 1994; Toyooka et al., 2000). PGCs undergo epigenetic reprogramming and finally enter mitotic arrest in the male and the prophase of meiosis I in the female (McLaren, 2003; Surani et al., 2007).