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 derivatives 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+

1. Introduction

Infertility affects approximately 1 in 10 couples of reproductive age; epidemiological studies suggest an increasing incidence of infertility is not 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 gametocytes (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).

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Abbreviations: ALP, alkaline phosphatase; BM-MNC, bone marrow mononuclear cell; BMP4, bone morphogenic protein 4; DAPI, 4',6-diamidino-2-phenylindole; DAZL, deleted in Azoospermia-like; DMEM, Dulbecco’s modified Eagle’s medium; EB, embryoid body; ESC, embryonic stem cell; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GDF9, growth differentiation factor 9; GFAP, glial fibrillary acidic protein; MACS, magnetic-activated cell sorting; Mvh, mouse vasa homologue; PE, phycoerythrin; PGC, primordial germ cell; RT–PCR, reverse transcription–PCR; SSEA-1, stage-specific embryonic antigen-1; ZP3, zona pellucida 3; β-FGF, β-fibroblast growth factor; VSEL, very small embryonic-like.
We have isolated pluripotent SSEA-1 (stage-specific embryonic antigen-1) positive stem cells from an adult tissue, mouse bone marrow, and followed their differentiation into germ cell lineage/PGCs.

2. Materials and methods

2.1. Collection of bone marrow cells

Bone marrow was collected from the femur and tibia of 2–4-week-old C57BL/6 mice (Pasteur Institute, Iran) by vigorous flushing with DMEM (Dulbecco’s modified Eagle’s medium; Invitrogen). Mononuclear cells were by the protocol of Soleimani and Nadri (2009). Erythrocytes were eliminated using erythrocyte lysing solution (Dako).

2.2. Purification of SSEA-1 positive cells from bone marrow

MACS (magnetic-activated cell sorting) was used to separate suspension of murine BMNMCs (bone marrow mononuclear cells) according to the expressed cell surface marker, SSEA-1. BMNMCs were resuspended in cold MACS buffer (Miltenyi Biotec) and incubated with microbead-conjugated anti-SSEA-1 antibody (Miltenyi Biotec) for 45 min at 4°C on a plate-shaker. The cells were washed three times with MACS buffer. For sorting and selection of SSEA-1+ cells, labelled cells were loaded on to a sterile LS column (Miltenyi Biotec) installed in a magnetic field (Miltenyi Biotec). The column was rinsed several times with MACS buffer and negative unlabelled cells (flow-through or negative fraction) were passed through and collected. Trapped cells (positive or labelled fraction) were eluted with MACS buffer after removal of the column from the magnetic field. To achieve higher purity, the positive fraction was reloaded on to a second column and positive selection repeated, with the sample finally being collected by centrifugation. Viability and purity of isolated SSEA-1+ cells were evaluated by Trypan Blue (Sigma) exclusion and flow-cytometric analysis (FACSCalibur™, Becton Dickinson) respectively.

2.3. Cultivation of purified SSEA-1 positive cells

Bone-marrow-derived SSEA-1+ cells were cultured on a mitomycin C-inactivated feeder layer cells, C2C12 cells (Pasteur Institute), in DMEM/F12 (Invitrogen) supplemented with 20% FBS (fetal bovine serum), non-essential amino acids (Invitrogen), 50 μM 2-mercaptoethanol (Invitrogen), 2 mM L-glutamine (Invitrogen) and 103 units/ml LIF (leukaemia inhibitor factor) (Sigma) at 37°C in an air-5% CO2 incubator. SSEA-1+ cells were cultured in parallel with the same medium.

2.4. RNA isolation and RT–PCR analysis

SSEA-1+, SSEA-1− and BMP4-treated SSEA-1− cells were washed and immediately transferred to microtubes containing TRIzol® reagent (Qiagen). Tubes were vortexed to lyse the cells and kept at −20°C until analysis. Total RNA was extracted using the Nucleospin RNA II kit (Macherey-Nagel). DNase treatment was given to clean up possible genomic DNA contamination. cDNA synthesis was done with RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas). A sample of 2.5 μl of cDNA, 1 × PCR buffer (AMS TM, Sinagen), 200 μM dNTPs, 1.5 μM MgCl2, 0.5 μM of each of forward and reverse primers and 1 unit Taq DNA polymerase (Fermentas) was used to prepare reaction mixtures for PCR. PCR was performed with an optimized programme for each primer. Amplified DNA fragments were electrophoresed on 2% agarose gel. The gels were stained with ethidium bromide (10 μg/ml) and the amplified fragments photographed under UV transilluminator (Uvidoc). GAPDH (glyceraldehyde-3-phosphate dehydrogenase) amplification was used as an internal gene control. Negative controls were mock RT (reverse transcription) with DEPC (diethyl pyrocarbonate)-treated water instead of RNA or cDNA samples. RNA from embryonic stem cell line, CGR-8, and germ cells was used as positive control. To avoid the possibility of amplifying contaminating DNA and unprocessed mRNA, primers (see Table 2) were designed as exon–exon junctions.

2.5. Alkaline phosphatase activity

Alkaline phosphatase activity was assessed for colonies formed from cultured SSEA-1+ stem cells on the feeder layer. The cells were washed with PBS and fixed with 10% formaldehyde for 10 min at 4°C. They were washed twice with 0.2 M Tris buffer (pH 8.9) and the activity was measured with freshly prepared substrate reagent (0.01% naphthol-AS-MX phosphate and 0.06% Fast Violet salt in 0.1 M Tris buffer, pH 8.9), which was released after incubation for 30 min at 37°C. Cells were washed with H2O, mounted and observed for red dye development under bright field microscopy.

2.6. Differentiation assays

2.6.1. Induction of SSEA-1 positive cells to differentiate into Schwann cells

Bone-marrow-derived SSEA-1+ cells were cultured along with feeder layer cells (C2C12) in DMEM/F12 (Invitrogen) containing 1 mM 2-mercaptoethanol (Invitrogen) without serum for 1 day. The culture medium was replaced with DMEM/F12 containing 10% FBS and 35 ng/ml RA (retinoic acid) (Sigma). Three days later, cells were finally transferred to inducer medium containing DMEM/F12, 10% FBS and trophic factors 5 μM FSK (forskolin; Calbiochem), 10 ng/ml β-FGF (β-fibroblast growth factor; Peprotech), 5 ng/ml PDGF (platelet-derived growth factor)-AA (Peprotech) and 200 ng/ml heregulin-b1-EGF (epidermal growth factor)-domain (HRG) (R&D systems) and cultured for 10 days. Differentiating medium was changed every second day.

2.6.2. Induction of SSEA-1 positive cells to differentiate into osteoblasts

Purified SSEA-1+ cells were cultured along with feeder layer cells (C2C12) and differentiated with osteogenic medium consisting of

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DMEM/F12 with 10% FBS, 100 μg/ml ascorbate, 0.1 mM dexamethasone and 10 mM β-glycerophosphate for a period of 3 weeks. Differentiating medium was changed every second day.

### 2.6.3. Induction of SSEA-1 positive cells to differentiate into pancreatic cells

To induce sorted SSEA-1+ cells to differentiate into pancreatic cells, the cells were cultured along with feeder layer cells (C2C12) in DMEM/F12 medium with 4 mM L-glutamine, 4.5 g/l glucose, 1% FBS and 50 ng/ml of recombinant human Activin A (R&D Systems). After 2 days, the medium was changed and induction was carried out in DMEM/F12 medium with 4 mM L-glutamine, 4.5 g/l glucose, 5% FBS in the presence of 10 mM nicotinamide (Stem Cell Technologies). Differentiating medium was replaced every second day.

### 2.6.4. Generation of Mvh positive cells/PGCs from SSEA-1 positive cells

Bone-marrow-derived SSEA-1+ cells cultured on feeder layer cells were induced to differentiate toward PGCs by adding 20 ng/ml BMP4 (Millipore) to the medium for 4 days. BMP4 was added every day and the medium was replaced every second day.

### 2.6.5. Flow-cytometric analysis

Flow-cytometric analysis was carried out on purified SSEA-1+ cells before and after cultivation and differentiation by the standard procedure. Briefly, cells were washed and suspended in PBS containing 0.5% BSA followed by incubation with the optimal concentrations of PE (phycoerythrin)-conjugated anti-SSEA-1 (BD Pharmingen) and rabbit anti-Mvh (Abcam) for 45 min at 4°C. Labelled cells were processed directly after being washed. In the case of a second marker, cells were washed with PBS–BSA and incubated with PE-conjugated anti-rabbit IgG (Abcam) for 20 min at 4°C. Cells were washed as mentioned above and analysed by flow cytometry (FACScalibur™, Becton Dickinson) using WinMDI 2.8 software (J. Trotter 1993–1998, U.S.A.). Appropriate isotype-matched controls (BD Pharmingen and Abcam, eBioscience) were used for each primary antibody.

### 2.6.6. Immunostaining

Cells were cytospinned on to glass slides and allowed to dry for 15 min. Cells were then washed with PBS and fixed with 4% ice-cold paraformaldehyde for 10 min. In the case of intracellular markers, permeabilization by 0.4% Triton X-100 (Sigma) was performed as the next step. Non-specific binding sites were blocked by incubation of cells with 10% serum containing secondary antibody species. Immunostaining procedure was the same as for flow cytometry, except that cells were incubated with optimal dilutions of primaries overnight and with secondaries for 1 h. Briefly the cells were stained with antibodies to SSEA-1 (1:400, PE-conjugated anti-SSEA-1, BD Pharmingen), Oct4 (1:500, unconjugated anti-Oct4; Abcam) and Mvh (1:200, rabbit anti-Mvh; Abcam). Appropriate secondary antibodies, FITC-conjugated anti-goat IgG (1:500; Abcam) and PE-conjugated anti-rabbit IgG (1:300; Abcam) were used. After three washes with PBS, nuclei were counterstained with DAPI (4′,6-diamidino-2-phenylindole; Roche). Slides were mounted with 50% (v/v) glycerol in PBS and checked under an Olympus BX 60 fluorescent microscope. For negative controls, equivalent concentrations of normal immunoglobulin of the primary species were used instead of primary antibodies. For SSEA-1 staining, primary antibody was substituted by irrelevant PE-conjugated isotype-matched antibody.

### 3. Results

#### 3.1. Bone marrow as a home of pluripotent SSEA-1 positive cells

SSEA-1+ cells isolated from mononuclear cells suspension using MACS system comprised 0.02% of the total population. To check the purity of SSEA-1+ cells, flow-cytometry analysis was done immediately after sorting. SSEA-1+ cells accounted for 55 ± 7% of the labelled fraction of MACS system (Figure 1). Further evaluation after 3 weeks culturing (3 passages) of sorted cells over feeder layer cells (C2C12, murine sarcoma cells) under undifferentiated

![Figure 1](image_url)  
**Figure 1** Flow-cytometric analysis of bone-marrow-derived SSEA-1+ cells revealed purity of approximately 55 ± 7% (a) which increased to 87 ± 4% (b) after cultivation over feeder layer cells.
condition gave a higher purity (87 ± 4%) of SSEA-1+ cells (Figure 1).

Expression of specific transcripts of pluripotent stem cells was assessed by RT–PCR with reference to an established pluripotent murine ESC line, CGR-8. In contrast with the SSEA-1 negative fraction, SSEA-1 positive cells expressed markers showing pluripotency properties, including Oct4, SSEA-1, Nanog, Dppa3 (Stella), fragilis, Rex-1, SOX-2 and ALP (alkaline phosphatase; Figure 2a). Immunofluorescence staining showed that purified SSEA-1+ cells expressed ESC markers Oct4 and SSEA-1 at the protein level (Figure 2b).

To confirm the pluripotency capability of SSEA-1+ cells purified from bone marrow, these cells were differentiated in vitro into all three germ layer derivatives. Purified SSEA-1+ cells were co-cultured with feeder layer cells (C2C12) in differentiating media of Schwann-like cells (ectoderm), osteoblasts (mesoderm) and pancreatic cells (endoderm). RT–PCR results showed that bone-marrow-derived SSEA-1+ cells could differentiate into Schwann-like cells [P75, S100 and GFAP (glial fibrillary acidic protein)], osteoblasts (fibronectin, Runx2 and Osx) and pancreatic cells (insulin, glucagon and Sox17), i.e. the different cells of three germ layers, a characteristic shared by all pluripotent stem cells (Figure 3).

3.2. Clonogenicity of bone-marrow-derived SSEA-1 positive cells

Cultured SSEA-1+ cells on a gelatinized dish only produced small aggregations and irregular sphere structures (Figure 4a). The cells survive in vitro for few days before starting to degenerate (Figure 4b). Purified SSEA-1+ cells could proliferate and form structures such as embryoid bodies when plated over feeder layer cells (C2C12, murine sarcoma cells) (Figure 4c). ALP activity showed positive colonies with strong and uniform ALP staining (Figure 4d).

3.3. Induction of SSEA-1 positive cells to differentiate into germ cell lineage/PGCs

Bone marrow may already contain PGCs. To test this hypothesis, the expression of germ cell markers – particularly some PGCs and

Figure 2  Expression of pluripotency markers in SSEA-1+ cells

(a) Gene expression analysis of pluripotency markers in bone-marrow-derived SSEA-1+ cells. Expression of specific transcripts for pluripotency markers in SSEA-1+ and SSEA-1- cells were assessed by RT–PCR. CGR-8, an established mouse ESC line, was used as a positive control. GAPDH amplification served as internal control. (b) Immunostaining of pluripotency markers in SSEA-1+ cells. Expression of Oct4 (green) and SSEA-1- (red) in purified SSEA-1+ cells were evaluated by immunofluorescence staining. Nuclei were counterstained with DAPI (× 400).
germ-cell-specific markers such as DAZL (deleted in Azoospermia-like), Mvh, c-kit, stra8 and SYCP3 – were investigated in negative flow-through and positive-labelled fractions after MACS. RT–PCR data showed both fractions were negative for germ cell markers (Figure 5a).

To test the ability of bone-marrow-derived SSEA-1+ cells to differentiate into germ line cells, BMP4 was added to the culture medium. Gene expression of germ cell markers related to different stages was assessed (see Table 1). The differentially expressed genes were c-kit, DAZL and Mvh (Figure 5b). Common markers between ESCs/bone marrow-derived pluripotent SSEA-1+ cells and germ line cells, such as Oct4, Nanog, SSEA-1, ALP, Stella and Fragilis, were expressed in BMP4-treated cells (Figure 5b).

To explore whether SSEA-1+ cell-derived germ cells could undergo also further differentiation in vitro, expression of known molecular markers of pre-meiotic (Stra8, Piwil2 and SYCP3) germ cells were analysed. The characteristics of differentiated cells clearly demonstrated that in vitro derived germ cells are comparable to PGCs. In vitro derived PGCs could not further differentiate along germ cell lineage by the impression of BMP4 (Figure 5b). More evidence to confirm PGCs development from bone-marrow-derived SSEA-1+ cells and prove sexually undetectable differentiated germ cells was provided by assessing male and female meiotic and post-meiotic germ cell markers [Pgk2, TP2 (transition protein 2), GDF9 (growth differentiation factor 9) and ZP3 (zona pellucida 3)] (Figure 5b).

To confirm the effect of BMP4 on the differentiation of bone-marrow-derived SSEA-1+ cells into PGCs, immunofluorescence (Figure 6a) and flow-cytometric (Figure 6b) analyses were carried out for Mvh. Figure 6(a) shows that Mvh was expressed cytoplasmically in SSEA-1+ derived germ cells.

### 4. Discussion

Germ cells are the only cells to meiotically divide and halve their genetic material in generating haploid cells (Tedesco et al., 2009). In the female mice, germ cells begin meiosis during fetal development, whereas in the male the onset of meiosis is postponed until after birth. The attainment of fertilization and...
infertility in both male and female animals requires the proper development and functioning of gametes (oocyte and sperm). The factors that operate to interfere with any of gametogenesis processes can disturb the normal pattern and lead to infertility (Surani, 2001; Hajkova et al., 2002). The possibility of generating germ cells (PGCs/gametes) from stem cells and other sources instead of gonads is an exciting area of investigation, since it may contribute to our understanding of gametogenesis and give new methods for the treatment of sterility by overcoming gamete donation problems (Tilgner et al., 2008).

Since PGCs are the first specialized cells that appear in gametogenesis process which initiate meiosis to give rise to the well-developed germ line cells, the process that forms the gametes (Kimura and Nakano, 2011), derivation of PGCs from stem cells in vitro would be of value in producing them. We were able to purify pluripotent SSEA-1+ stem cells from mice bone marrow using the MACS system and could successfully differentiate them into PGCs by adding BMP4 to the culture medium. PGC formation in vitro could be a first step in differentiating stem cells into more developed gametes and inducing them to initiate meiosis. Toyooka et al. (2003) obtained Mvh+ cells (PGCs) from mouse ESCs through EB (embryoid body) formation, which was greatly enhanced by the inductive effect of BMP4-producing cells (Eguizabal et al., 2009; Park et al., 2009). The inductive effect of BMP4, notably BMP4, to induce hESCs to differentiate into germ line cells; up-regulation in expression of Mvh was observed in human ESCs during EB formation and confirmed the emergence of a PGC population in vitro. The differentiated cells did not necessarily enter meiosis. In addition to the differentiation of pluripotent ESCs into PGCs reported in other studies (Wei et al., 2008; Bucay et al., 2009; Eguizabal et al., 2009; Park et al., 2009), investigators could show the first PGC-like cell generation from the induced differentiation of skin-derived stem cells (Linher et al., 2009). Mazaheri et al. (2011) also showed the expression of Mvh, a PGC-specific marker, in the bone-marrow-derived mesenchymal stem cells in the presence of BMP4 in the culture medium. There was no more evidence to prove PGC derivation from mesenchymal stem cells in their study (Mazaheri et al., 2011). Our immunostaining and gene expression analyses confirmed the pluripotency characteristic of highly purified SSEA-1+ cells from mouse bone marrow. Other data showed that SSEA-1+ cells express markers such as ALP, Stella and fragilis that are the same in ESCs and PGCs. Purification of a very rare population of VSEL (very small embryonic-like) stem cells from murine bone marrow and other adult tissues and organs (brain, liver and kidney) showed that VSEL cells are 0.02–0.03% of total bone marrow nucleated cells and express many pluripotency markers such as Oct4, Nanog, Rex-1 and SSEA-1 and differentiate into cells belonging to three germ layers in an appropriate media (Kucia et al., 2006; Ratajczak et al., 2009a). We obtained similar results regarding the ability of highly purified SSEA-1+ stem cells to differentiate into various cells belonging to the three germ layers. In addition to pluripotency markers and some common markers of ESCs and PGCs (such as fetal-type ALP, Stella and fragilis), it has been shown that VSEL stem cells express Mvh, a specific marker of PGCs (Ratajczak et al., 2009b). The existence of special stem cells in bone marrow that express germ cell markers has also been claimed by Johnson et al. (2005). In contrast with a basic dogma of reproductive biology that most mammalian female lose the capability of germ cell production before or immediately after birth, these authors proposed that bone marrow contains progenitors for oocytes in adult ovarian tissue and that bone marrow transplant helps to preserve or recover ovarian function of recipient females in mice (see also Lee et al., 2007). Eggan et al. (2006) challenged these
findings and clearly showed that there is no proof for the contribution of bone-marrow-derived germ cells or any other normally circulating cells to the formation of oocytes. Bone-marrow-derived pluripotent SSEA-1+ stem cells in our study also failed to express Mvh, a specific PGC marker that is specifically expressed throughout germ cell development. Mvh (DDX4) is a member of the DEAD-box family of genes encoding an ATP-dependent RNA helicase, which is specific for differentiating germ cells. Negative results from the analysis of expression of pluripotency and germ cell markers in SSEA-1 negative cells also confirmed the non-existence of germ cell population (BMNCs). Since PGCs originating from pluripotent epiblast cells cannot give rise to other cell types and are described as ‘nullipotent’ or ‘monopotent’ germ line cells (Donovan and de Miguel, 2003; Durcova-Hills et al., 2008), and because differentiation of pluripotent SSEA-1+ stem cells into cells of three germ layers can occur as shown in our study, it can be concluded that the purified cells have not entered into germ cell lineage yet. That they are able to differentiate into germ cells in response to an exogenous growth factor such as BMP4 in vitro or probably an appropriate stimulation in vivo. SSEA-1+ cells in bone marrow and inductive effect of BMP4 to differentiate the pluripotent stem cells into germ cells showed that bone marrow is a potential source for extragonadal gametes and can supply effective cells to migrate

<p>| Table 1 Gene expression pattern in BMP4-treated SSEA-1+ cells |</p>
<table>
<thead>
<tr>
<th>Stage</th>
<th>Genes</th>
<th>BMP4-treated SSEA-1+ cells</th>
<th>Untreated SSEA-1 cells</th>
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<tr>
<td>Pre-migratory PGCs</td>
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<tr>
<td></td>
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<td>Fragi1s</td>
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<td>Migratory PGCs</td>
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<td></td>
<td>DAZL</td>
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<td>-</td>
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<tr>
<td>Post-migratory PGCs</td>
<td>Mvh</td>
<td>+</td>
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<tr>
<td>Pre-meiotic</td>
<td>Stra8</td>
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<td></td>
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<tr>
<td></td>
<td>SYCP3</td>
<td>+</td>
<td>-</td>
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<tr>
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<td></td>
<td>TP2 (male)</td>
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<p>| Table 2 Primers used for RT–PCR analysis |</p>
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<tr>
<th>Genes</th>
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towards gonads affected in response to an appropriate stimulatory signal. There are two reports indicating the possibility of PGC formation from somatic stem cells (Linher et al., 2009; Mazaheri et al., 2011) and the existence of different cell population within somatic stem cells (Zuba-Surma et al., 2009), but it is not clear what is the real source and origin of differentiated cells. Our study reports the new finding therefore of the differentiation of PGCs from well-defined pluripotent stem cells derived from an adult tissue/organ, bone marrow. Using adult tissue derived pluripotent stem cells can overcome the difficulties of ESC application and introduce novel possibility in the stem cell research.

In conclusion, high potential of bone-marrow-derived SSEA-1$^+$ cells to differentiate into PGCs, make this cell population a very attractive therapeutic alternative to ESCs in regenerative medicine for in vitro development of functional gametes. In vitro generated germ cells could represent a desirable model and potential strategy for treating infertility. It is obviously clear that stem cell-derived germ cells also provide an in vitro model for the study of germ cell development, the associated epigenetic modification and mammalian gametogenesis.

**Author contribution**


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**Figure 6** Expression of germ cell-specific marker in BMP4-treated cells (a) Immunostaining of Mvh, a specific PGC marker in BMP4-treated SSEA-1$^+$ cells. Mvh was expressed cytoplasmically in SSEA-1$^+$ derived germ cells ($\times$ 400). (b) Flowcytometric analysis of Mvh, a specific PGCs marker in BMP4-treated SSEA-1$^+$ cells.

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**References**


