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Comparison of Human Amniotic, Chorionic, and Umbilical Cord Multipotent Mesenchymal Stem Cells Regarding Their Capacity for Differentiation Toward Female Germ Cells

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Abstract

Placenta harbors a plentiful source of various cells with stem cells or stem-like cell properties, which can be used in therapeutic procedures and research. Mesenchymal stem cells (MSCs) have attracted much attention due to their specific differentiation potential and tolerogenic properties. MSCs have been isolated from different parts of placenta; however, in this study, we isolated MSCs from amnion and chorion membrane, as well as umbilical cord (Wharton's jelly [WJ]) and compared their capacity regarding differentiation toward female germ cells under influence of 10 ng/mL BMP4. All placenta samples were collected from delivering mothers by normal cesarean section and cells were isolated by different methods. Results showed that all isolated cells were mostly positive for the MSC markers CD73, CD166, and CD105, and minimally reacted with CD34 and CD45 (hematopoietic markers). After differentiation induction using third passage cultured cells, immunocytochemistry staining showed that cells were positive for germline cell-related genes *Ssea4, Oct4,* and *Ddx4,* and oocyte-related gene *Gdf9*. RT-qPCR results indicated that human chorion MSCs (hCMSCs) had a greater potential to be differentiated into female germline cells. Moreover, the results of this study indicate that human umbilical cord MSCs originated from either male or female umbilical cord have the same differentiation potential into female germline cells. We recommend that for presumptive application of MSCs for infertility treatment and research, hUMSCs are best candidates due to their higher differentiation potential, ease of proliferation and expansion, and low immunogenicity.

Keywords: umbilical cord, germ cells, mesenchymal stem cells, differentiation potential, oocyte, amnion membrane

Introduction

The first organ formed in human life is placenta (Schmidt, 1992). It has been established that placenta, the tissue routinely discarded postpartum, harbors various cells with stem cells or stem-like cell properties (Fauza, 2004; Matikainen and Laine, 2005). Placenta-derived stem cells include mesenchymal (Fukuchi et al., 2004), hematopoietic (Amos and Gordon, 1995), trophoblastic, and multipotent/pluripotent stem cells (Miki et al., 2005; Tamagawa et al., 2004; Yen et al., 2005).

Embryonic and adult stem cells have potential clinical applications in the repair of tissue damage caused by diseases such as diabetes, neurodegenerative disorders, ischemia and spinal cord injury, and for infertility management in patients undergoing cancer chemotherapy. However, among the stem cells with therapeutic potential, mesenchymal stem cells (MSCs) have attracted much attention due to their specific differentiation potential and tolerogenic properties. MSCs have been isolated from different tissues, including bone marrow (Pittenger et al., 1999), umbilical cord blood (Erices et al., 2000, 2003), peripheral blood (Villaron et al.,

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2004), and adipose tissue (Rodriguez et al., 2005; Zuk et al., 2001), but mesenchymal stromal/stem cells isolated from fetal membranes (amnion and chorion) are particularly useful because of their fewer ethical dilemmas, availability, abundant source, and noninvasive isolation procedure (Matikainen and Laine, 2005; Miki and Strom, 2006; Parolini and Soncini, 2006).

Over this decade, different independent research groups worldwide have reported successful isolation of MSCs from various placental components such as unfractionated placenta (Miao et al., 2006; Yen et al., 2005; Zhang et al., 2004), villous stroma of the paraumbilical area (Wulf et al., 2004), internal area of placental lobules (Fukuchi et al., 2004), amniotic and chorionic membranes (Bailo et al., 2004), amniotic fluid (De Coppi et al., 2007), Wharton's jelly (Mitchell et al., 2003), deciduas parietalis (Scherjon et al., 2004), decidua basalis (Scherjon et al., 2004), and chorionic villi (Zhang et al., 2006).

One of main targets for the clinical application of stem cells is infertility treatment and many research groups are investigating on the differentiation potential of stem cells into germ cells (Marques-Mari et al., 2009). Germ cells are highly differentiated and specialized cells in mammalian developmental biology, which contribute to fertilization process and retain their pluripotent properties, which give rise to a new individual.

Over the past years, there have been several reports on the successful derivation of germ cells from different stem cell sources (Marques-Mari et al., 2009; Nagano, 2007), including human umbilical cord, Wharton's jelly-derived MSCs (Huang et al., 2010), embryonic stem cells (Hübner et al., 2003; Lacham-Kaplan et al., 2006; Toyooka et al., 2003), fetal- and adult-derived induced pluripotent stem cells (Panula et al., 2011), bone marrow stem cells (Nayernia et al., 2006), fetal skin stem cells (Dyce and Li, 2006), bone marrow and peripheral blood (Johnson et al., 2005), pancreatic stem cells (Danner et al., 2007), and recently from human menstrual blood-derived endometrial MSCs (Lai et al., 2016).

Although presumptive gametes derived from these stem cells have similar molecular aspects to true gametes, their accurate functions still need to be demonstrated. The abovementioned stem cells have different potentials to differentiate into germ cells, for instance, some studies indicated that Wharton's jelly-derived MSCs have a greater potential to differentiate into germ cell lineages (Amidi et al., 2015; Carlin et al., 2006). In this study, we compared the differentiation potential of MSCs into female germ cells from three different sources; human umbilical cord (Wharton's jelly) and amniotic and chorionic membrane. We intend to determine which of the stem cells, that is, human umbilical cord MSCs (hUMSCs), human amniotic MSCs (hAMSCs), and human chorionic MSCs (hCMSCs) has a greater potential to differentiate into female germ cells.

Material and Methods

Collection of human umbilical cords and placentas

Human sampling was approved by the Tehran University of Medical Sciences ethics committee, under signed informed consent form. In this study, to reduce contamination risk, all human samples were collected from delivering mothers by normal cesarean section performed by the faculty of the Department of Obstetrics and Gynecology, Mohebe Yas Hospital, Tehran University of Medical Sciences. Then, samples were transferred into sterile phosphate-buffered saline (PBS) from the hospital to laboratory on ice in a container ($n = 10$). In the laboratory, the samples were immediately washed several times with sterile PBS. Amniotic and chorionic membranes were mechanically peeled off from each other by blunt dissection. The arteries and veins of umbilical cords were also removed and the remaining tissue, Wharton's jelly, was transferred into a sterile Petri dish containing PBS.

Isolation and culture

Enzymatic digestion was performed for the isolation of stem cells from amniotic and chorionic membranes. The human samples were cut into small 3 cm fragments and washed thrice with sterile PBS. All fragments were transferred into two 15 mL centrifugation tubes containing 400 U/mL collagenase IV (Worthington) and 1 mg/mL DNase I (Sigma), and digestion was carried out in a shaking water bath at 37°C for 20 minutes. Cell suspension was filtered through, first, a 100 μm cell strainer (Thermo Fisher Scientific, Waltham, MA) and then a 40 μm cell strainer (Thermo Fisher Scientific) to remove contaminating tissue debris, and centrifuged for 5 minutes in 300 g.

The supernatant was discarded and cells were resuspended in DMEM supplemented with 10% FBS, 1% penicillin and streptomycin mixture, and 1% GlutaMAX (Gibco), and seeded in 25 cm^2 flask in the culture medium mentioned above. Explant method was used for the isolation of stem cells from umbilical cord as described below. Amniotic membrane, arteries, and veins were removed from the umbilical cord and the remaining Wharton's jelly was minced into fine pieces of 1 mm^2. Wharton's jelly fragments were explanted into 25 cm^2 flask in the culture medium mentioned above and maintained at 37°C humidified incubator with 5% CO_2.

Fragments were left undisturbed for 3 weeks to allow migration of cells from the explants. At confluency of 80%–90%, cells were harvested by 0.05% Trypsin-EDTA solution (Gibco) and replated at a ratio of 1:3. At each passage, a small portion of cells was frozen in a cryomedium containing DMED medium supplemented with 10% DMSO (Sigma) for future evaluation. Differentiation studies were performed on cells from passages 3 to 4.

Flow cytometry analysis

For flow cytometric analysis of cell surface marker, 1×10^5 cells from passages 3 to 4 were used for a panel of MSC antibodies. Cells were dispersed with 0.25%- trypsin-EDTA (Gibco) and resuspended in PBS supplemented with 0.5% FBS. The cells were then aliquoted into several parts and incubated at 4°C for 20 minutes in dark, with monoclonal antibodies (All antibodies are from Dako and BD) against the hematopoietic cell markers CD34-PE and CD45-FITC, and MSC markers CD73-PE, CD166-PE, and CD105-FITC. Negative control samples were incubated with mouse IgG1-FITC/PE isotype antibodies to help differentiate nonspecific background signals from specific antibody signals. The samples were analyzed on a Partec cytometer (German) and the resulting data were processed using FloMax software.

Differentiation induction into female germ cells

In this study, cells from third passage were used for differentiation induction studies. Cells were seeded at a density of 2×10^5 per well in 48-well plates and were treated with 10 ng/mL bone morphogenetic protein 4 (BMP4) (R&D system) in the above-mentioned medium for 21 days. Half of the medium was replaced every 3–4 days. Cells were observed for morphological changes during 21 days of induction, after which immunocytochemistry and RT-qPCR studies were performed.

Immunocytochemistry

For Immunocytochemistry analysis of the specific germ cell markers SSEA4, OCT4, DDX4, and GDF9, 2.5×104 cells from three different sources and at third passage were cultured on 4-well plates treated with 10 ng/mL BMP4 for 21 days. After 21 days of induction, the cells were washed twice with sterile PBS and fixed in 4% paraformaldehyde in PBS for 20 minutes at room temperature. For intracellular marker analysis, the membrane was permeabilized with a 0.1% Triton X-100 solution in PBS for 20 minutes.

Nonspecific binding sites were blocked with 5% goat serum for 45 minutes without washing, followed by incubation with anti-C-kit (Rabbit polyclonal anti-human, 1:100; Abcam), anti-SSEA4 (Mouse monoclonal anti-human, 1:100; Abcam), anti-DDX4 (Rabbit polyclonal anti-human, 1:100; Abcam), and anti-GDF9 (Rabbit polyclonal anti-human, dilution 1:200; Abcam) antibodies overnight at 4°C. The cells were then washed with PBS and incubated with FITC-conjugated Goat Anti-Rabbit (1:50; Abcam) or Goat Anti-Mouse (1:200; Abcam) for 1 hour at room temperature. Nuclei were counter-stained with DAPI (Sigma) for 5 minutes and observed using a fluorescent microscope (Olympus, IX71).

RT-qPCR (quantitative transcription polymerase chain reaction)

To isolate whole RNA, Tri-Pure reagent (Roche) was directly added to the cells and processed as per manufacturer's instructions. Genomic DNA contamination was removed by digestion using RNase-free DNase I (Thermo Scientific) for 30 minutes at 37°C. RNA concentration and purity were determined using spectrophotometric method (WPA spectrophotometer, Biochrom, UK). RNA was reversely transcribed by random Hexamer and 1000 ng of DNA-free RNA using a Transcriptor First Strand cDNA Synthesis kit (Roche). TaqMan® Gene Expression Assays (Life Technologies) were used to study the expression of *Ssea4*, *Ddx4*, *Gdf9*, and *Zp3*, which normalized against 18 seconds

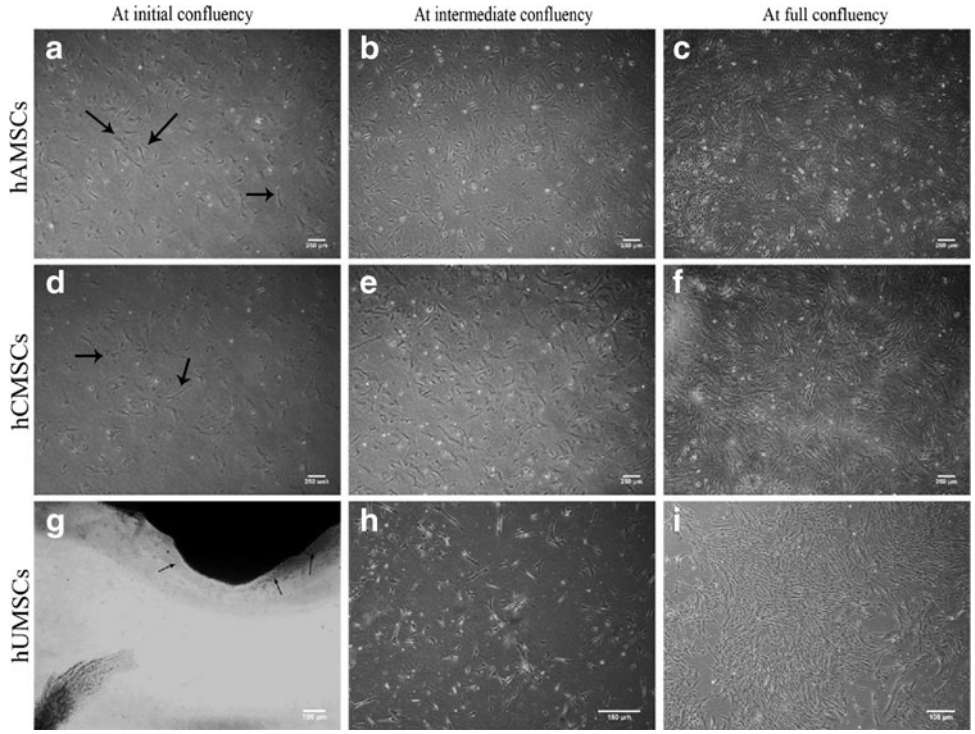

FIG. 1. Cell morphology of hAMSCs, hCMSCs, and hUMSCs at different confluency stages. All cells had a spindle-like morphology and in some cases possessed some long and short processes. **(a–c)** hAMSCs at different confluency stages. hAMSCs had a shorter doubling time and almost reached full confluency after 4–5 days following each subculture. Most hAMSCs had a spindle-like morphology and some found wide structure with some long and short processes (*black arrows*) at initial confluency. **(d–f)** hCMSCs at different confluency stages. hCMSCs had a spindle-like morphology and in some cases possessed some long and short processes (*black arrows*). Doubling time was longer in these cells and usually needed 6–7 days to reach full confluency. **(g–i)** hUMSCs at different confluency stages. hUMSCs migrated from tissue inserts after 3 weeks **(g**, *black arrows***)** and after each subculture needed 6–7 days to reach full confluency. hAMSCs, human amniotic mesenchymal stem cells; hCMSCs, human chorionic mesenchymal stem cells. Scale bar: 250 μm for **a–f**; 100 μm for **g** and **i**; 150 μm for **h**.

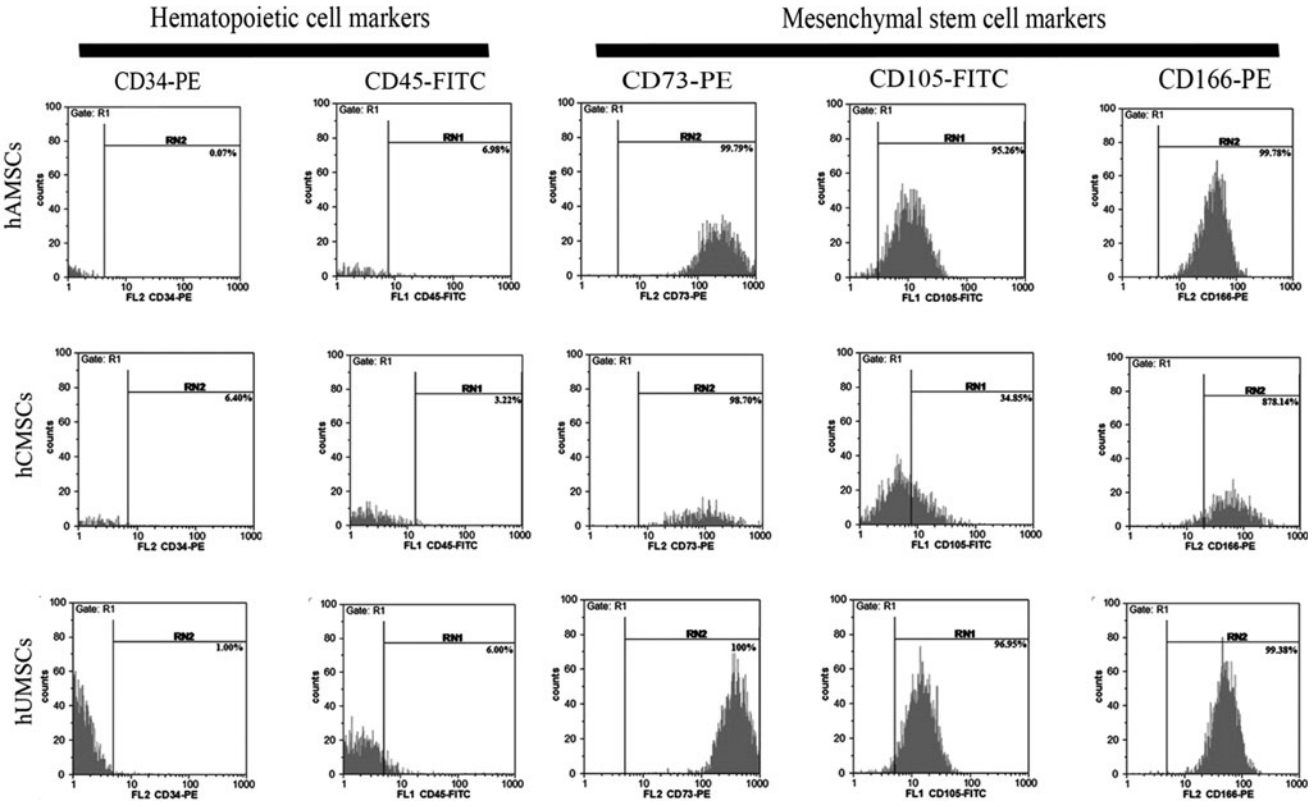

FIG. 2. Flow cytometry results of surface markers expression in isolated cells at third passage. The cells were mostly positive for the mesenchymal stem cell markers CD73, CD166, and CD105 and minimally reacted with CD34 and CD45 (hematopoietic markers). Data were representative of three independent experiments. Negative control samples were incubated with mouse IgG1-FITC/PE isotype antibodies to help differentiate nonspecific background signals from specific antibody signals.

expression as a housekeeping gene. PCR reaction components were mixed to obtain a final volume of 20 μL.

The following components were used: 10 μL TaqMan Universal Master Mix, 1 μL TaqMan Assay reagent, 0.5 μL (25 ng) cDNA, and 8.5 μL distilled water. PCR cycling parameters were set as follows: 10 minutes at 95°C (polymerase activation), then 40 cycles at 95°C for 15 seconds, and 60°C for 1 minutes using a Rotor-Gene Q instrument (Qiagen). Using $\Delta\Delta$Ct method, relative expression of targets was calculated by normalizing Ct values of targets against 18 seconds.

To examine whether hUMSCs originated from either male or female umbilical cord have the same differentiation potential, we compared the relative gene expression of the above-mentioned genes between hUMSCs originated from male and female umbilical cord.

Statistical analysis

All data are presented as mean ± SD (standard deviation) and were analyzed using Prism 5 software. One-way ANOVA followed by Bonferroni *posttest* were used for comparison between the groups. p-values <0.05 were considered statistically significant in this study.

Results

Cell characterizations

Cells from the three sources were successfully cultured up to 7 passages. We observed no significant changes in proliferation rate and morphology between cells from the three different sources. Proliferation rate was, however, relatively higher in hAMSCs so that they reached full confluency after 4–5 days following each passage (with doubling time estimated 24–36 hours) (Fig. 1a–c), whereas hCMSCs (Fig.1d–f) and hUMSCs needed 6–7 days to reach such confluency (with doubling time estimated 36–48 hours) after establishing the culture system. All cells from three sources had spindle-like morphology and in some cases possessed some long and short processes. In each passage, splitting rate was 1:3 and one third of each passage was freezed for future use with successfully reestablishing the culture system.

To establish hUMSCs, after 5–6 days from primary culture initiation, adherent fragments of Wharton's jelly were observed. Spindle-like cells started to migrate out from the Wharton's jelly fragments (Fig. 1h–j). After every 3–4 days, half of the medium was replaced until full confluency was attained. In this study, the time needed to reach complete confluency in the hUMSCs was 9–10 days.

Flow cytometry findings

To examine the multipotent potential of cultured cells at passage 3, we assessed the expression of a number of markers associated with ESCs using flow cytometry. The results depict MSC characteristics of the cells and rule out hematopoietic origin of the isolated cells (Fig. 2). The cells were mostly positive for the MSC markers CD73, CD166, and CD105, and minimally reacted with CD34 and CD45 (hematopoietic markers).

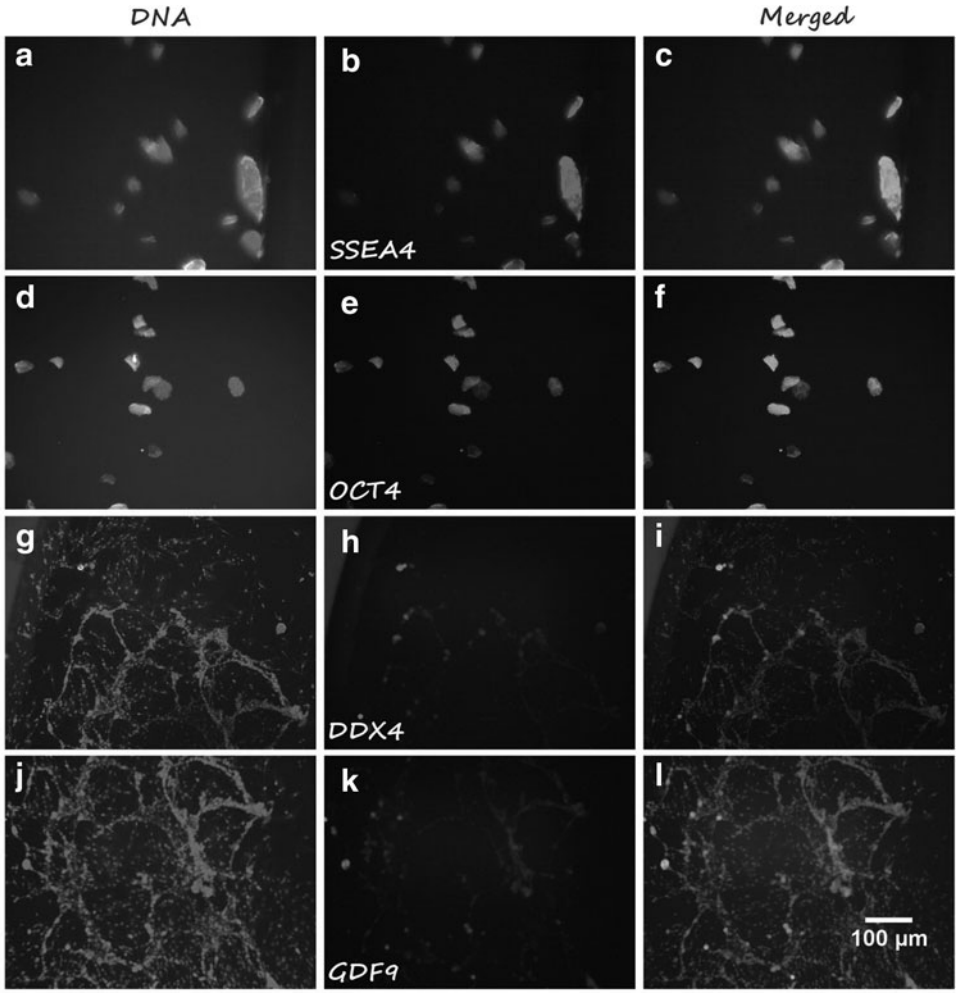

FIG. 3. Immunocytochemistry staining of hAMSCs for specific female germ cells and oocyte markers [Ssea4 (**a, b, c**); Oct4 (**d, e, f**); Ddx4 (**g, h, i**); and Gdf9 (**j, k, l**)] after 21 days differentiation induction. Nuclei were stained with DAPI (4′, 6′-diamidino-2-phenylindole) staining.

Morphological changes in cells after induction

After differentiation induction, the cells were treated with BMP4 for 21 days, and observed, each day, for any morphological changes under a phase-contrast microscope. In addition, one of the 24-well plates was left as negative control containing the basic medium. During this period, no significant morphologically important changes were observed, but some cells formed a tadpole-like shape. However, density of the treated cells was significantly lower than the negative control, which did not receive BMP4.

Immunocytochemistry staining of specific germ and oocyte markers

Twenty-one days after differentiation induction, the expression of germ cell-related genes *Ssea4*, *Oct4*, and *Ddx4* and oocyte-related gene *Gdf9* in all isolated cells, including hAMSCs (Fig. 3), hCMSCs (Fig. 4), and hUMSCs (Fig. 5), was detected using immunocytochemical analysis. The results of this experiment confirm the fact that all cells from the three different sources have a differentiation potential into germ- and oocyte-like cells; however, the quality and quantity of differentiation are dependent on the source of isolated cells. Differentiation potential was quantified using RT-qPCR.

RT-qPCR results after induction

RT-qPCR results show that all the cells expressed primordial germ cell- and oocyte-like cell genes after 21 days of induction. However, the quality and quantity of each expression were different among isolated cells from the three different sources (Fig. 6a). In this experiment, hCMSCs showed a greater potential to be differentiated into female germline cells: we observed a significantly higher relative gene expression level of *Zp3* and *Gdf9* genes in hCMSCs compared with the hAMSCs ($p \leq 0.05$).

In addition, the level of transcripts of *Oct4* and *Ddx4* genes in the hCMSCs was lower than in the other cells; however, the decrease was not significant ($p \leq 0.05$). These results show that hCMSCs may have a higher degree of differentiation, and their pluri/multipotent characteristics could be reduced under the influence of 10 ng/mL BMP4. The results of this study also show that hUMSCs have higher potential for differentiation into female germ cells compared with hAMSCs due to a higher relative expression of *Zp3* and *Gdf9* genes. However, higher expression of *Ddx4* in the hUMSCs indicates that these cells maintain more primitive germ cell characteristics.

Moreover, the results of this study indicate that hUMSCs originated from either male or female umbilical cord have the same differentiation potential into female germline cells,

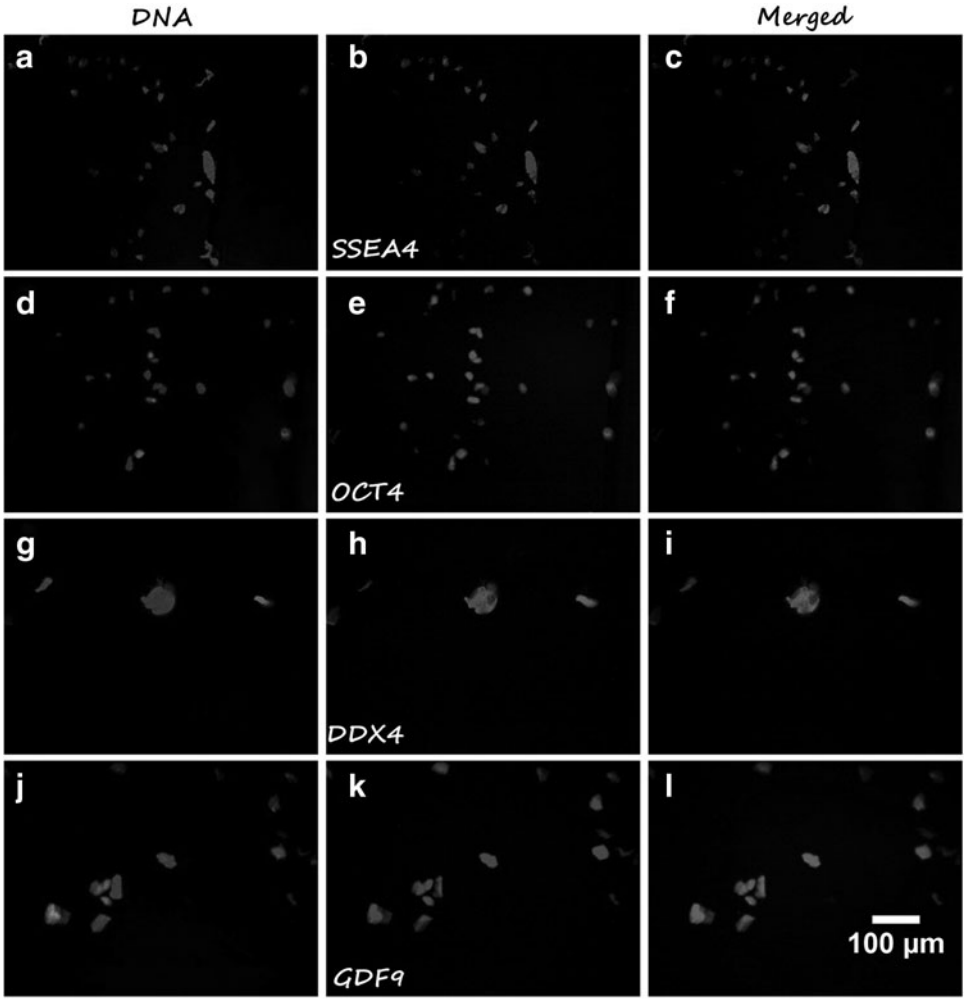

FIG. 4. Immunocytochemistry staining of hCMSCs for specific female germ cells and oocyte markers [Ssea4 (**a, b, c**); Oct4 (**d, e, f**); Ddx4 (**g, h, i**); and Gdf9 (**j, k, l**)] after 21 days of differentiation induction. Nuclei were stained with DAPI (4′, 6′-diamidino-2-phenylindole) staining.

and there was no significant difference between them regarding their relative germ- and oocyte-like gene expression (Fig. 6b).

Discussion

Presence of BMP4, a mesoderm inducer, during gastrulation is vital such that its expression is needed for the allocation of primordial germ cells (PGCs) and allantois (Fujiwara et al., 2001; Lawson et al., 1999). BMP4 is widely used as an inducer for the differentiation of stem cells from different sources into germline cells, especially PGCs (Amidi et al., 2015; Hamidabadi et al., 2011; Latifpour et al., 2014). In this study, we showed that human amniotic, chorionic, and umbilical cord multipotent MSCs have different capacities to differentiate into germline cells and oocyte-like cells. The results of our study show that hCMSCs have a higher capacity to differentiate into germ cells. Differentiation potential of hCMSCs into germ-like cells was higher than the other two MSCs, and after induction, they gained a higher degree of differentiation compared with the other cell groups.

It has been established that quantitative expression of *Oct-3/4* defines differentiation, dedifferentiation, or self-renewal of ES cells (Niwa et al., 2000). Various studies have indicated that relative gene expression of pluripotency-related genes is reduced following differentiation of stem cells. For instance, in a study conducted by Filliers et al. (2012), it was demonstrated that the level of transcript of *Oct4* was significantly decreased in *in vitro* blastocysts. Some studies indicated that *Oct4* might be a specific gene marker for totipotency or a gene required for totipotency (Deyev and Polanovsky, 2004; Pesce and Schöler, 2001). Tai et al. (2005) also reported that *Oct4* gene and protein are expressed in several adult pluripotent stem cells, as well as several human and rat tumor cells, but not in normal differentiated daughters of these stem cells.

There have been many researches showing that germ cells can be generated from ESCs and somatic stem cells under a suitable culture medium (Danner et al., 2007; Dyce and Li, 2006; Johnson et al., 2005; Nayernia et al., 2006; Parvari et al., 2015); however, no standard culture system has been defined for the expansion of germline progenitor cells and their differentiation into mature gametes *in vitro* (Parvari et al., 2016; Yazdekhasti et al., 2016; Childs et al., 2008).

In our previous study, we showed that hUMSCs are able to differentiate into germ-like cells under coculture condition with placental cells. We suggested that placental cells (chorion or amnion) supplemented with transforming growth factor (TGF α, β) and basic fibroblast growth factor

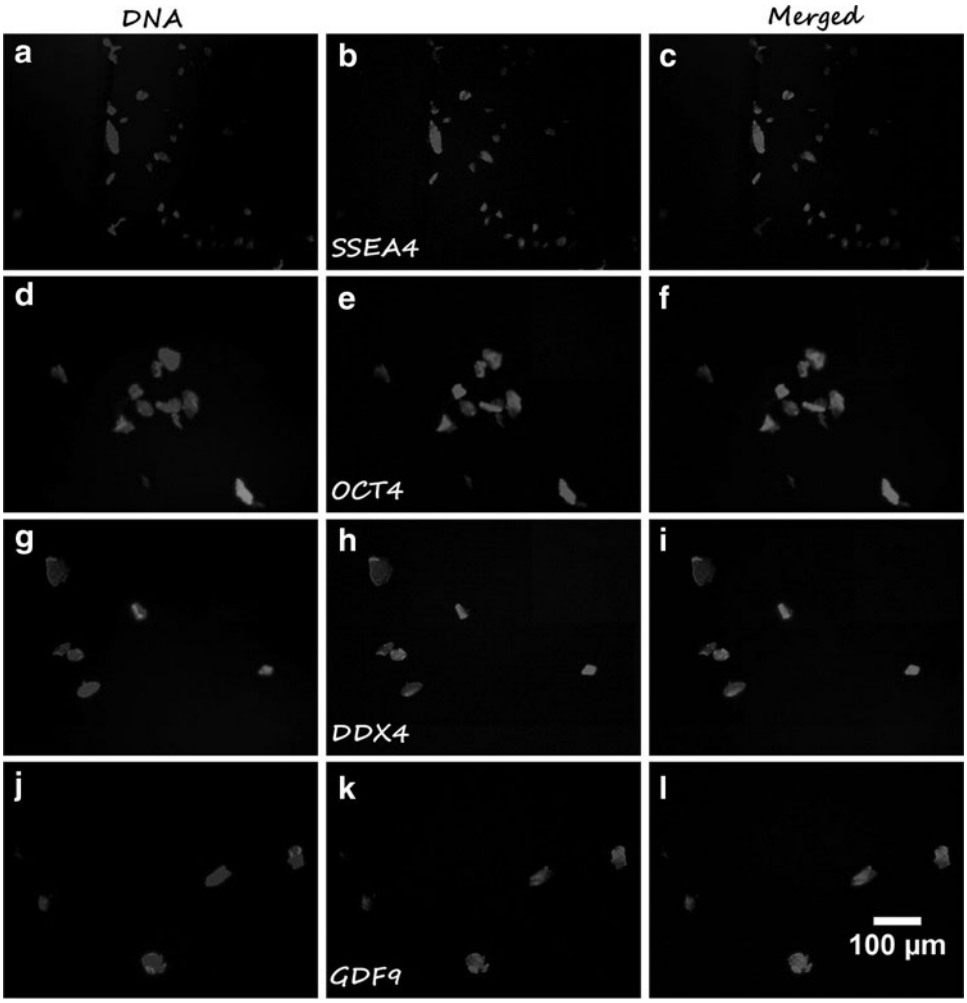

FIG. 5. Immunocytochemistry staining of hUMSCs for specific female germ cells and oocyte markers [Ssea4 (**a, b, c**); Oct4 (**d, e, f**); Ddx4 (**g, h, i**); and Gdf9 (**j, k, l**)] after 21 days of differentiation induction. Nuclei were stained with DAPI (4′, 6′-diamidino-2-phenylindole) staining.

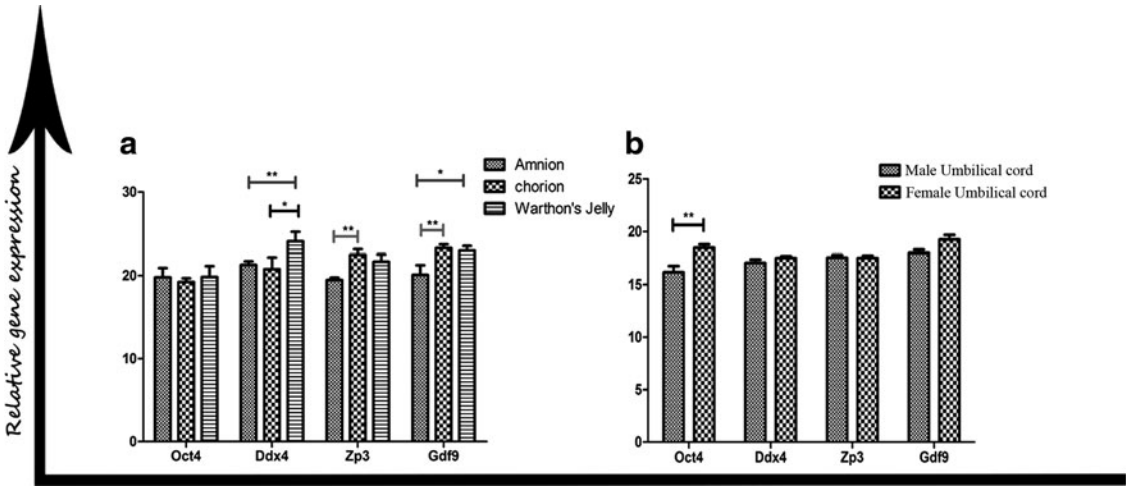

FIG. 6. RT-qPCR analysis of isolated cells after differentiation induction under influence of 10 ng/mL BMP4 for 21 days. **(a)** Results showed that hCMSCs showed a greater potential to be differentiated into female germline cells due to significantly higher relative gene expression level of *Zp3* and *Gdf9* genes in hCMSCs compared with the hAMSCs. In addition, the level of transcripts of *Oct4* and *Ddx4* genes in the hCMSCs was lower than in the other cells; however, the decrease was not significant. hCMSCs may have a higher degree of differentiation, and their pluri/multipotent characteristics could be reduced under the influence of 10 ng/mL BMP4. **(b)** Meanwhile, results indicate that hUMSCs originated from either male or female umbilical cord have the same differentiation potential into female germline cells, and there was no significant difference between them regarding their relative germ- and oocyte-like gene expression. Values are mean ± SD; $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$.

in a coculture model can provide a proper environment for the induction of hUMSCs into PGCs and expression of oocyte-like markers (Asgari et al., 2015).

However, we only evaluated hUMSCs, but in this study, we have shown, for the first time, that MSCs originated from chorion membrane have a higher potential to differentiate into germ- and oocyte-like cells compared with hAMSCs and hUMSCs during 3 weeks incubation with BMP4. Ataie nejad et al. showed that in the presence of BMP4 and retinoic acid (RA), in association with placenta cell coculture system, hUMSCs differentiate into male germ-like cells. They indicated that after 3 weeks of treatment, morphology of hUMSCs changed to shiny clusters, and germ cell-specific markers (*c-Kit*, *Ddx4*, *piwil2*, *Dazl*, $\alpha 6$ integrin, and $\beta 1$ integrins) in mRNA were upregulated in both placental feeder + RA and BMP4 + RA groups compared with control and placental feeder cells + RA (Amidi et al., 2015). BMP4 causes expression of PGC-specific genes such as *Dpp3a* (*Stella*), *Fragilis*, and *Ddx4* (Fujiwara et al., 1994; Nagano, 2007) through SMADs 1 and 5 intracellular cascades (Hiller et al., 2010).

Our flow cytometry results confirmed adequate purity obtained by the isolation method. Immunocytochemistry and RT-qPCR results showed that MSCs from all the three sources can be differentiated into germ- and oocyte-like cells with different potential. Our findings indicate that hCMSCs are the best among the various MSC candidates for infertility treatment procedures due to their higher capacity to differentiate into germ- and oocyte-like cells. We observed that not only was the relative expression of germ cell-related genes upregulated in the hCMSCs under the influence of 10 ng/mL BMP4 but also downregulation of pluripotency-related gene (Oct4). This result shows that following differentiation induction and expression of germ cell markers in MSCs, there is a reduction in pluripotency-related characteristics, and this finding is consistent with other existing studies (Deyev and Polanovsky, 2004; Filliers et al., 2012; Niwa et al., 2000; Pesce and Schöler, 2001; Tai et al., 2005).

Conclusion

This study indicates that the derivation of mammalian germ cells, both female and male, is possible *in vitro* from placenta-derived MSCs. Such *in vitro* studies can be used to improve our knowledge about germ cell development. *In vitro*-derived germ cells are very useful tools for understanding molecular and cellular mechanisms of female infertility, establishment of therapeutic approaches for female infertility, and providing testing systems to examine toxicological effects of drugs on human germ cells. MSCs originated from birth-associated tissues are best candidates for therapeutic procedures due to ease of proliferation and expansion, low immunogenicity, high differentiation capacity into other cell lineages useful in treating neural, spinal, and somatic diseases, absence of moral and ethical problems, high immune tolerance in transplantations, and interestingly, harvesting without any pain or discomfort.

The study of *in vitro* germ cell induction is still at a stage where we apply knowledge obtained in studies *in vivo*. During formation of germ cells and localization into gonads, three critical steps occur in germ cell development (specification, migration/proliferation, and sex-specific pre- and postnatal development), and this is hard to be determined if the *in vitro* process faithfully recapitulates normal germ cell development (Nagano, 2007). Thus, very detailed experiments are needed to fully understand cellular and molecular aspect of germ cell differentiation in *in vitro* conditions and use them as a presumptive source for cell therapy procedures and female infertility treatment.

Author Disclosure Statement

The authors declare that no conflicting financial interests exist.

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