Review Article

Strategies for Mammalian Mesenchymal Stem Cells Differentiation into Primordial Germ Cell-Like Cells: A Review

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Abstract

Primordial germ cells develop into oocytes and sperm cells. These cells are useful resources in reproductive biology and regenerative medicine. The mesenchymal stem cells (MSCs) have been examined for in vitro production of primordial germ cell-like cells (PGLCs). This study aimed to summarize the existing protocols for MSCs differentiation into PGLCs. In limited identified studies, various models of MSCs, including those derived from adipose tissue, bone marrow, and Wharton's jelly, have been successfully differentiated in to PGLCs. Although the protocols of specification induction are basically very similar, they have been adjusted to the mesenchymal cell type and the species of origin. The availability of MSCs has made it possible to customize conditions for their differentiation into PGLCs in several models, including humans. Refining germ cell-related signaling pathways during induced differentiation of MSCs will help to define extension to the protocols for PGLCs production.

Keywords: Adult Stem Cells, Cytological Techniques, Gametogenesis, Germ Cells, Retinol

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Introduction

Successful fertility in mammals is dependent on various biological processes, including oocyte maturity, ovulation, embryo formation, and implantation (1). In majority of multicellular organisms, germ cells are the origin of new organism, which transfer the genetic and epigenetic information to the next generation. Furthermore, these cells are the main source of totipotency to create a new organism (2). Two important phases of gamete development are i. Primordial germ cell (PGC) formation during early embryogenesis and active migration to gonadal ridge and ii. Receiving distinct environmental signals for controlled cell meiosis division, in oogenesis and spermatogenesis processes (3). Considering the unique capabilities of PGCs in the production of gametes, these cells would be precious resources in reproductive biology and regenerative medicine. Thus, laboratory production of primordial germ cell-like cells (PGCLCs) has been a growing trend for years.

Stem cells can build specialized cells of human body, and exert self-renewal and differentiative capacity (4). In general, stem cells are categorized as "embryonic" and "adult". Pluripotent embryonic stem cells (ESCs) can generate all cell lineages of human tissues through differentiation into more specialized multipotent stem cells with ectodermal, mesodermal, or endodermal origin.

ESCs were first isolated from mouse embryo in 1981 (5). The pluripotent characteristics of ESCs have given treatment hope in patients suffering from different diseases including infertility (6). However, the use of ESCs has been associated with tumorigenesis and ethical concerns, and researchers have tried to replace these cells with adult stem cells (7, 8).

Adult stem cells, also known as "somatic" stem cells, are found both in developing and adult tissues (9). Mesenchymal stem cells (MSCs) are multipotent stromal cells which are highly found in adipose tissue, umbilical cord blood and bone marrow; however, they can also be identified in other tissues and regions requiring wound healing (10). MSCs can be differentiated into different cell types with mesodermal and non-mesodermal origin such as adipocytes, osteoblasts, and chondrocytes (11). The easy harvesting of some MSCs types, including umbilical or adipose tissue derived MSCs, immunosuppressive properties, fewer ethical concerns, as well as simple and cost-effective culture and differentiation methods have made them a suitable alternative for ESCs and induced pluripotent stem cells (iPSCs) (12).

Remarkably, PGCLCs can be differentiated not only from ESCs, but also reprogrammed pluripotent stem cells (PSCs) generated either through somatic cell nuclear transfer or induced pluripotency (13). Differentiation induction in transdifferentiated cells is a newer strategy for *in vitro* production of PGCLCs. iPSCs are obtained by reprogramming mature somatic cellsthat have been used successfully to produce PGCLCs (14). In

transdifferentiation process, somatic stem cells are reprogrammed into cells of other germ layers and tissues, which is characterized by losing germ layer properties. The process of transdifferentiation to PGCLCs has been successfully induced in several somatic stem cell types such as skin-derived stem cells (15). The application of reprograming and transdifferentiation prospectively circumvents the strict ethical limitations associated with obtaining PSCs from human embryos. In addition, genetically modified gametes can be obtained eventually using gene editing. However, somatic cells have rather different pattern of mutations (16) and epigenetic status (17) than that of germ cells. These differences can only be identified and characterized by strict monitoring at the genetic level, which have not yet been well developed.

Induction of PSCs into PGCLCs has been examined in two-dimensional culture (18) and embryoid bodies (19). Despite inherent differences, in both mouse and human PSC types, bone morphogenetic proteins (BMPs) have been identified as essential inducers of PGC specification. Using cytokines such as retinoic acid, co-culture with somatic cells and conditioned media, successful experiments have been performed. Further, in other studies by manipulation of transcriptional regulators expression deleted in azoospermia-like (*Dazl*) family genes (20) or using small molecules such as kinase inhibitors, successful PGCLC meiosis induction have been achieved in human iPSCs (21).

Wnt signaling plays an important role in gastrulation process especially in mesoderm and endoderm differentiation. It has also been suggested that Wnt signaling inhibition stabilizes the undifferentiated state of PSCs. Moreover, during induction of human iPSCs in defined conditions through Wnt signaling, an initial differentiation stage to mesoderm-like cells has been identified (14). These cells express genes such as Eomesodermin promoting commitment to PGCLCs of iPSCs through *Sox17* upregulation (22). Such a phenomenon has also been shown in PSCs (23). Subsequently, using BMP4, mesoderm-like cells were differentiated into PGCLC (24). In this process, B lymphocyte-induced maturation protein 1 (Blimp1) suppresses the "neuronal differentiation" program and its expression is as a key feature of the PGC specification (14). Shirzeily et al. (25) in their study also demonstrated the differentiation ability of mouse adipose tissue and bone marrow-derived MSCs into primordial germ cells by expressing Mvh, Dazl, Stra8, and Scp3 specific markers.

Based on the findings, it is hypothesized that the mesoderm-like cells might be efficient precursors to form germ cell line with fewer ethical considerations than ESCs and iPSCs (26).

This review aims to overview the utilized MSC types and differentiation protocols for *in vitro* germ cell production with a focus on human and certain other mammalian models. Initially, the background of PGC development in the mouse model will be reviewed. Then, the existing studies on the production of PGCLCs from MSCs will be discussed by cell origin. Finally, the relevant information on all-trans-retinoic acid (RA) and BMP4 participation, as common factors used in the PGC specification, will be outlined.

The search for published records was carried out in the PubMed, EMBASE (Elsevier) and Google Scholar in January and August 2021 without limiting the search by date of publication and geographical region. The search terms were "mesenchymal stem cell", "mesenchymal stromal cells", "Wharton Jelly cells", “mesenchymal progenitor cells", "germ cells", "oogonial stem cells", "germline stem cells", "primordial germ cells", and "primordial germ cell-like cells". In addition, the operators “AND" and "OR" were applied for "primordial germ cells" or "primordial germ cell-like cells" and the other terms.

Authors independently screened the records by reading the title and abstract. Only peer reviewed full-length records covering the mammalian MSCs differentiation into primordial germ cell-like cells were included by experimental models.

In initial search, we retrieved 159 potentially relevant records, from which 50 were duplicates. Of the papers screened by reading the title and abstract, 77 studies were included (Fig.1).

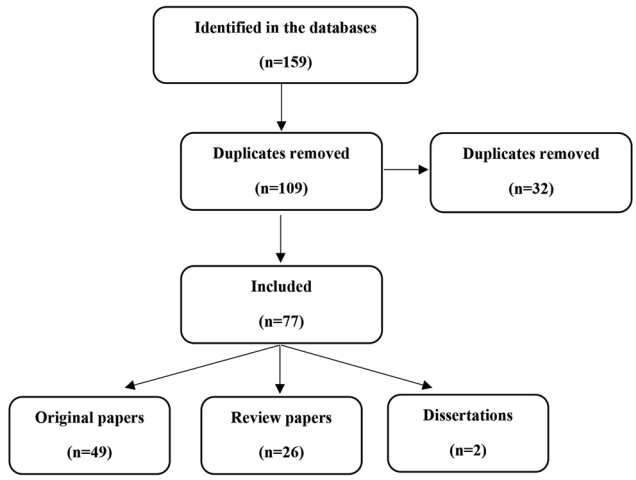

Fig.1: Flow chart of the review records selection.

Overview of mice primordial germ cell generation

Wnt3A from trophoblasts together with BMP4 and BMP8b from amniotic adjacent mesenchyme inducing PGC specification. PGCs are the first population of germ cells which are established during the development (Fig.2) and are the immediate precursors of both oocytes and spermatogonia. In mice, PGCs are initially identified as an approximately 40-cell cluster in the incipient allantois

based on day 7.25 (E7.25) of embryonic life. These cells then migrate to the developing hindgut endoderm and mesentery at E7.75 and E9.5, and colonize the genital ridges at E10.5, respectively (2).

An important event during the proliferative phase of PGCs is epigenetic reprogramming (27), especially a genome-wide DNA demethylation (28). Blimp1 and Prdm14 promote PGC specification via repressing somatic genes. PGCs retain remarkable pluripotential capabilities as shown by the ability to generate teratomas and pluripotent cell lines. Despite partial differences, both mouse and human PGCs express a group of 'naïve' and 'general' pluripotency factors. A transcription program involving the expression of the RNA-binding protein Dazl subsides PGCs pluripotential capabilities and prime them toward germline commitment (29).

In female XX embryos, PGCs will continue the proliferation until E13.5 to reach a 25000 cell population. Afterwards, they enter to the prophase I of meiotic division and will be arrested in diplotene stage of meiosis prophase I. In contrast, XY PGCs enter the mitotic arrest upon entry into the genital ridges, and stay silent in the G0/G1 phase of the cell cycle for the remaining embryonic period. Around day 5 postpartum many of these cells resume proliferation, whereby some of them will be recruited as spermatogonial stem cells (30).

Strategies for germ cell differentiation from mesenchymal stem cells

As summarized in Table 1, several studies have been reported that MSCs originally show germ cells characteristics, and in the presence of certain chemicals, they can also be differentiated into PGCLCs, potentially applicable as a therapeutic approach for infertility (31-33).

Table 1: Studies using mesenchymal stem cells to produce germ cell-like cells

Cell source	Cytokines		Additional strategy	Differentiation time (day)	Final cell type	Reference
	RA (µM)	BMP4 (ng/ml)				
BM-MSC						
Rat	1	-	-	21	PGCLC	(11)
Mouse	-	20	-	4	PGCLC	(32)
Mouse	-	1-25	-	4	PGCLC	(33)
Mouse	1	-	-	2	PGCLC	(25)
Ram	-	100	TGF-β1 treatment	21	Male GCLC	(34)
WJ-MSC						
Human	1	-	Co-culture with placental cells	14	PGCLC	(35)
Human	1	10	-	21	PGCLC	(36)
Human	10	-	-	14	PGCLC	(37)
Human	-	12.5	Overexpressed CD61	7	Male GCLC	(38)
Human	-	-	Follicular fluid, cumulus cells conditioned medium	21	PGCLC	(39)
Human	2	-	-	14	PGCLC	(40)
Ad-MSC						
Mouse	1	-	-	2	PGCLC	(25)
Human	10	-	-	21	PGCLC	(41)
Human	1	-	-	7-21	Male GCLC	(42)
Human	10	-	Co-culture with Sertoli cells	21	Male GCLC	(43)
Human	-	25	Overexpressed miR-106b	4	PGCLC	(44)
Canine	-	-	Overexpressed CD61	2 and 10	PGCLC	(45)
FF-MSC						
Human	-	-	BMP15 treatment	21	Female GCLC	(46)

Ad; Adipose tissue, BM; Bone marrow, FF; Follicular fluid, GCLC; Germ cell-like cell, PGCLC; Primordial germ cell-like cell, WJ; Wharton's jelly, and MSC; Mesenchymal stem cell.

Bone marrow-derived mesenchymal stem cells

BMP4 is one of the most frequently used factor for stem cell differentiation (47). Using BMP4, several protocols have been established to differentiate MSCs, including PGCLCs from bone marrow-derived (BM)-MSCs. Leading studies have used feeder layer, respectively and fetal bovine serum to support cell proliferation. For example, the mouse myoblast cell line C2C12 feeder layer in the presence of an interleukin 6 class cytokine was used to support proliferation (32). The cells obtained from rat, mouse, and human have been examined and various BMP4 concentrations have been employed from 1 to 100 ng/ml. In one study, RA was used as the PGCLCs differentiation inducer (11).

In the study by Ghasemzadeh-Hasankolaei et al. (34), although transforming growth factor (TGF)-β1 was more effective than BMP4, BMP4 could significantly boost the male germ cell markers. In addition to inducing differentiation, BMP4 had a positive effect on cell proliferation and survival (33). In three studies, the induction period was shorter than 5 days (25, 31, 33), while, in two studies the induction period was 21 days (11, 34). In two studies, mouse vasa homologue (Mvh) marker was employed to determine the extent of differentiation into germ cells (32, 33). This marker is expressed in germ cells up to the post-meiotic stage in both males and females (48).

In only two studies, isolated MSCs were characterized before induction of differentiation (11, 33). For this purpose, $CD90^{+}$, $CD105^{+}$, $CD34^{-}$ and $CD45^{-}$ molecular pattern was used to isolate and detect the cells. After inducing differentiation, several germ cell markers including c-Kit, Dazl, Stella, and Fragilis (also known as interferon induced transmembrane protein 3) were assessed. Fragilis is highly expressed in mouse PGCs at E6.5-7.25 together with Stella (Fig.2). In two studies, serum concentrations of 20% (32) and 10% (11) were used to support cell growth and proliferation. In the study of Shirazi et al. (32), comparison of migrating cells with migrated cells revealed a pattern of differentiation markers similar to PGCLCs. In one study the differentiation capabilities of BM-MSCs and ADSCs were compared. While both cell populations had the potential to become PGCLCs, BM-MSCs indicating a greater potential (25).

Wharton's jelly-derived mesenchymal stem cells

Since BM-MSCs isolation is an invasive and complicated process, the use of umbilical cord or Wharton's jelly (WJ)-MSCs is considered more convenient. The isolation of these cells from umbilical cord or placenta of a newborn is non-complicated, and non-invasive with a lower risk of contamination and ethical concerns (35). In addition to multipotent properties of these cells, which are between adult cells and ESCs, they seem to have a high potential to be differentiated into germ cells (49).

The strategies used to differentiate PGCLCs from WJ-MSCs are more diverse than BM-MSCs. Co-culture and genetic manipulation methods have been employed as supporting or adjuvant factors with RA and BMP4 treatments.

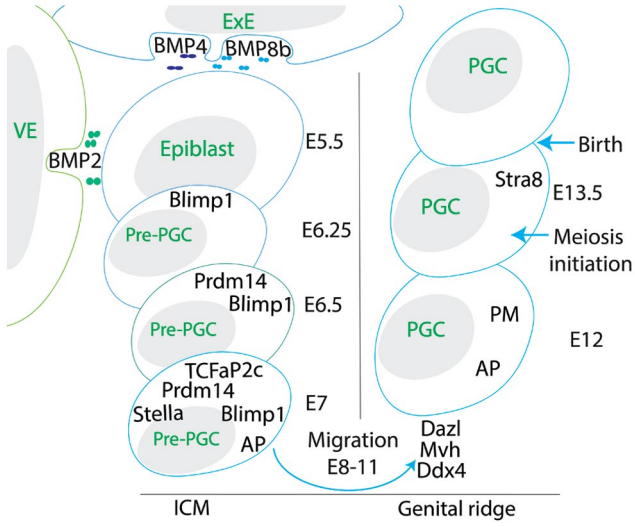

Fig.2: Female mice primordial germ cell (PGC) generation. The extraembryonic endoderm (ExE) layer, which is in contact with epilblast, secretes BMP4. Signals generated by ExE and visceral endoderm (VE) play important roles in inducing differentiation to PGCs. B lymphocyte-induced maturation protein 1 (Blimp1) is the first expressed marker of PGCs precursor at embryonic day (E) 6.25. BMP4 and BMP8b secreted by ExE and BMP2 secreted by proximal VE induce differentiation to $Blimp1^{+}$PGC precursors in the posterior proximal epiblast at E6.25. Initial PGCs at E6.5 and E7 express the transcription factors Prdm14 and Stella, respectively. By the fate determination of the germ cells, they begin to proliferate and migrate to the base of the yolk sac and show strong alkaline phosphatase (AP) activity. Then, they start migrating from inner cell mass (ICM) to the genital ridge. These cells express the pluripotency gene markers (PM) *Oct4*, *Nanog*, and *Sox2*, which are important for PGC growth and germination. Deleted in azoospermia-like (Dazl), mouse vasa homologue (Mvh) and ATP-dependent RNA helicase Ddx4 expression lead to germ cell commitment and indicate formation of post-migrating gonocytes. PGCs express the signaled by retinoic acid 8 (Stra8) from E13.5 which initiates the transition between mitosis and meiosis in oogenesis (26).

Compared with RA induction, the placental cell co-culture method further increased the early germ cell markers, Oct4, and ATP-dependent RNA helicase Ddx4. However, no significant differences were observed for specific cell markers such as growth differentiation factor 9 (GDF9) and zona pellucida glycoprotein 3 (Zp-3). GDF9 is a growth factor from the TGF-β family. This factor is expressed in large amounts in eggs that plays an important role in folliculogenesis plus ovulation (50). Zp-3 as a receptor mediates the initial binding of sperm to the egg (51).

Based on these results, it seems that in MSCs differentiation induction into PGCLCs, BMP4 is not a substitutive factor, but a complementary or amplifying factor of RA. In support of this hypothesis, initial treatment

of human WJ-MSC with BMP4 and RA outperformed BMP4 alone. In addition, co-culture with placental cells and RA also showed fewer germ cell-specific markers than BMP4 and RA combination (36).

Human follicular fluid and cumulus cells-conditioned medium could induce expression of oocyte specific genes and proteins (39). These factors also induced morphological changes matchingoocyte-like cell differentiation. The observed effect has been related to potent growth factors of cumulus cells secretome such as epidermal growth factor. The potential of cells derived from follicular fluid to differentiate into oocyte-like cells further demonstrate the possibility of developing germ cells from adult stem cells (46).

In optimizing the development of PGCLCs, genetic manipulation is a potential strategy. Through overexpression and suppression of selected genes, the differentiation pathway can be oriented in the relevant direction. This approach can also facilitate *in vitro* induction of differentiation into PGCLCs. For this purpose, the genes involved in embryonic development are prioritized. In a study, obtaining male PGCLCs, CD61 or Integrin beta-3 was overexpressed in human placental MSCs. This manipulation alone increased the PGCLC markers such as c-Kit, sex determining region Y-Box 2 (*Sox2*), and *SSEA1*. Treatment of manipulated cells with BMP4 enhanced male-PGCLC differentiation, which was characterized by an increase in the signaling through retinoic acid 8 (*Stra8*) marker (38).

Adipose-derived stem cells

It has recently been shown that MSCs derived from adipose tissue can be differentiated into PGCLCs with BMP4 treatment or transfection by miR-106b (44). BMP4 has not been used in any of the other five studies on adipose-derived stem cells (ADSCs). Nevertheless, RA has been used alone or in combination with co-culture. Genetic manipulation of ADSCs has also been described as a successful means to induce PGC specification.

The Sertoli cell co-culture (43), testicular cell-conditioned medium (42), and testosterone (43) were used to induce male PGCLCs with RA. In addition to direct differentiation induction, treatment with RA and testosterone indirectly enhanced the differentiation of ADSCs into male PGCLC by promoting the viability and secretory activity of sertoli feeder cells. Similar to human WJ-MSCs (48), increased CD61 expression in Canine ADSCs alone significantly elevated PGC specification and stem cell markers compared to control cells (42). Examination of TGF-β signaling showed that CD61 expression significantly enhanced the level of Smad2 phosphorylation, without affecting the level of phosphorylated Smad2/3 and Smad3.

Retinoic acid and bone morphogenetic proteins pathways

All-trans-retinoic acid

Retinol is obtained as retinyl ester from plants β-carotene. RA is one of the main retinol metabolites with potent biological capabilities related to proliferation and differentiation (52). On the cell surface, RA is taken up by retinol binding protein encoded by steroidogenic acute regulatory protein (StAR) 6 (53). Lecithin:retinol acyl transferase is also required for retinol uptaking and esterification (Fig.3A). Inside the cell, the transferase and dehydrogenase enzymes convert retinol to retinyl ester, retinaldehyde, and then RA. RA binds to cellular RA binding protein 2that is transferred into the nucleus (54). In the presence of RA, the retinoid-X receptor (RXR)/RA receptor (RAR) heterodimer complex interacts with DNA and activates the transcription of RA "primary response" genes (55).

Transcription activation is one of the primary steps of RA-associated differentiation process occuring during several minutes to hours after RA addition to the culture media. A number of "immediate early" genes or "primary response" genes are the direct targets of RA (Fig.3B) (56).

RA-mediated gene expression regulation often involves polycomb group (PcG) proteins (Fig.3C). PcG proteins can form a complex of gene-silencing proteins, which play a central role in embryogenesis, patterning, and differentiation (57). Following Ra in addition to stem cell culture medium, a fast dissociation of PcG proteins occurred, leading to the induction of differentiation-related genes expression, including *Stella*, *Fragilis,* and *Stra8* (58). Thus, retinoids provide an essential early signal to induce a certain cascade for totipotent and lineage restricted stem cell differentiation (55).

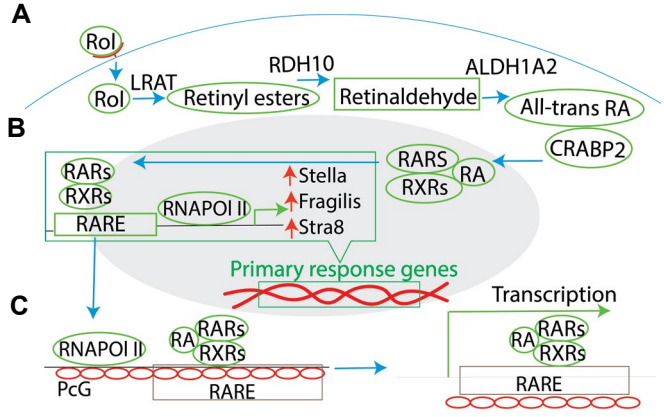

Fig.3: Retinoic acid signaling pathway in the regulation of germ-cell related gene expression. **A.** On the cell surface, retinol (Rol) by binding to retinol binding protein enters the target stem cell and is converted to all-trans retinoic acid (RA) following the lecithin:retinol acyl transferase (LRAT), retinol dehydrogenase 10 (RDH10) and aldehyde dehydrogenase 1a2 (ALDH1A2) reactions. Then, RA is transferred to the nucleus by binding to cellular retinoic acid binding protein 2 (CRABP2). Once in the nucleus, RA is initially bound to retinoic acid receptors (RARs) and the RA-RARs complex by binding to retinoid X receptors (RXRs) will interact with RA primary response genes (53). **B.** RA primary response genes have some enhancers known as RA response element (RARE) which RARs/RXRs complex binds to, leading to RNApol II activation and increased PGCs-related genes, including Stella, Fragilis, and Stra8, expression (55). **C.** PcG proteins form a gene-silencing complex for gene expression regulation. RA causes the dissociation of these proteins and activates the differentiation-related genes expression (57, 59).

bone morphogenetic proteins

PGCLC production from MSCs has been studied in both human and mouse models. Studies on the mouse and rat models have predominantly applied BM-MSCs. Despite promising early results on the differentiation of mouse BM-MSCs into PGCLCs, no follow-up data has been published on human BM-MSCs. MSCs of WJ and adipose tissue origin used in the human model studies (Table 1). However, the current knowledge on the mechanism of MSCs differentiation into PGCLCs is not persuasive enough. As the isolation of MSCs in humans is far easier than the obtaining ESCs, establishing suitable conditions for them to differentiate into PGCLCs will progress more rapidly. The recent studies that have efficiently differentiated MSCs are good models for future mechanistic studies, though failure to control the key variables remains a major limitation. Figure 4 illustrates the BMPs signaling pathway in the regulation of germ-cell related gene expression.

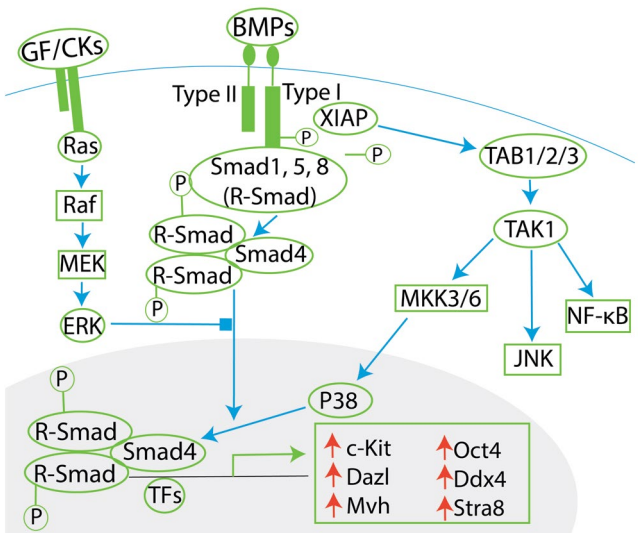

Fig.4: BMPs signaling pathway in the regulation of germ-cell related gene expression. BMPs act via type I and type II receptors. These receptors interaction results in receptor I-mediated phosphorylation of Smad1, Smad5, and Smad8 (R-Smad). Two phosphorylated R-Smads in combination with Smad4 form a heterotrimeric complex which is transmitted into the nucleus and regulates the PGCs differentiation-related target genes (such as *c-Kit*, *Dazl*, *Mvh*, *Oct4*, *Ddx4* and *Stra8*) expression together with the other transcription factors (TFs). X-lined inhibitor of apoptosis (XIAP) also links the BMP receptor signals to TGFh1 activated tyrosine kinase 1 (TAK1). Then, TAK1 activates Map kinase kinase isoforms 3/6 (MKK3/6), JNK and NF-κB. Mitogen-activated protein kinase (MAPK) activated by growth factors (GFs) or cytokines (CKs) through Ras/Raf/Mek can inhibit the Smad nuclear translocation and consequently inhibit the BMP signaling pathway (60).

In vitro differentiation of MSC using RA and BMP4 has provided a customizable approach for improving conditions (Fig.5).

The available methods in this area have a number of limitations. Defining standard functional assays in this area will enable us to improve the conditions for producing PGCLCs. Evidence suggests that the mouse PSC is not very similar to the human PSC in terms of its pluripotency nature, making the PGCLC properties obtained from these two species different. For example, in very similar differentiation induction protocols, human PGCLCs, unlike mice PGCLCs, are negative for Ddx4 and Dazl genes, analogous to early stage PGCs (14, 61). Refining germ cell-related signaling pathways will enhance preferential differentiation of somatic MSCs into PGCLCs.

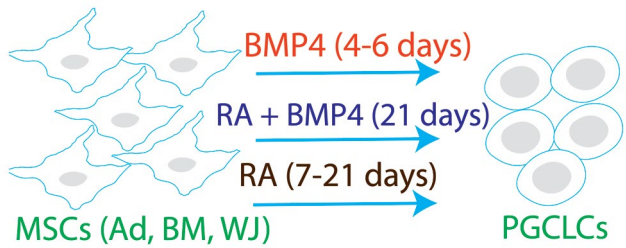

Fig.5: Induction of germ cell-like cells (PGCLCs) from mesenchymal stem cells (MSCs). The cells can be differentiated into PGCLCs from adipose tissue (Ad), bone marrow (BM), and Wharton jelly (WJ) cultured in the presence of BMP4. Alternatively, or in combination with BMP4, retinoic acid (RA) can also be used as an inducer.

Discovering gene expression signatures using RNA sequencing methods will allow determining the functional potential of PGCLCs without the need for further differentiation. In determining such characteristics, the intrinsic differences between cell types from different species must be taken into account. The difference in the initial pluripotency state is attributed to the limited response of rat ESCs compared to mice ESCs to PGCLCs differentiation stimuli. Indeed, induced expression of the PGC transcriptional repressor Blimp1 by genetic manipulation increased differentiation towards cells expressing PGCLC markers (62). Moreover, human PGCs, unlike mice PGCs, do not express the pluripotency factor Sox2, while requiring Sox17 for PGC specification (24). Thus, depending on the studied species, additional strategies may be needed to obtain more mature PGCLCs, such as co-culture with somatic gonadal cells.

A reasonable similarity between ESC-induced PGCLCs and *in vivo* PGCs has previously been reported (63). However, no study has yet compared the transcript of PGCLCs obtained from MSCs with *in vivo* PGCs or PGCLCs from ESCs, reprogrammed cells, or transdifferentiated cells. The use of a CRISPR/Cas9 screening platform to identify the factors required for PGC development in a new study (64) is a good example of a mechanistic study of the PGC specification.

The development of safe and efficient protocols in this field will provide useful resources for the PGCLCs in fertility science. Certainly, a fully defined condition cannot be provided with somatic cell co-culture strategy

or serum supplementation. Nevertheless, using co-culture techniques, signaling and environmental cues related to PGC specification can be investigated. With these techniques, the effect of different types of somatic cells can be tested comparatively. In this way, more effective cells are identified during screening, after which the potent player can be identified. In that framework, the indirect effect of nutrients on cell development through somatic cells can be determined (65). Further, the bioactive components might be identified by serum or follicular fluid pre-fractionation (66, 67). For instance, follicular fluid has shown preserving effects on the stemness characteristics of human granulosa cells (68). In the approach of employing MSCs to produce PGCLCs, there is a great opportunity for manipulation before differentiation. So far, two studies (48, 52) have shown direct MSCs towards PGCLCs through genetic manipulation. With this strategy, the activity of related signaling pathways has been studied. Gene therapy can be performed once optimal PGCLCs are achieved. Thus, as with cases of reprogramming and transdifferentiation, genetically modified PGCLCs can be obtained from MSCs in genetic diseases.

At present, *in vivo* PGCLC grafting is mainly used to obtain gametes, as no suitable in vitro condition has yet been established for this purpose. For instance, Hayashi and Surani (63) successfully used somatic cells from mice embryonic ovaries to direct oogenesis from PGCLC, but the efficiency of oocytes derived from PGCLC to produce zygotes was only 53%. Providing appropriate conditions for the proliferation and maintenance of PGCLCs is a challenge in this regard. Many factors such as cell species, age, and sex affect MSC's ability to eventually differentiate into gametes. Initial identification and purification of MSCs using exclusive and robust markers is an essential requirement for achieving optimal and defined conditions. Another important factor is the optimal duration of differentiation induction. Accordingly, it is necessary to determine the dynamics of markers in an integrated way. In addition, the appropriate stage of differentiation needs to be determined according to the ability of proliferation and subsequent differentiation into gamete lineages.

Conclusion

The availability of MSCs has made it possible to customize conditions for their differentiation into PGCLCs in several models, including humans. Umbilical cord, adipose tissue, and bone marrow are prospective sources of MSCs for germ cell line regeneration. Refining germ cell-related signaling pathways during induced differentiation of MSCs will help define extension to the protocols for PGCLCs production.

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Authors' Contributions

Sh.F.; Contributed to the conception, design, and drafting. P.F., Z.N.; Contributed to the literature review. A.M.; Was responsible for overall supervision. All authors read and approved the final manuscript.

References

1. Kahnamouyi S, Nouri M, Farzadi L, Darabi M, Hosseini V, Mehdizadeh A. The role of mitogen-activated protein kinase–extracellular receptor kinase pathway in female fertility outcomes: a focus on pituitary gonadotropins regulation. Ther Adv Endocrinol Metab. 2018; 9(7): 209-215.
2. Chen D, Sun N, Hou L, Kim R, Faith J, Aslanyan M, et al. Human primordial germ cells are specified from lineage-primed progenitors. Cell Rep. 2019; 29(13): 4568-4582.
3. Larose H, Shami AN, Abbott H, Manske G, Lei L, Hammoud SS. Gametogenesis: a journey from inception to conception. Curr Top Dev Biol. 2019; 132: 257-310.
4. Suman S, Domingues A, Ratajczak J, Ratajczak MZ. Potential clinical applications of stem cells in regenerative medicine. Adv Exp Med Biol. 2019; 1201: 1-22.
5. Eguizabal C, Aran B, Chuva de Sousa Lopes SM, Geens M, Heindryckx B, Panula S, et al. Two decades of embryonic stem cells: a historical overview. Hum Reprod Open. 2019; 2019(1): hoy024.
6. Zhao YX, Chen SR, Su PP, Huang FH, Shi YC, Shi QY, et al. Using mesenchymal stem cells to treat female infertility: an update on female reproductive diseases. Stem Cells Int. 2019; 2019: 9071720.
7. Wuputra K, Ku CC, Wu DC, Lin YC, Saito S, Yokoyama KK. Prevention of tumor risk associated with the reprogramming of human pluripotent stem cells. J Exp Clin Cancer Res. 2020; 39: 1-24.
8. Volarevic V, Markovic BS, Gazdic M, Volarevic A, Jovicic N, Arsenijevic N, et al. Ethical and safety issues of stem cell-based therapy. Int J Med Sci. 2018; 15(1): 36.
9. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther. 2019; 10(1): 1-22.
10. Mohr A, Zwacka R. The future of mesenchymal stem cell-based therapeutic approaches for cancer–From cells to ghosts. Cancer Lett. 2018; 414: 239-249.
11. Kumar K, Das K, Madhusoodan A, Kumar A, Singh P, Mondal T, et al. Rat bone marrow derived mesenchymal stem cells differentiate to germ cell like cells. Available from: biorxiv.org/content/10.1101/418962v1.full (24 May 2021).
12. Lee SH. The advantages and limitations of mesenchymal stem cells in clinical application for treating human diseases. Osteoporos Sarcopenia. 2018; 4(4): 150.
13. Easley CA, Latov DR, Simerly CR, Schatten G. Adult somatic cells to the rescue: nuclear reprogramming and the dispensability of gonadal germ cells. Fertil Steril. 2014; 101(1): 14-19.
14. Sasaki K, Yokobayashi S, Nakamura T, Okamoto I, Yabuta Y, Kurimoto K, et al. Robust in vitro Induction of human germ cell fate from pluripotent stem cells. Cell Stem Cell. 2015; 17(2): 178-194.
15. Sun R, Sun YC, Ge W, Tan H, Cheng SF, Yin S, et al. The crucial role of Activin A on the formation of primordial germ cell-like cells from skin-derived stem cells in vitro. Cell Cycle. 2015; 14(19): 3016-3029.
16. García-Nieto PE, Morrison AJ, Fraser HB. The somatic mutation landscape of the human body. Genome Biol. 2019; 20(1): 1-20.
17. Rahbari R, Wuster A, Lindsay SJ, Hardwick RJ, Alexandrov LB, Turki SA, et al. Timing, rates and spectra of human germline mutation. Nat Genet. 2016; 48(2): 126-133.
18. Gell JJ, Liu W, Sosa E, Chialastri A, Hancock G, Tao Y, et al. An extended culture system that supports human primordial germ cell-like cell survival and initiation of DNA methylation erasure. Stem Cell Rep. 2020; 14(3): 433-446.
19. Mitsunaga S, Shioda K, Isselbacher KJ, Hanna JH, Shioda T. Generation of human primordial germ cell-like cells at the surface of embryoid bodies from primed-pluripotency induced pluripotent stem cells. J Vis Exp. 2019; (143).
20. Gulimiheranmu M, Wang X, Zhou J. Advances in female germ cell induction from pluripotent stem cells. Stem Cells Int. 2021: 8849230.
21. Fang F, Li Z, Zhao Q, Ye Z, Gu X, Pan F, et al. Induced pluripotent stem cells derived from two idiopathic azoospermia patients display compromised differentiation potential for primordial germ cell fate. Front Cell Dev Biol. 2020; 8: 432.
22. Kojima Y, Sasaki K, Yokobayashi S, Sakai Y, Nakamura T, Yabuta Y, et al. Evolutionarily distinctive transcriptional and signaling pro-

grams drive human germ cell lineage specification from pluripotent stem cells. Cell Stem Cell. 2017; 21(4): 517-532.
23. Pierson Smela M, Sybirna A, Wong FCK, Surani MA. Testing the role of SOX15 in human primordial germ cell fate. Wellcome Open Res. 2019; 4: 122.
24. Yokobayashi S, Okita K, Nakagawa M, Nakamura T, Yabuta Y, Yamamoto T, et al. Clonal variation of human induced pluripotent stem cells for induction into the germ cell fate. Biol Reprod. 2017; 96(6): 1154-1166.
25. Shirzeily MH, Pasbakhsh P, Amidi F, Mehrannia K, Sobhani A. Comparison of differentiation potential of male mouse adipose tissue and bone marrow derived-mesenchymal stem cells into germ cells. Int J Reprod Biomed. 2013; 11(12): 965.
26. Saitou M, Miyauchi H. Gametogenesis from pluripotent stem cells. Cell Stem Cell. 2016; 18(6): 721-735.
27. Rassoulzadegan M, Cuzin F. Epigenetic heredity: RNA-mediated modes of phenotypic variation. Ann N Y Acad Sci. 2015; 1341: 172-175.
28. Zeng Y, Chen T. DNA methylation reprogramming during mammalian development. Genes. 2019; 10(4): 257.
29. Nicholls PK, Schorle H, Naqvi S, Hu YC, Fan Y, Carmell MA, et al. Mammalian germ cells are determined after PGC colonization of the nascent gonad. Proc Natl Acad Sci USA. 2019; 116(51): 25677-25687.
30. Wang JH, Li Y, Deng SL, Liu YX, Lian ZX, Yu K. Recent research advances in mitosis during mammalian gametogenesis. Cells. 2019; 8(6): 567.
31. Nayernia K, Lee JH, Drusenheimer N, Nolte J, Wulf G, Dressel R, et al. Derivation of male germ cells from bone marrow stem cells. Lab Invest. 2006; 86(7): 654-663.
32. Shirazi R, Zarnani AH, Soleimani M, Abdolvahabi MA, Nayernia K, Kashani IR. BMP4 can generate primordial germ cells from bone-marrow-derived pluripotent stem cells. Cell Biol Int. 2012; 36(12): 1185-1193.
33. Mazaheri Z, Movahedin M, Rahbarizadeh F, Amanpour S. Different doses of bone morphogenetic protein 4 promote the expression of early germ cell-specific gene in bone marrow mesenchymal stem cells. In Vitro Cell Dev Biol Anim. 2011; 47(8): 521-525.
34. Ghasemzadeh-Hasankolaei M, Sedighi-Gilani M, Eslaminejad M. Induction of ram bone marrow mesenchymal stem cells into germ cell lineage using transforming growth factor-β superfamily growth factors. Reprod Domest Anim. 2014; 49(4): 588-598.
35. Asgari HR, Akbari M, Abbasi M, Ai J, Korouji M, Aliakbari F, et al. Human Wharton's jelly-derived mesenchymal stem cells express oocyte developmental genes during co-culture with placental cells. Iran J Basic Med Sci. 2015; 18(1): 22.
36. Amidi F, Hoseini MA, Nia KN, Habibi M, Kajbafzadeh AM, Mazaheri Z, et al. Male germ-like cell differentiation potential of human umbilical cord Wharton's jelly-derived mesenchymal stem cells in co-culture with human placenta cells in presence of BMP4 and retinoic acid. Iran J Basic Med Sci. 2015; 18(4): 325.
37. Dissanayake D, Patel H, Wijesinghe P. Differentiation of human male germ cells from Wharton's jelly-derived mesenchymal stem cells. Clin Exp Reprod Med. 2018; 45(2): 75-81.
38. Li B, Liu W, Zhuang M, Li N, Wu S, Pan S, et al. Overexpression of CD61 promotes hUC-MSC differentiation into male germ-like cells. Cell Prolif. 2016; 49(1): 36-47.
39. Zolfaghar M, Mirzaeian L, Beiki B, Naji T, Moini A, Eftekhari-Yazdi P, et al. Wharton's jelly derived mesenchymal stem cells differentiate into oocyte like cells in vitro by follicular fluid and cumulus cells conditioned medium. Heliyon. 2020; 6(10): e04992.
40. Alifi F, Asgari HR. Alteration in expression of primordial germ cell (PGC) markers during induction of human amniotic mesenchymal stem cells (hAMSCs). J Reprod Infertil. 2020; 21(1): 59.
41. Liu H, Chen M, Liu L, Ren S, Cheng P, Zhang H. Induction of human adipose-derived mesenchymal stem cells into germ lineage using retinoic acid. Cell Reprogram. 2018; 20(2): 127-134.
42. Bräunig P, Glanzner W, Rissi V, Gonçalves P. The differentiation potential of adipose tissue-derived mesenchymal stem cells into cell lineage related to male germ cells. Arq Bras Med Vet Zootec. 2018; 70(1): 160-168.
43. Luo Y, Xie L, Mohsin A, Ahmed W, Xu C, Peng Y, et al. Efficient generation of male germ-like cells derived during co-culturing of adipose-derived mesenchymal stem cells with Sertoli cells under retinoic acid and testosterone induction. Stem Cell Res Ther. 2019; 10(1): 91.
44. Mahboudi S, Parivar K, Mazaheri Z, Irani S. Mir-106b cluster regulates primordial germ cells differentiation from human mesenchymal stem cells. Cell J. 2021; 23(3): 294.
45. Fang J, Wei Y, Lv C, Peng S, Zhao S, Hua J. CD61 promotes the differentiation of canine ADMSCs into PGC-like cells through modulation of TGF-β signaling. Sci Rep. 2017; 7(1): 1-9.
46. Taheri M, Saki G, Nikbakht R, Eftekhari AR. Bone morphogenetic protein 15 induces differentiation of mesenchymal stem cells derived from human follicular fluid to oocyte-like cell. Cell Biol Int. 2021; 45(1): 127-139.
47. Li Q, Zhang S, Sui Y, Fu X, Li Y, Wei S. Sequential stimulation with different concentrations of BMP4 promotes the differentiation of human embryonic stem cells into dental epithelium with potential for tooth formation. Stem Cell Res Ther. 2019; 10(1): 1-8.
48. Toyooka Y, Tsunekawa N, Takahashi Y, Matsui Y, Satoh M, Noce T. Expression and intracellular localization of mouse Vasa-homologue protein during germ cell development. Mech Dev. 2000; 93(1-2): 139-149.
49. Hass R, Kasper C, Böhm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): a comparison of adult and neonatal tissue-derived MSC. Cell Commun Signal. 2011; 9(1): 12.
50. Otsuka F, McTavish KJ, Shimasaki S. Integral role of GDF-9 and BMP-15 in ovarian function. Mol Reprod Dev. 2011; 78(1): 9-21.
51. Han L, Monné M, Okumura H, Schwend T, Cherry AL, Flot D, et al. Insights into egg coat assembly and egg-sperm interaction from the X-ray structure of full-length ZP3. Cell. 2010; 143(3): 404-415.
52. Mongan NP, Gudas LJ. Diverse actions of retinoid receptors in cancer prevention and treatment. Differentiation. 2007; 75(9): 853-870.
53. Kawaguchi R, Yu J, Honda J, Hu J, Whitelegge J, Ping P, et al. A membrane receptor for retinol binding protein mediates cellular uptake of vitamin A. Science. 2007; 315(5813): 820-825.
54. Schug TT, Berry DC, Shaw NS, Travis SN, Noy N. Opposing effects of retinoic acid on cell growth result from alternate activation of two different nuclear receptors. Cell. 2007; 129(4): 723-733.
55. Gudas LJ, Wagner JA. Retinoids regulate stem cell differentiation. J Cell Physiol. 2011; 226(2): 322-330.
56. Li G, Margueron R, Hu G, Stokes D, Wang Y-H, Reinberg D. Highly compacted chromatin formed in vitro reflects the dynamics of transcription activation in vivo. Mol Cell. 2010; 38(1): 41-53.
57. Simon JA, Kingston RE. Mechanisms of polycomb gene silencing: knowns and unknowns. Nat Rev Mol Cell Biol. 2009; 10(10): 697-708.
58. Kashyap V, Gudas LJ. Epigenetic regulatory mechanisms distinguish retinoic acid-mediated transcriptional responses in stem cells and fibroblasts. J Biol Chem. 2010; 285(19): 14534-14548.
59. Amat R, Gudas LJ. RARγ is required for correct deposition and removal of Suz12 and H2A. Z in embryonic stem cells. J Cell Physiol. 2011; 226(2): 293-298.
60. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-β family signalling. Nature. 2003; 425(6958): 577-584.
61. Irie N, Weinberger L, Tang WW, Kobayashi T, Viukov S, Manor YS, et al. SOX17 is a critical specifier of human primordial germ cell fate. Cell. 2015; 160(1-2): 253-268.
62. Taylor RM. Investigation into germ cell fate determination of rat embryonic stem cells. Presented for the Ph.D., Edinburgh. University of Edinburgh. 2020.
63. Hayashi K, Surani MA. Self-renewing epiblast stem cells exhibit continual delineation of germ cells with epigenetic reprogramming in vitro. Development. 2009; 136(21): 3549-3556.
64. Oliveira GPMSda. The identification of novel factors required for the development of primordial germ cells. Presented for the Ph.D., Cambridge. University of Cambridge. 2020.
65. Fayezi S, Ghaffari Novin M, Darabi M, Norouzian M, Nouri M, Farzadi L. Primary culture of human cumulus cells requires stearoyl-coenzyme A desaturase 1 activity for steroidogenesis and enhancing oocyte in vitro maturation. Reprod Sci. 2018; 25(6): 844-853.
66. Mardomi A, Nouri M, Farzadi L, Zarghami N, Mehdizadeh A, Yousefi M, et al. Human charcoal-stripped serum supplementation enhances both the stearoyl-coenzyme a desaturase 1 activity of cumulus cells and the. Hum Fertil (Camb). 2019; 22(3): 212-218.
67. Eyvaznejad E, Nouri M, Ghasemzadeh A, Mehdizadeh A, Shahnazi V, Asghari S, et al. Steroid-depleted polycystic ovarian syndrome serum promotes in vitro oocyte maturation and embryo development. Gynecol Endocrinol. 2018; 34(8): 698-703.
68. Yousefi S, Soleimanirad J, Hamdi K, Farzadi L, Ghasemzadeh A, Kazemi M, et al. Distinct effect of fetal bovine serum versus follicular fluid on multipotentiality of human granulosa cells in in vitro condition. Biologicals. 2018; 52: 44-48.