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Review

Intra-ovarian stem cell transplantation in management of premature ovarian insufficiency: towards the induced Oogonial Stem Cell (iOSC)

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Abstract: The specialized resident-stem cells in gonads are tasked with restorating damaged ovarian cells following injury to maintain sequential reproductive events. When we talk about premature ovarian insufficiency (POI) we accept the existence of decreased stem cell and their regenerative abilities. The present study was to explain how restorating damaged ovarian cells following injury to maintain sequential reproductive events in evidence-based medicine indexed in PubMed and Web of Science. The exact mechanism is unclear stem cells transfer may improve compromised ovarian function and fertility outcome in women with POI. Soluble factors secreted by stem cell may rescue impaired mitochondrial function in oogonial stem cells, enhance metabolic capacity of resident stem cells, induce local neovascularization in the ovary, and activate gene shifting between transferred stem cells and germ cell precursors. This review may provide insight into how stem cells show some of their beneficial effects on compromised ovarian microenvironment and germ cell niche and paves the way for clinical trials for improving ovarian function of women with POI. We also had the opportunity to share our hypothesis about the design and development of induced oogonial stem cell (iOSC) and its use in POI.

Key words: Oogonial stem cell; Stem cell transplantation; Premature ovarian insufficiency.

Introduction

Premature ovarian insufficiency (POI), which affects approximately 1% of women, is defined as the termination of menstruation before the age of 40 (1). The rapid depletion of the egg pool due to decreased follicle's vital functions and increased atresia is considered to be the underlying mechanism of the POI. However, the main mechanism of this uncontrolled reduction in the follicle pool is unknown. Although intermittent ovarian function and spontaneous pregnancy have been reported POI treatment is one of the most troublesome topics in reproductive biology, especially in populations where egg donation is not legal. Therefore, studies suggesting alternative methods to treat POI cases continue to be published. During the first of the proposed treatment methods are to transplant the patient's own stem cells to the ovaries (2,3). Conflicting results regarding stem cell therapy have been reported in experimental POI models and in patients with diminished ovarian reserve (DOR) (4,5). However, to date, no stem cell therapy has been performed in patients diagnosed with POI. For this reason, the comments we will make during the review will be evaluated either on the basis of the experimental POI model or the results of DOR patients. Because of these limitations, we may sometimes have to make speculative sentences with scientific basis.

Self-renewal and multipotency properties allow stem cells to keep their numbers in gonads within normal limits. In this way, stem cells can replace lost germ cells with new ones. However, this regeneration is not a sustainable method. Host stem cells do not have the potential to continually regenerate reduced germ cells in the ovary (4,5). Similar to somatic cells, stem cell needs a suitable and sufficient amount of oxygen, growth factors and a well-developed neural network to survive and reproduce. It is not unusual for the stem cell to continue its vital activities in compromised and aged POI ovaries. For this reason, it should be ensured that the ovarian tissue should have optimum conditions before stem cell transplantation. Another point that is wrongly known about stem cell applications is that the stem cells

can easily mimic the cells in the host tissues. If a stem cell is transferred to a tissue without being differentiated to a certain stage in vitro environment, it is not reasonable to expect it to turn into host cells. If the stem cell is differentiated up to one stage by germ cell signaling and growth factors and then transferred to the ovary, it can turn into germ cell precursors (4,5).

Formation of new follicle in POI or DOR women following intraovarian stem cell transplantation depends upon the adaptation and repopulation properties of transferred stem cells (5). However, the molecular basis of stem cell differantiation and mechanisms underlying their enlodgement in ovarian niches have not been identified. In this comprehensive review, we have discussed in detail the behavioral patterns of different stem cell types after intraovarian transplantation and their interactions with host cells. We have also tried to explain our hypothesis about the design and development of induced oogonial stem cell (iOSC) and its use in POI cases.

What is the logic of stem cell transplantation to the ovaries of women with POI?

Reports of whether the transferred stem cells form a new gamete are conflicting. Many studies have showed successful differentiation of transferred stem cells to gamete-like cells in vitro (4,5). Although the in vivo results of stem cell administration are not as clear as in vitro data one can think that why in vitro results do not occur in in vivo applications. The successful results obtained in experimental animals increased our expectations from stem cell applications. Through the stem cell trasnplantation or infusion we hope the following will happen in the ovaries (2,3,6,7).

- Correction of compromised germ cell niche.
- Replacement of insufficient soluble growth factors.
- Correction of failed microvascularization.
- Recovery of dying germ cells or follicles via gene or mitochondria transfer (in vivo resuscitation).
- Differentiation of transplanted stem cells and formation of new germ cell precursors (Figure 1).

All of the above-mentioned adverse conditions can be corrected by stem cell transplantation except for the last mentioned (2-4). Although different type of stem cell application promotes failed follicle function and ended with the birth of offspring whether stem cell transplantation develops a completely new and functional follicle in vivo is unclear. Germ cell precursors may continue to live in the ovaries of POI subjects but they cannot become functional due to damaged germ cell niche and failed microvasculature. Although spontaneous activation is rarely seen, there is no complete recovery in ovarian function of POI women. External stem cells application may restore ovarian function by adjusting the compromised environment. They can be administered directly into the ovary, or they can reach the ovary following infusion of stem cells through the veins (2,3). This improvement in ovarian function may be due to the healing of germ cells and their niches with the help of stem cell derived growth factors. Moreover, the given stem cells may restore compromised ovarian microvascularity. In accordance with our aboves suggestions infusion of stem cells allowed return of menses in the animal model of POI (3). Furthermore, spontaneous pregnancies were achieved after bone marrow derived stem cells (BMDSCs) infusion in experimental animals their gonads were damaged by chemotherapy to simulate poor responders or ovarian failure. Increased cellular proliferation, follicular density, estradiol production, ovarian neoangiogenesis, ovarian weight, primordial and antral follicle counts and decreased granulosa cell apopitosis have been reported during histopathological and hormonal evaluation of animal receiving BMDSCs (3). In same study, animals xenografted with human ovarian tissue of poor responders were given human BMDSCs. Histopathological evaluation of ovaries showed transferred stem cells were located near the vessels and granulosa cells. Moreover, increased ovarian stroma proliferation and blood vessel formation were noted (3).

Basic futures of POI ovary

Prior to stem cell transplantation, it should be determined whether the ovaries of POI cases have optimal conditions for stem cell seeding. It should be kept in mind that POI ovaries cannot be considered physiologically and morphologically as a healthy organ. The impairment in ovarian vascularization due to aging, gonadotoxines, or autoantibodies may lead to diminished ovarian reserve or low ovarian response (8). In accordance with this, women with DOR, low response or POI might be associated with a reduced ovarian blood flow (9). Hence, to obtain a successful result from stem cell transplantation POI ovaries must have a number of conditions such as good blood flow, sufficient amount of soluble factors, sufficient ovarian volume, good neural network, a healthy cortex and surface epithelium. All these allow the transferred stem cells to survive. In the absence of these conditions, the transferred cells will die within a short time of period.

Do the ovaries of POI or poor responder patients undergoing intraovarain stem cell transplantation (IOSCT) meet the above-mentioned conditions? Since the etio-

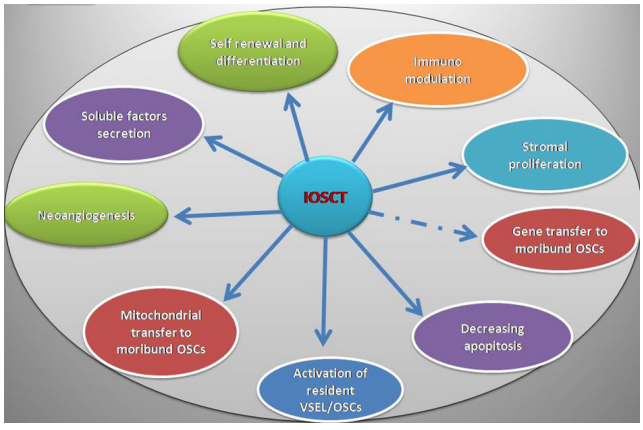

Figure 1. Schematic representation of possible healing effects of intraovarian stem cell transplantation (IOSCT) in gonads. When the stem cell is transplanted into the ovaries, they cause the above-mentioned changes (in circles). Most of the features other than gene transfer are believed to be performed by the stem cell therapy (dashed arrow). More details are given in the text.

logy of POI differs between patients, their ovaries are different from each other in terms of function and morphology. In accordance with this, two histopathological types of POF have been described (10). Afollicular form of POI (type 1) is characterized by complete depletion of whole follicle pool. In type 1 POI primordial germ cells either fail to develop or not present (11). In follicular POI (type 2), follicles or germ cell precursors are preserved somewhat, so either an induced or spontaneous ovulation is possible. It is bear in mind that the follicular form of POI can slowly or rapidly progress into the afollicular POI. Although numerous primordial follicles in their ovaries some women can show POI-like clinical condition (resistant ovary syndrome). In most cases of POI, the ovaries are often smaller than expected and cannot be palpated in the pelvic examination. Palpabl ovaries could be found uncommonly a small group of women with POI having large luteinised follicular cyst (12). Autoimmune oophoritis or 17-20 desmolase deficiency should be considered in this cases. Preserved granulosa cell with theca cell destruction and inflammation of follicle are main histopathological findings of autoimmune oophoritis (13,14). Due to many underlying defects, it is not logical to give stem cells directly to the POI ovaries. Therefore, correcting the impaired ovarian microenvironment with the use of growth factors (ovarian priming) and then performing stem cell transplantation will help to homing and proliferation of the transplanted stem cells (Figure 2 and 3).

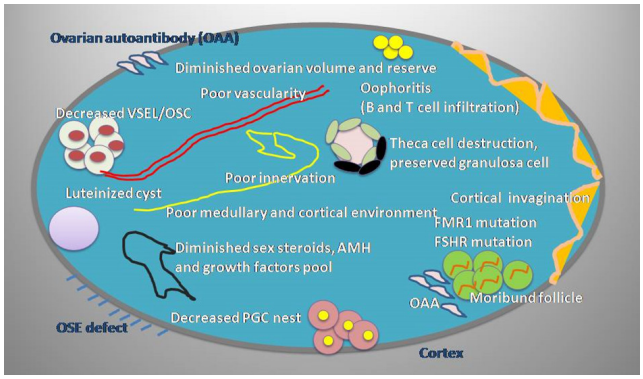

Figure 2. Schematic representation of morphological and histological changes in the ovaries of POI patients. Ovaries of POI patients are not normal morphologically and histologically. Decreased ovarian volume, decreased germ cell nest, dying germ cells, granulosa cell apopitosis, T and B lymphocyte infiltration, isolated lutein cysts and impaired vascular structure are the most basic findings.

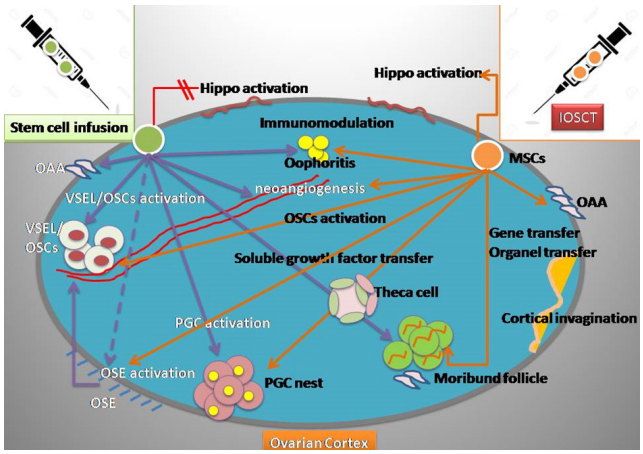

Figure 3. Schematic representation of possible curative properties of stem cell transplantation in POI patients. Stem cells can be administered into the ovary or intravenously. In both methods, stem cells reach the ovary and show similar healing properties. However, if the stem cell is introduced into the ovary, it will have a higher density than the infusion. This allows continuous release of soluble factor. Needle puncture of the ovaries during IOSCT may also activate the hippo signaling pathway and stimulate follicle development.

Ovarian priming with soluble factors before stem cell transfer

Infusion of stem cell increases neoangiogenesis and cell proliferation in different reproductive tissues. Concordantly, when stem cell was transferred to the endometrium of patients with Ashermen's syndrome, it increases endometrial vascularization (15). Likewise, basic impact of stem cell treatment in human and mouse ovaries, mimicking chemotherapy-induced poor responder and POI conditions, is the development of local vascular network (2,3). Local increase in vascularization may occur either due to the direct effect of the stem cell or the factors it secretes (15). Concordantly, soluble factors secreted by stem cells have a critical role in inducing ovarian neoangiogenesis (2,3,16). Decreased GCs apoptosis after stem cell transfer has also been reported (3).

Although the ovaries of women with POI contain OSCs, their stem cells niche get compromised with autoantibodies, gonadotoxines, and age. Therefore the spontaneous activation of residuel stem cells is rarely seen. Providing a healthy niche and microenvironmet by given soluble and growth factors before stem cell transplantation will allow transferred stem cells to live and differentiate to the germ cell precursors easily in the POI ovaries (17). We have some options for making the ovaries of POI women available before stem cell transplantation such as; intra-ovarian soluble factors transfusion (i), intra-ovarian platelet-rich plasma (PRP) transfusion (ii), small intestine submucosa (SIS) transplantation to ovary (iii). Transplanted stem cells in the ovary provide a sustained release of soluble factors into the systemic circulation (2,16). Hence, it is more logical to give soluble factors together with stem cells rather than pure ones. It is possible to give soluble factors to the ovaries by intraovarian PRP.

PRP is defined as plasma with a high platelet content (18). Cytoplasmic granules of platelet contain different kinds of proteins and growth factors including platelet derived growth factor, the epidermal growth factor, the insulin-like growth factor, the transforming growth factor beta, the vascular endothelial growth factor, the hepatocyte growth factor, the adenoside diphosphate, and the adenoside triphosphate (19). All these factors found in granules may act synergistically on host cells to promote compromised ovarian microenvironment. Another way of transporting growth factors to the ovaries is the use of small intestinal submucosa. Decellularized porcine SIS is a FDA-approved biological material used for tissue repair. Its collagen, glycoprotein, and glycosaminoglycan content may restorate surgically damaged ovarian tissues (20,21). Due to decellularized nature of SIS material the risk of immune rejection againist SIS is

negligible. One of the oldest studies of ovarian rejuvenation and new follicle formation belongs to our group. Unfortunately, our study did not attract enough attention. In our study conducted in 2009, we implanted porcine SIS in rabbit ovaries undergoing wedge resection. At the end of 16th week, we found that new ovarian tissue was formed on SIS bioscaffold. At 28th week, scattered primordial follicles were formed on the SIS graft (21). Because of the aforementioned properties, SIS can be applied to the ovaries of POI patients by grafting or homogenized manner. In addition to growth factors, its biological scaffold feature allows healing of damaged ovarian tissue and may contribute to the attachment and differentiation of transferred stem cells (Figure 2 and 3).

In cases of follicular POI, POR, or DOR, we can grow the follicles by giving some soluble factors because of small amount of follicles are present on the ground. On the other hand, infusion of some soluble factors into a follicle-free ovaries (afollicular POI, menapouse) does not meet our expectations. Since there are not enough follicles on the ground, it is not sufficient to give only growth factor to ovaries of afollicular POI and menopause patients. In order to generate new follicle on the ground in afollicular POI patients, stem cell transplantation is also required after soluble factor transfer, or growth factors and stem cells should be given together. Transporting sufficient amount of growth factors to the ovaries prior to stem cell transplantation may allow stem cells to better hold and proliferate in this primed ovarian environment.

In terms of our expectations from IOSCT, subjects with POI or DOR should not be put in the same pool. IOSCT can be done in these two groups of women, but the results are quite different. Because the ovaries of patient with DOR are better in morphological and physiological aspects than those in the POI patients, transplanted stem cells may live inside the ovary of DOR patients and transform to the germ cell precursors. There are already some healthy or dying germ cells in patients with DOR. By release of cell signaling factors transferred stem cell may allow host cells cling to life (22). Moreover, stem cells promote of host tissue repair through the transfer of healthy organelles including mitochondria (23). Since no germ cells or follicles are present in the afollicular POI, the transferred stem cell must be differentiated into the host's own stem cell and form new germ cells containing a haploid number of chromosome. Because follicular POI cases have a few germ cell precursors and follicles on the ground, in vivo resuscitation may be possible through transferred stem cells.

Stem cell mediated rescue of compromised germ cells

Mesenchymal stem cells (MSCs) have the potential to transform into many different cell types (24). They have capacity for self-renewal and proliferation in culture medium (25). Several molecules such as retionic ascid and bone morphogenic protein-4 have been used to differentiate MSCs into germ cell precursors (4). Excess and rapid follicle loss is a main reason for the occurence of POI caused by different etiologies. Accelerated follicle loss occurs through the inability to manage follicular selection and programmed cell death. It has been postulated that MSCs can transform into the cells equal with the cells of transferred tissue (4). The main mechanism of action how MSCs can stimulate dormant follicles in women with POI or POR remains unknown. The regenerative ability of MSCs may be related to soluble factors secreted by stem cells. Various growth factors and signaling molecules produced by transferred MSCs may be involved in the ovarian rejuvenation. MSCs therapy may also restorate compromised host tissue via gene shifting and metabolic rearrangement. It has been reported that stem cells can transfer their genes and some organelles to injured cells (6,7). In good agreement with this, recent study has demonstrated that MSCs can deliver mitochondria to damaged vascular cells (6). We, therefore, hypothesized that the stem cell-mediated mitochondrial transfer may be a possible path involved in restoration of compromised germ cell niche and ovarian microvasculature of POI women. If the MSCs transfer their healthy genes to the host cells, an increase in euploid embryo rates would be expected. Inconsistent with our expectation, study conducted by Herraiz et al. (2) reported that stem cell infusion did not modify embryo euploidy rates. This discrepancy may be due to short-term follow up period of patiens after MSCs transplantation. Since the follow-up period of patients ranged from 31 to 109 days, the low rates of euploid embryos should not mean that there is no mitochondria or gene transfer. If patients had been followed up for long periods after stem cell transplantation, an increase in the number of euploid embryos could be detected.

Paracrine and autocrine impuls in the gonads are necessary for normal folliculogenesis. Circulating levels of some soluble factors following stem cell infusion are correlated with ovarian reserve and IVF outcomes in poor responders (2). Since embryonic stem cells (ESCs), induced pluripotent stem cells (IPS) or MSCs possess the ability to differentiate many cell types one may think that intraovarian transfer of these cells may rejuvenate ovaries of POI women through residual stem cells via paracrine or autocrin communication. Oophoritis, gonadotoxines, autoantibodies and aging appear to compromise autocrine signals. In accordance with this, the ovaries of patients with POI are exposed to many autoantibodies, and gonadotoxines. As a result, the vascularization, stromal support and innervation required for the continuation of normal folliculogenesis are compromised in POI. Although the clinical symptoms of POI occur before the age of 40, we can add the destructive effects of advancing age. On the other hand, whether the transplanted stem cells transform into germ cell precurors, activate regeneration of unhealty or dying dormant follicles through paracrine or autocrine signals remained unresponsive (4,5). Soluble growth factors released by stem cells were accepted as a paracrine regulators for tissue regeneration (26). This hypothesis was supported a study conducted by Herraiz et al. They reported that circulating fibroblast growth factor (FGF)-2 and thrombospondin (THSP)-1levels were significantly increased in poor-responder patients who underwent stem cell therapy (2). POR women with a positive response to stem cell infusion had higher FGF-2 and THSP-1values than those without a positive response. Both molecules involve in the folliculogenesis and neovascularization

(2,16). A little more, FGF-2 is expressed in human follicles and stimulates estrogen production (27). Similar to FGF-2, THSP-1 promotes ovarian neoangiogenesis and follicle growth (28). On the other hand, other stem cell derived soluble factors such as PDGF and IGF-1 levels were not changed after stem cell transplantation (2).

The outcome of transferred stem cells

When stem cells transferred to the ovary they can behave in four different ways. They may die within a short time in the area of transfer (i). They live after the transfer, begin to multiply and differentiate to a certain stage (ii). Transfer the their genes and some organelles into the healthy or dying follicles or germ cell precursors (in vivo resuscitation) (iii). They can differentiate into the stem cell format (OSC) of the ovary (iv). The most important difference between transferring the stem cell to the ovary and transfer to other tissues is the need to halve the number of chromosomes in the ovary. If a stem cell does not perform haploidization, it is unlikely that the cell will not differ in the germ cell direction. If the stem cell transforms into OSC after transfer, one of the cells will be 2n and the other will have n chromosomes. While n-chromosome cell (haploid) provides conditions for new oogonia and follicle formation, oocyte transformation is not possible for 2n chromosome cell (diploid). If the transferred stem cells do not turn into OSC, it will not be possible to differentiate these cells in the direction of germ cells because all of the new cells to be formed will have 2n chromosomes (Figure 4).

Is it possible to design induced OSC (iOSC) for POI therapy?

In order for a cell to become a germ cell, it must fulfill a number of conditions. Half the number of chromosomes, surround itself with a certain number of somatic cells, initiate meiosis and complete the epigenetic shift. Stem cells transplanted into the ovary must perform each of these steps flawlessly to form a new germ cell. With our current knowledge, we do not know which stem cell type is performing these steps exactly. We know that new germ cells are formed after ESC or IPS cell transplantation. However, we do not know whether ESCs or IPS cells are transformed directly into germ cells or into host stem cells. Although the transfer of ESCs or IPS cells to the ovary may result in new germ cells, these cells must be differentiated in the germ cell direction before transplantation. New oocytes may only occur after directed ESC or IPS transfer, but these cells can induce tumor growth (Figure 5).

Oocyte-forming stem cells within the ovary of female mice were named as OSCs. Zou et al. (29) isolated OSCs from postnatal mouse ovaries and transplanted them into ovary of recipient female mice. This process allowed the birth of live offspring (29). Three years later, White et al. (30) generated new oocyte by using germ cell precursors obtained from ovaries of reproductive-age women. In addition to OSCs, the presence of small group of stem cells named very small embryonic-like stem cells (VSELs) in adult human ovary has been reported (31). VSELs are pluripotent in nature and located within the ovarian surface epithelium (OSE) (32). VSELs contain receptors for follicle stimulating hormone. The size of the VSELs is 2-4 µm and slightly smaller than the OSCs. They can be produced in cultures made from ovarian surface epithelial swabs of both POF and postmenopausal patients (33,34). Similar to primordial germ cells (PGCs), VSELs/OSCs are the ideal candidate cells for getting gametes from adult female gonads. VSELs give rise to primordial germ like cells

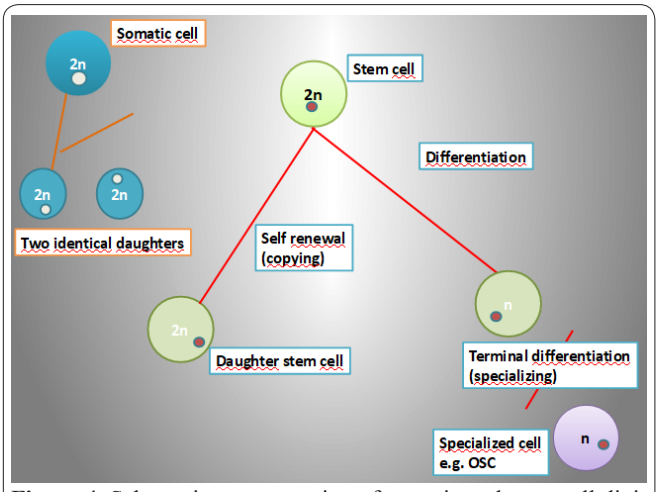

Figure 4. Schematic representation of somatic and stem cell division and differentiation. Resident stem cells have the capacity to propagate themselves through symmetric divisions and to divide asymmetrically to engender new cells that can progress to differentiate into tissue-specific, terminal cell types such as oogonial stem cells (OSCs).

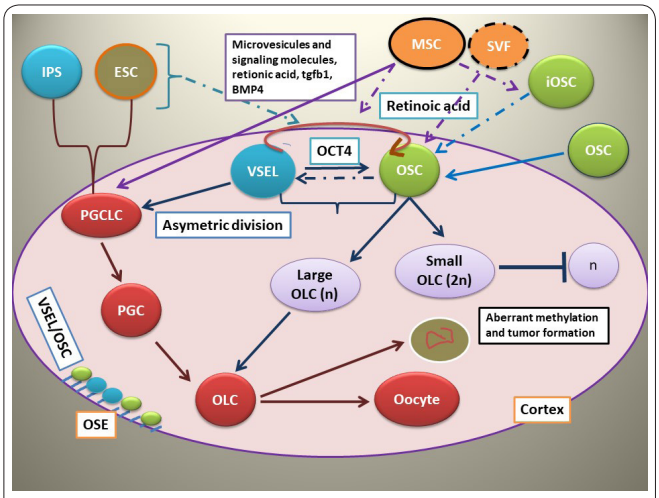

Figure 5. Schematic representation of possible differentiation pathways of different stem cell types after transplantation into the ovaries. We can transplant any type of stem cell into the ovary. However, not every stem cell creates a new egg cell. While ESCs and IPS cells form a new egg cell, MSCs can be transformed into host stem cells with the help of factors such as retionic ascid and BMP-4. ESCs and IPS cells convert to primordial germ cell like cell (PGCLC) to form new oocytes. OSCs are first transformed into large oocyte like cells (OLC), followed by new oocytes. VSELs and OSCs have the potential to differentiate among themselves. VSELs can be converted to both OSCs and PGCLC. There is a risk of tumor development and methylation defects after ESCs and IPS cells transplantation. iOSCs will probably exhibit an OSC-like differentiation. Dashed lines indicate unclear paths of differentiation. This drawing is made in the light of the information in the text. More details are given in the text.

(PGLCs) via asymmetric cell division. When cultured in suitable conditions VSELs/OSCs undergoe proliferation and differentiation into oocyte-like structures (OLC) (35). However, the main function of VSELs-derived oocyte-like structures is not known (Figure 5).

Haploidization stage of the transferred stem cell in the ovary is the most difficult stage for obtaining new germ cell precursor. We do not have clear scientific evidence that MSCs have halved the number of chromosomes. The only cell type that meets these requirements is OSCs. It can reduce the number of chromosomes in half and does not lead to tumor formation. When MSCs are divided two new cells with "2n" chromosomes occur. In contrast to MSCs, when OSCs are divided, one cell with "n" chromosome and one cell with "2n" chromosomes consist. While cells with "2n" chromosomes do not differ in the direction of germ cells, cells with "n" chromosomes are transformed into germ cells (Figure 4). Limitations in the use of ESC, IPS cells and MSCs due to different handicaps intraovarian transfer of the host's own stem cells such as VSELs/OSCs seem to make more sense. However, there are a number of problems related to VSELs/OSCs isolation and reproduction. Although the ovarian surface epithelium of POI patients contains VSELs/OSCs, numerical scarcity and scattered placement of these cells make them difficult to obtain (4,5,30,36,37).

We have two alternatives to get VSELs/OSCs. The main option we have to obtain OSCs is to take the ovarian cortical tissue or scraping from the surface epithelium via laparoscopy (L/S) and generate OSCs from the obtained samples in vitro (33,34). Laparoscopy is both invasive and difficult to repeat and recurrent L/S may lead to some complications. Alternatively, ovarian cortex tissue can be obtained via transvaginal needles. Although this method is less invasive than L/S, its effectiveness in tissue harvesting is not clear. Actually, we do not know how effective the transvaginal needles are in taking a biopsy in a small and hardened POI ovary. Moreover, ovaries of women with POI may be unsuitable for differentiation of VSELs/OSCs to form new follicle. For all these reasons, it is obvious that it is necessary to produce OSCs in a repeatable, easy and inexpensive way. We can solve this problem by producing induced OSC (iOSC). iOSC is a hypotetic concept that we put forward. Similar to IPS cells, we can generate iOSC by transferring some specific genes to somatic cells or MSCs. The most important scientific support behind this suggestion and/or hypothesis is the design and development of IPS cell shortly before (38). If we can reprogram the human somatic cells or MSCs in the direction of the VSELs/OSCs, the new cells formed will be the induced OSC (iOSC).

Directed differentiation of iOSCs

Although the mechanism we propose in the production of iOSC is somewhat speculative, it has been written in the light of the scientific data used to generate IPS cells. Similar to the acquisition of IPS cells, iOSCs may be produced by reprogramming somatic cells or MSCs. Following isolation, somatic cells or MSCs are cultured in vitro and genes specific to OSC can be transferred to MSCs to produce iOSC. The basic question we encounter at this point is what factors we can transfer to the MSCs or somatic cells to generate iOSC?

To find the answer to this question, we need to know the OSCs specific transcription factors. DEAD-box polypeptide 4 (DDX4) is a germ cell protein (36,39,40). All germ cells carry DDX4 protein in their cytoplasm. However, OSCs carry the DDX4 protein as externalized manner (41). In addition, gene profiling-based characterization of oocytes originating from OSCs showed the existence of meiosis-specific DNA recombinase, dosage suppressor of mck1 homolog (DMC1), and the meiotic recombination protein, SYCP3, in the human OSCs cultures (30). Another candidate transcription factor to generate iOSC is OCT-4. There are two types of stem cells, VSELs and OSCs, in ovary surface epithelium. While VSELs contain nuclear OCT-4, OSCs contains cytoplasmic OCT-4 (32). VSELs have also ability to differentiate the OSCs. For this reason, at least five transcription factors (ecDDX4, DNA recombinase, DMC1, OCT-4, and SYCP3) should be transferred into somatic cells or MSCs to produce iOSC. Expression of these five genes induces events of silencing in somatic cells or MSCs (Figure 6). White et al. (30) showed OSCs of human or mouse can be transformed into haploid (n) cells in vitro. Hence, intraovarian transfer of iOSCs may generate oocyte like cell with haploid chromosome.

Which type of stem cell should be preffered in POI treatment? Why?

Stem cells can be obtained from embryonic, somatic organs or bone marrow. In addition, stimulated pluripotent cells can be obtained by transferring some genes to somatic cells. As a result, we have MSCs, BMDSC, ESCs, IPS cells, VSELs and OSCs type stem cells to transplant them to damaged ovaries of POI subjects (4,5). Although there is a developmental link between all stem cells, it should be noted that the differentiation potentials are quite different from each other (4,5).

When choosing which of these cells will be used for

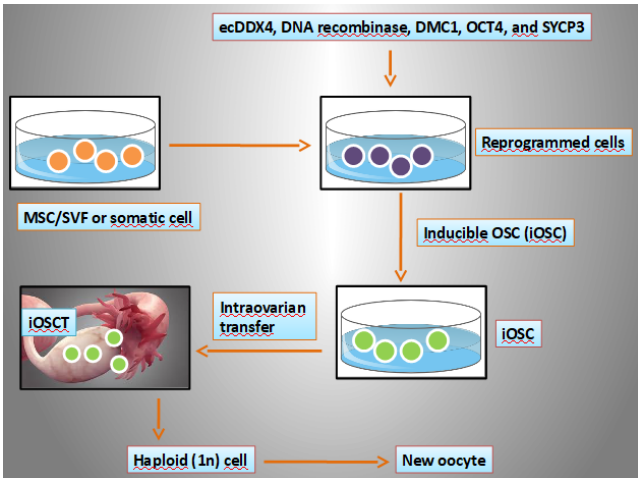

Figure 6. Schematic representation of the reprogramming of MSCs or somatic cells for the acquisition of induced oogonial stem cells (iOSCs). Similar to the acquisition of IPS cells, iOSCs may be generated by reprogramming adult somatic cells or MSCs. Following isolation, somatic cells or MSCs are cultured in vitro and transduced with expression vectors (ecDDX4, DNA recombinase, DMC1, OCT-4, and SYCP3) encoding transcription factors associated with OSCs. More details are given in the text.

transplantation to the ovaries of POI or POR subjects we should consider the following features:

Is the method of obtaining stem cells easy and cost effective?

Are the homing and differentiation capacity of transferred stem cell sufficient?

Can stem cells be transplanted without differentiation?

Does the transplanted stem cell lead to aberrant methylation?

Does the transplanted stem cell lead to tumor growth?

Can the transplanted stem cell have ability to transform into the host's stem cell to form a new germ cell?

Although compramised ovarian tissue contains germ cell precursors they may not be functionally active. Adequate and well differentiated stem cell transplantation may correct the damaged areas to some extent and enable the ovary to become functional again. In limited number of studies, the compromised microenvironment was corrected by the transplantation of different kinds of stem cell and successful results were obtained (2-5,42). Treatment with ESC, IPS and BMDCs contribute to the formation of functional gamets in animals with chemotherapy-induced ovarian damage. Likewise MSC treatment reverses the impaired follicular development (4,5).

The most logical approach to obtain new gametes is to use the host's own germ cell precursors or their own stem cells. The main purpose of using these cells is that they do not require differentiation. The biggest handicap related to these cells is the difficulty in obtaining them and their small number. VSELs, multilineage differentiating stress stem cells and OSCs are types of stem cells in the ovaries that have the potential to turn into gamete. Despite their different names, these cells are very close to each other in terms of their genetic content. For example while VSELs contain nuclear OCT-4 OSCs contain cytoplasmic OCT-4 (32). MSCs are capable of migrating and settling in damaged gonads (3,4). Production of competent oocytes and viable offsprings following MSCs treatment have paved the way for these cells to be used in treatment of women with POI and animal model of POI (4). Transplanted MSCs may differentiate into VSELs/OSCs that allow for the regeneration of new gametes. MSCs have also been reported to stimulate VSELs through secreted factors and paracrine signals. However, we do not know clearly whether MSCs make germ cells either directly or by differentiating into host stem cells. Since VSELs contain FSH receptors, their number may increase with rFSH treatment. Because VSELs cells are detected in both POI, postmenopausal ovaries and the ovaries of women using chemotherapy, it seems to be the first preferred cell type for POI treatment (33-37).

It is a well known fact that ESCs and IPS cells are transformed into many cell types including oocyte. Therefore, instead of OSCs, the transplantation of ESCs or IPS cells directly to the ovary can also be considered. However, ESCs or IPS cell transplantation may not be as innocent as it may seem. Successful differentiation of human ESCs and iPS cells to germ cell precursors has been reported (29,43). Because of their preserved epigenetic memories and somatic nature, the conversion of IPs cells into germ cells was reported to be better than ESCs (44). Unfortunately, both risk of aberrant methylation and making germ cell tumor use of intraovarian transplantation of ESCs are restricted (45). The advantages of intraovarian OSCs/VSELs transplantation instead of ESCs or IPS cells are as follows: (i) Because VSELs/OSCs are germ cell-derived they do not need to be guided in the germ cell direction prior to transplantation. In contrast, ESCs or IPS cells must be differentiated in the germ cell direction before transplantation. (ii) During the generation of germ cells from VSELs/OSCs, the mtDNA bottleneck is preserved. In contrast, during differentiation of ESC or IPS cells into germ cells, the process of mtDNA bottleneck may not be preserved. (iii) Since VSELs/OSCs are unipotent, there is little risk of tumor transformation in the region where they are transplanted (31-37).

Conclusion

ESCs or IPS cell transplantation to ovary may be a promising approach but embryonic cell-like properties of these cells can lead to tumor development in the transplanted tissue. In addition to potential for tumor development defect in epigenetic arrangement and normal meiosis can occur in the germ cells derived from transplated stem cells. Tumor development is not expected after intraovarian OSCs/VSELs transplantation. MSCs or iOSC may be a candidate stem cells for POI treatment since they can be obtained from the POI women themselves. Unlike ESCs or MSCs, iOSCs are more easily obtainable for therapy, and their generating and use in POI do not carry the ethical obstacle. If their design is possible, intraovarian transfer of iOSCs may open up a new era in the treatment of patients with POI or DOR. This treatment strategy may also allow therapy of azoospermia patients.

Conflicts of interests

The authors declare that there are no conflicts of interest.

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