Rewiew

Ovarian Stem Cell Nests in Reproduction and Ovarian Aging

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Key Words

Ovarian stem cells • Nests • Reproduction • Aging

Abstract

The fixed primordial follicles pool theory, which monopolized reproductive medicine for more than one hundred years, has been broken by the discovery, successful isolation and establishment of ovarian stem cells. It has brought more hope than ever of increasing the size of primordial follicle pool, improving ovarian function and delaying ovarian consenescence. Traditional view holds that stem cell aging contributes to the senility of body and organs. However, in the process of ovarian aging, the main factor leading to the decline of the reproductive function is the aging and degradation of ovarian stem cell nests, rather than the senescence of ovarian germ cells themselves. Recent studies have found that the immune system and circulatory system are involved in the formation of ovarian germline stem cell niches, as well as regulating the proliferation and differentiation of ovarian germline stem cells through cellular and hormonal signals. Therefore, we can improve ovarian function and delay ovarian aging by improving the immune system and circulatory system, which will provide an updated program for the treatment of premature ovarian failure (POF) and infertility.

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Introduction

Stem cell niche is also called stem cell micro-environment. Scientists have thoroughly researched the structure and functions of the stem cell niche in the hematopoietic system, intestinal system, neuronal cells, spermatogonia and the *Drosophila* ovary system [1-4]. However, further research remains to be conducted on the impact of mammalian ovarian stem cells and their aging on the ovarian nest functions. This paper discusses the composition of the mammalian ovarian stem cell nests, their characteristics and their influence on senescence of the female reproductive function.

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Research background and characteristics of ovarian germ stem cells

The traditional view, which has lasted more than half a century states that the oocytes in major follicle pools of female mammals are fixed after birth and begin to decrease after puberty until they are depleted [5, 6]. However, in 2004, Johnson et al. [7] observed that the atresia follicles have a higher occurrence rate than reduction rate when they measured the number of the mouse follicle atresia conditions on different days. Therefore, he speculated that the ovary contains proliferative cells, which challenges the fixed follicle pool theory that monopolized reproductive medicine for a long time. Since then, scholars have used different methods to verify this discovery independently. Although there are a few scholars who doubt the mammalian ovarian stem cells theory [8, 9], researchers have verified the existence of ovarian stem cells in several mammals including humans using methods including stem cell isolation and purification, *in vitro* and *in vivo* transplantations, transgenic lineage tracing method, etc [10-18].

Ovarian germ stem cells are derived from bi-potential stem cells in the ovarian cortex and can produce new stem cells by symmetric division or differentiate into ovarian reproductive cells and primitive granular cells via asymmetrical division [19]. Ovarian germ stem cells appear round under a light microscope, with a diameter between 10-15 μm . They are extremely rare and can be seen as groups or clusters when subcultured (Fig. 1). Additionally, they can express MVH, OCT4, Nango, Fragilis, Stella, C-kit and many other markers of reproductive cells and stem cells as determined via PCR. After obtaining training in Professor Ji Wu's laboratory of Shanghai Jiao Tong University and improving our culture techniques, our group has been able to expertly separate, purify and culture female ovarian stem cells, resulting in a solid foundation for female reproductive stem cells research [20-22].

The composition and the biological characteristics of ovarian stem cell nests

The stem cell niche is the micro-environment where the surrounding stem cells survive, a concept that was proposed by Schofield [23] during a study of hematopoietic stem cells. He later found similar niche structures in the digestive system, nervous system, hair follicle glands and the sexual glands [24].

Fig. 1. Immunofluorescence of germ cells cultured *in vitro* with MVH and OCT-4. (Green: MVH; Red: OCT-4; Blue: Hoechst; Black: Bright field; Purple: Merge; Scale bars: 10 μm).

The stem cell niche is composed of niche cells, extra-cellular matrix and cytokines [25], and plays an important role in the histogenesis of the body's early development and tissue repair after birth. Ovarian stem cell nest, one of the indispensable components of ovarian stem cell niche, which is mainly composed of granulosa cells, vascular endothelial cells, immunologically relevant cells and molecules, surrounds the ovarian reproductive stem cells and regulate their functions (Fig. 2). Bukovsky [26] observed that the ovarian stem cell nest has been formed in the early embryonic development period and is composed of non-site specific ovarian Monocyte-derived cells (MDCs), T cells and vascular endothelial cells whereas the adult ovarian reproduction stem cell nest is made up of primary MDCs (CD14+ MDC), activated MDCs (HLA-DR+ MDC), T cells, etc.

Relevant immunological cells and molecules in the ovarian stem cell nest are involved in the OGSCs' asymmetric division, contributing to the production of germ cells and regulating their symmetrical division, migration, the formation of new granule cells, as well as fetal and adult primordial follicles, which promote the selection and growth of the primordial follicles, including the formation of dominant follicles and subsequently, maintain the normal functions of ovaries. The conclusions are based on the following phenomenon of ovarian [27-29]: the immune relevant cells in the ovary rete tunnels, are necessary for the formation of ovary by OGSCs. Human embryonic ovary is determined by the evolution of the ovarian stem cells in the genital ridge peritoneal mesothelium, formed from the rete ovary in the mesonephric duct. Follicular development usually begins in the innermost layer of the ovarian cortex near the ovary rete, which is necessary for the development of ovary. Additionally, the ovarian network channels contains many immune system related cells such as some small and not presented mononuclear cells (MDC), which can be divided into a large number of activated MDC, responsible for the formation of ovary from the ovary rete with T cells. The immune cells are an important part of the OGSC nest and trigger the asymmetric fission of OGSCs. Fetal ovarian germ stem cell nest is composed of primordial germ cells, non-presenting monocytes and T lymphocytes. OGSCs (uncommitted OGSCs, u-OGSCs) are produced during the sixth week of pregnancy, before the arrival of the primordial germ cells (Primordial germ

Fig. 2. The composition and function of ovarian stem cells nests. Ovarian stem cell nest is mainly composed of granulosa cells, vascular endothelial cells, immunologically relevant cells and molecules, surrounds the ovarian reproductive stem cells and regulate their functions. A: Ovarian germ stem cells self-proliferation B: Ovarian germ stem cells differentiate into granulosa cells C: Ovarian germ stem cells differentiate into ovarian epithelial cells that can compensate for ovarian epithelial loss when ovulation D: Ovarian germ stem cells differentiate into primordial follicles E: Primordial follicular development to follicles at all levels.

cells, PGC) after which, the primordial germ cells invade the OGSC layer in the seventh week. OGSCs can only divide into secondary germ cells through asymmetric fission under the influence of both cellular signals (cellular signaling, CS; such as CD14 secreted by MDC and CD8 secreted by T lymphocytes) and hormonal signals (hormonal signaling, HS hCG; human chorionic gonadotropin and estradiol E2). Subsequently, the secondary germ cells enter the ovarian cortex and finally differentiate into oocyte cells (definitive oocytes, DO). The amount of OGSCs that are presented by MDCs and T cells determines the number of germ cells. The immune cells can also trigger the symmetrical fission of OGSCs and begin follicular growth until the decline of the immune system finally leads to the completion of follicle formation. When the primary CD14+MDC cells and T lymphocytes sometimes induce the OSE triggering the germ cells' symmetric division, the function of the immune system declined obviously between 35-40 years old and at the same time the follicles renewal stops.

The effects of the ovarian stem cell nests aging on the ovarian aging

Ovarian senescence is a complex physiological process involving the interaction and gradual accumulation of several factors, of which the most important aspects are the reduction in the number and quality of ovarian follicles [30, 31]. Ovarian aging occurs faster than the aging of other organs, which not only affects reproductive health but also causes serious perimenopausal conditions and many age-related diseases [32-34], such as cardiovascular disease, cancer, osteoporosis, Alzheimer's disease, obesity and so on.

The old view states that the aging of the body and organs is due to the senility of the stem cells [35-37]. However, in recent years, some scholars indicate that the aging problem is more closely connected with the ageing of the stem cell nests [38, 39]. In senescent individuals, in spite of the stem cells, the deterioration of the nest will finally result in the imbalance of the body's systems [40]. With increasing age, ovarian function cannot be maintained, despite the ovarian germ stem cell activity [41]. Niikura and his team discovered that the meiotic specific markers *Stra8* and *Dazl* are highly expressed in non-ovulating older female mice [42]. Other scholars have successfully separated ovarian germ stem cells from the ovaries of the old rats, infertile mice and mice with premature ovarian failure [12]. Therefore, ovarian functions have are not directly associated with the existence of ovarian stem cells. In addition, after transplanting ovarian tissue of ageing mice into the ovaries of young mice, the primordial follicles were positive for GFP expression in the receptor mice; however, transplanting ovarian tissue of young mice into elderly mouse reduced the number of immature follicles in the transplanted tissue of the young mice [43]. These results indicate that ovarian function decline is mainly due to the aging of the ovarian germ cell nests but not due to the aging of the ovarian germ stem cells.

Factors related to ovarian stem cell nests aging

Ovarian germ stem cells belong to the category of adult stem cells. Currently, the majority of scholars believe that there are two mechanisms that contribute to adult stem cell aging. One of them is the endogenous mechanism, whereas the other one is related to the exogenous micro-environment (Fig. 3), which includes factors such as DNA damage, telomere shortening, elevated levels of reactive oxygen species, epigenetic modifications, changes in cell polarity, metabolism, micro-environments aspects and so on [44-48]. The aging of the exogenous micro-environment plays important roles both in leading to and accelerating the ovarian reproductive aging. The ovarian stem cell nests, one of the indispensable components of ovarian stem cell niche, is composed of intervascular compartments that connect the stem cells with the immune system and the circulatory system [49]. Thus, any functional change in the immune and circulatory systems may have an effect on the aging problems of the ovarian stem cells nests.

Fig. 3. Schematic diagram of the mechanism of adult stem cell senescence. (Modified from Jung Y, Brack AS, Cellular mechanisms of somatic stem cell aging. *Curr Top Dev Biol*, 2014, 107: 405–438.).

In 2005, Johnson et al. [50] proposed the existence of circulatory system stem cells that can replenish the follicle pools in the bone marrow and peripheral blood of adult female mice. Once this concept was proposed, several scholars questioned it. Kevin et al. [51] did not find the phenomenon of egg chimerism via the circulatory system in mice. In addition, after the CT-treated mice underwent the bone marrow transplantation, they still could not produce eggs. Therefore, Kevin successfully argued against the theory proposed by Johnson. In 2007, Lee [52] noted that the bone marrow transplantation could recover the reproductive functions of the CT treated infertile mice, but the resulting offsprings originated from the recipient mice germ line. Subsequently, some scholars stated that the bone marrow transplantation could repair damages to the ovarian functions caused by the chemotherapy drugs [53, 54]. In recent years, researchers have found that ovarian stem cells are always accompanied by a few, very small, embryonic stem cells in the adult mammalian ovary [55–57], and these human embryonic stem cells are found in the peripheral blood of young, middle-aged and elderly people [58]. Virant et al. indicated that there is a potential connection between the VSELs and the germinal system as well as the hemapoiesis reconstruction process [59]. Veronique et al. [60] found that the “companion” blood cells are essential for the formation and the steady state of the ovarian germ stem cell nests. Therefore, we can conclude that the circulatory system is important for promoting ovarian functions.

The relationship between the immune system and the ovarian functions was discovered early, but little attention was paid to the effect of the immune system on the ovarian germ stem cell nests. Nishizuka et al. [61] found that mice developed ovarian hypoplasia after the removal of thymus in 2 to 4 day old, new-born mice. Russell et al. [62] discovered that thymus from normal female mice can suppress the superovulation induced by cyclophosphamide and X-rays. In 1979, Bukovsky [63] proposed for the first time, that the immune system played an important role in the regulation of ovarian function. In recent years, some scholars found that intramuscular injections of thymosin to new-born nude mouse can maintain the levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in the serum during the time when the mice developed into adults, and additionally, they can inhibit ovarian hypoplasia of the nude mice [64]. In addition, researchers also found that with increasing age, the immune system inevitably degraded. First, the thymus function declines followed by

a decline in ovarian function [27]. These results demonstrate that there is a close relationship between the immune system and the ovarian function. However, additional studies are needed to understand the exact changes in the ovarian stem germ cell nests brought about by the aging immune system as well as the specific mechanisms that are involved.

The clinical significance of regulating ovarian germ cell nests aging

People suffer from premature ovarian failure (POF) or perimenopause can improve the function of immune and circulatory system through diet, exercise and other health care products, which can indirectly provide a suitable micro-environment for aging ovaries. Therefore, the ovarian germ stem cells can remain active and continue to replenish the follicle pool. These measures can delay the female genital aging and relieve the symptoms associated with perimenopausal ovarian aging and other related senile diseases.

Through freezing eggs in the ovum bank before chemotherapy and radiotherapy, female adults can adopt assisted reproductive methods to remedy the infertility problems caused by cancer treatments. However, this method is not applicable for the pre-pubertal patients because oogenesis has not yet been initiated. For these patients, ovarian tissue can be partially resected before the cancer treatments and subsequently cryopreserved in the form of digested tissue suspension or single cell suspension. After these cancer patients completed their treatments, they can regain ovarian functions after puberty through transplantation of the frozen suspension liquid into the left ovary.

Summary and prospect

Aging is closely related to stem cell senility. In ovarian aging, the senility and degradation of the ovarian stem cell nests are thought to be the main factors leading to the decline of ovarian reproductive function, more so than the ovarian germ stem cells caducity. The immune and circulatory systems play vital roles in ovarian stem cell nests aging, however, the specific mechanisms need to be studied further. More recently, these factors have increasingly drawn people's attention to the decline in female reproductive function declining and perimenopausal related diseases caused by aging. Additionally, with the introduction of the two-child policy, it is of great significance to explore the mechanisms of ovarian aging. Furthermore, the study of delaying the ovarian aging process indirectly by improving immunity could provide us with useful information.

Acknowledgements

The authors would like to thank Dr. Liping Zheng and Dr. Yuehui Zheng for article mentoring, , We also thank the members in our lab who participated in this article. This work was supported by the National Nature Science Foundation of China (No. 81671455, 81360100, 81771583, 81260098, 81660245), the Natural Science Foundation of Jiangxi province (No. 20152ACB20023, 20161BAB205207, 20161BAB205213, 20153BCB23031).

Disclosure Statement

The authors declare no conflicts of interest.

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