

Ovarian stem cells and neo-oogenesis: A breakthrough in reproductive biology research

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Abstract

The concept of ovarian stem cells which can replenish the ovarian reserve in postnatal mammalian females is a revolutionary breakthrough in reproductive biology. This idea overturned the central dogma existed in female reproductive physiology. Contradicting the popular belief that oogenesis does not occur in post natal life, researchers proved the existence of putative stem cells in ovary, which can supply functional follicles in post natal ovaries. Even though the idea of neo-oogenesis in postnatal ovaries in normal conditions is controversial, the isolation and manipulation of ovarian stem cells have got tremendous application in medical, veterinary and animal production fields.

Key words: - Germline stem cells (GSC), Ovarian stem cells, Neo-oogenesis

Introduction

The central dogma of female reproductive biology is that female mammals are born with fixed number of non renewing pool of germ cells (Zuckerman S., 1951). According to this concept, oogenesis ceases around birth and the ovarian reserve of oocytes will deplete gradually by the process of atresia, where as male mammals continuously produce sperm from a store of stem cells. Interestingly, females of lower species, such as flies, fish and birds, has got the capacity to generate new oocytes during adult life. The ability of adult females to produce new eggs was believed to be lost during evolution prior to the emergence of mammals. In invertebrates like *Drosophila melanogaster*, germline stem cells maintain oocyte production in adult ovaries. However, in some primates, oogenesis continues after birth. In *Macaca fascicularis*, a marker, considered as exclusively expressed in pre-meiotic germ cells, could be detected 12 days after birth. In prosimian monkeys, the adult ovary contains proliferating oogonia and meiotic oocytes in medullary nests where in humans, structures observed up to the age of 3 years, disappearing thereafter. But the ability of males to produce sperm throughout adulthood was conserved through evolution from flies to man.

Neo-oogenesis in mammalian ovary

An innovative study was published in Nature by

a group of scientists from Harvard Medical School (Johnson et al., 2004). In this study, the authors claimed that in adult mouse ovary, neo-oogenesis takes place and new follicles originate from germline stem cells present in the ovarian surface epithelium. It was a provocative claim that challenged the central dogma in reproductive physiology. Johnson et al made their conclusions of oocyte and follicular renewal from existing germline stem cells (GSC) in the postnatal mouse ovary based on following observations. They noticed some discordance in follicle loss versus follicle atresia in the neonatal and adult ovaries. The rate of follicular atresia in mouse ovary is so high that mouse ovaries should be almost devoid of any healthy follicles with in 6 week itself. But ovaries supplies normal number of follicles in their reproductive life. They suggested occurrence of neo-oogenesis in ovaries to explain why ovaries are not completely depleted at this age despite of high rate of atresia. Additionally immunohistochemical detection of proliferating GSC with meiotic capacity using combined markers for meiosis, germline, and mitosis indicated germ cell proliferation and follicle renewal in ovaries. Finally experiments with ovarian chimeric grafted adult mice pointed the existence of neo-folliculogenesis.

Germ cell progenitors from bone marrow

One year later, Johnson et al modified their

earlier view that neo-oogenesis takes place during adult life in the mouse ovary. However, egg cells no longer arise from the ovarian surface epithelium but from bone marrow and other circulating cells. In their experiment, 36 h after a doxorubicine treatment, which destroy germinal cells within 24h, spontaneous regeneration of the immature follicle pool was observed. The team showed evidence for the existence of germ cell progenitors, putative stem cells, in mouse bone marrow and blood. In addition, the researchers also found these markers in human bone marrow and blood. Remarkably, they found that bone marrow or blood cell transplants appear to completely revive the ovaries of female mice sterilized by chemotherapy. Just 24 hours after a transplant, the sterilized mice had new egg cells and follicles, the nurturing group of cells that encloses each egg cell. Two months after bone marrow transplant, the ovaries of normal mice and mice that had undergone chemotherapy appeared nearly identical. They discovered expression of a germ cell marker in bone marrow fluctuated regularly with the female mouse's estrous cycle, much like the cyclical rise and fall of certain hormones. Further results suggest that the ovary itself is sending out a chemical signal to the bone marrow, readying the progenitor cells to travel to the ovary and restock its egg cell supply.

Controversies on Neo-oogenesis

Naturally this study evoked a huge wave of controversy in the following years. Laboratories around the world have taken up the challenge to dogma raised by this initial report, either to test this concept in an experimental basic science setting or give direction to clinical applications that could result. Byskov in 2005 questioned Johnson's arguments. He stated that, the cells, considered as germline stem cells by Johnson, are resting follicles leaving the ovary as a component of the resting pool depletion and provided evidence to suggest that an overestimation of atretic follicle number contributed to Johnson et al.'s conclusions.

Bristol-Gould et al in 2006 showed that follicle numbers gradually decline in the mouse ovary with age and used mathematical modelling to determine that de novo follicle production is not required to support fertility. Eggen et al in 2006 used parabiotic mice to show that ovulated oocytes do not come from circulating stem cells, and also indicated that chemotherapy does not destroy all oocytes which may resulted in false result of follicular renewal.

In 2005 Oktay et al reported spontaneous

pregnancy in presumably sterile human patient following chemotherapy, haematopoietic stem cell transplantation and ovarian tissue transplantation. At the same period Professor Bukovsky and his team at the University of Tennessee reported that they had obtained ovarian stem cells from five women aged 39 to 52. They cultured the cells for five days until they developed into eggs "suitable for fertilisation".

The study by Lee et al (2007) remains very intriguing in that it shows fertility rescuing in recipients treated by chemotherapy followed within one week by bone marrow transplantation and mating. When bone marrow transplanted and/or mating was delayed, the fertility rescuing was strongly decreased. Importantly, high doses chemotherapy did not allow substantial fertility rescuing. This study indicates the presence of unidentified factors/cells in BM, either stimulate resting follicles, not destroyed by the cytotoxic compounds, to enter growth phase, or initiate "putative" germinal stem cells, already present in the ovary, to undergo changes leading to a neo-oogenesis. Finally, one morphological study supports the neo-oogenesis by Kerr et al. 2006 Showed that follicle numbers remain constant in mouse ovaries from puberty to early mid-life, suggesting possible follicular renewal.

Liu Y et al in 2007 demonstrated the absence of early meiotic-specific or oogenesis-associated mRNA adult human ovaries and lack of early meiocytes and proliferating germ cells led to the conclusion that neo-oogenesis does not take place in the adult human ovary. According to a study by Irma et al in 2008 no evidence was found to support the hypothesis that progenitor cells from extra-ovarian sources can repopulate the adult ovary. The findings are consistent with the conventional view that a limited number of oocytes are formed before birth and declines with age. The study did not, however, rule out the possibility that germline stem cells may reside in the adult ovary. French scientist Alain Gougeon, said "I feel that in normal conditions, neo-oogenesis does not take place in the adult ovary, however, experimental conditions such as BM or peripheral blood transplantations, might induce some unexplained events leading to fertility rescuing in experimentally-induced premature ovarian failure".

Production of offsprings from ovarian stem cells

Ji Wu at Shanghai Jiao Tong University in China, in 2009, came with a fantastic result giving a boom in ovarian stem cell research. They produced

offspring from a germline stem cell line derived from neonatal ovaries. First, they identified candidate stem cells by testing ovarian tissue for mouse vasa homologue (MVH), a hormone exclusively found in germ cells. Next, they fluorescently tagged the MVH-containing cells, extracted them, and cultured them. They then implanted the cells from adult mice into the ovaries of mice whose eggs had been chemically destroyed. Mice with the implanted cells formed new eggs and went on to have healthy pups from those eggs.

Though the experimental results are fantastic it is not clear that adult mammals actually form eggs as part of their natural life cycle. Some researchers doubted the cell culture process can change the developmental potential of cells so the cells they implanted could be fundamentally different from those that spent all their time in a mouse.

Applications of ovarian stem cell technology

The outcome of overall research works on ovarian stem cells are supposed to create revolutionary changes in female infertility treatment. Ovarian stem cells will provide opportunity to develop oocytes from patients whose follicle reserve has been depleted by anti cancer chemotherapy or normal menopausal changes. In the field of animal reproduction, as the works all over the world progress, the knowledge on ovarian stem cells will be more lucid enough to cause emergence of technologies for identification, extraction and manipulation of ovarian stem cells which have awesome number of applications. Definitely, the production of infinite number of offsprings from a stock of ovarian stem cells from a superior dam through IVF will be possible with less labour and high efficiency. Isolation of ovarian stem cells from a superior dam will enable production of unlimited superior ova in vitro which bypasses the requirement of maintaining animal stock, multiple ovulation and ovum pick up procedures.

Conclusion

Considering the overall picture of ovarian stem cell research, we can see how science and its methods sail its journey from basic facts to more evident facts. Johnson and his co-workers came with a novel idea of existence of ovarian stem cells and neo-oogenesis in post natal female mammals. Later they suspected stem cells may be from bone marrow. Even though existence and requirement of neo-oogenesis in post natal ovary in 'normal conditions' was disproved, this study incited queries for identification of stem cells in ovary which 'almost' became a success story. Certainly, researches in the field of ovarian stem cells give a light of hope in female infertility treatment along with its tremendous scope in the field of animal reproduction.

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