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INSTITUTIONEN FÖR KEMI OCH MOLEKYLÄRBIOLOGI

Molecular pathways mediating the development of female germ cells

Sanjiv Risal

Institutionen för kemi och molekylärbiologi
Naturvetenskapliga fakulteten

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Abstract

In mice, primordial germ cells (PGCs) dramatically increase in their number from 200 cells at 9.5 dpc to 10 000 cells at 12.5 dpc with doubling time of 12.6 hours. Thus, cell proliferation of PGCs seems a unique compared to proliferation of other cell types. How Mastl (microtubule-associated serine/threonine kinase-like) is involved in the rapidly dividing PGCs is not known. PGC-specific deletion of *Mastl* resulted in a significant loss of PGCs by interfering with cell cycle progression beyond metaphase. This mitotic defect further led to the activation of DNA damage and the apoptotic cell death of *Mastl*-null PGCs by 12.5 dpc. Therefore, indicating that Mastl-mediated molecular events are indispensable for cell cycle progression in PGCs. However, the metaphase-anaphase transition defects observed in the *Mastl*-null PGCs is rescued by simultaneous deletion of *Ppp2r1a* (α subunit of PP2A). Collectively, our results indicated that the Mastl-PP2A axis plays a fundamental role in controlling PGCs proliferation. At 13.5 dpc, PGCs started to differentiate into female germ cells by entering prophase I of meiosis. Around the time of birth, the primordial follicles assembly occurs in mammalian ovary. In mice, there exist two classes of primordial follicles: the first wave of primordial follicles develops immediately after birth, whereas the second wave of primordial follicles activated later in adult life. To label and trace the *in vivo* development and activation of two waves (or classes) of primordial follicles, we used two different mouse models: *Foxl2-CreERT*^{2} and *Sohlh1-CreERT*^{2}. Our study showed that the first wave of primordial follicles get exhausted around three months of age in mice. Additionally, this class of primordial follicles contributes to the onset of puberty and to early fertility in mice. On the other hand, the primordial follicles in the cortical region of the ovary are activated after three months of age of mice contributing to fertility until the end of reproductive life. One of the hallmarks of the activation of primordial follicles is a squamous-cuboidal transition of primordial follicle granulosa cells (pfGCs). However, how pfGCs–oocytes dialog regulate the activation of primordial follicles and determines their developmental fates are still unclear. To study this, we used *Foxl2-CreERT*^{2} mouse model to show that for maintenance of quiescence of primordial follicles, inhibition of mammalian target of rapamycin complex 1 (mTORC1) signaling in pfGCs is imperative. For this purpose, we deleted *Tsc1* and *Rptor* from pfGCs, which caused over-activation and suppression of activation of primordial follicles respectively. Furthermore, our study also demonstrated that mTORC1-KITL cascade trigger the awakening of dormant oocytes in primordial follicles as initiated by cellular and molecular changes in pfGCs. Mammalian females are endowed with fixed number of primordial follicles in the ovaries, which are depleted gradually and menopause ensued with the exhaustion of pool of primordial follicles. However, many ovarian pathological conditions lead to premature ovarian failure due to early depletion of follicles pool. Therefore, the existence of OSCs (oogonial stem cells) in ovaries generated excitement in many females suffering from infertility resulting from various reproductions related ailments. This also changed the notion that OSCs might contribute to renewable of follicles as shown by OSCs purified from human and mouse ovaries by DDX4 antibody-based FACS. However, technically the isolation of OSCs by DDX4 antibody-based FACS is in controversy. To verify this claim, we followed the published reported method to isolate OSCs from human and mouse ovaries. Our results showed that isolated DDX4 positive cells from human ovary did not express any *DDX4* mRNA as shown by single-cell mRNA sequencing analysis. Additionally, when these DDX4-positve cells were injected into SCID mice, no follicle formation was noted, thus refuting the reported evidence. Similarly, Ddx4-antibody based FACS sorted mouse ovarian cells also displayed an absence of *Ddx4* mRNA and unable to generate follicles. Our results claimed that previously reported DDX4 positive ovarian cells purified from human and mouse are neither DDX4 expressing cells nor are they functional OSCs.

Keywords: Ovary, primordial follicles, primordial germ cells, Oogonial stem cells, metaphase-anaphase transition