Ovarian germline stem cells (OGSCs) and the hippo signaling pathway association with physiological and pathological ovarian aging in mice

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The Hippo signaling pathway plays an important role in stem cell maintenance in a variety of tissues and has thus implications for stem cell biology. Key components of this recently discovered pathway have been shown to be associated with primordial follicle activation. Recently, we observed that the Hippo signaling pathway plays a role in the development of Ovarian Germline Stem Cells (OGSCs) during physiological and pathological ovarian aging in mice. The Hippo signal pathway and MVH/OCT4 genes were observed in the mouse ovarian cortex. The level and co-localization of LATS2, MST1, MVH, and OCT4 were obviously decreased with increased age, but YAP1 was more prevalent in the mouse ovarian cortex of 2M mice than 7D mice and was not observed in 20M mice. Furthermore, YAP1, MST1, MVH, and OCT4 were gradually decreased after Tripterygium wilfordii polycopride tablets (TPT) and cyclophosphamide/busulfan (CY/BUS) treatment, and LATS2 up-regulation persisted in TPT- and CY/BUS-treated mice. In addition, pYAP1 protein showed the highest level in the ovarian cortices of 7D mice compared with 20M mice, and the value of pYAP1/YAP1 decreased from 7D to 20M. Moreover, pYAP1 decreased in the TPT- and CY/BUS-treated groups, but the value of pYAP1/YAP1 increased in these groups. Altogether; we demonstrated that the Hippo signaling pathway may be involved in the development schedule of OGSCs.

Keywords: Ovarian germline stem cells; Hippo signaling; Ovarian aging; Mice

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Previous studies have shown that primordial follicle depletion is the fundamental reason for physiological and pathological ovarian aging in mammalian females [1]. Historically, it was believed that the majority of the primordial follicle pool remains in a dormant state and declines with age under physiological conditions [2].

In recent years, this cornerstone understanding of a constant primordial follicle pool remains in a dormant state and declines with age under physiological conditions [2]. In recent years, this cornerstone understanding of a constant primordial follicle pool was challenged by the study of Johnson et al. [3], who isolated mitotically active germ cells from juvenile and adult mouse ovaries. Currently, several study groups have demonstrated the isolation of germline stem cells from the ovarian surface epithelium (OSE) of adult and even menopausal mouse and human ovaries [4-6] and have also successfully isolated and characterized long-term cultured ovarian germline stem cells (OGSCs) from neonatal and adult mouse ovaries [7-13]. However, the OSE layer has also been confirmed to be a normal, naturally present source of oocytes by producing stem cells in the embryonic or fetal period of life, and it is commonly referred
to as the “germinal” epithelium[14]. Therefore, some of these recently published studies have shown that OGSCs might be present in the OSE layer.

The Hippo signaling pathway is a recently discovered novel signaling pathway [15]. In mammals, the core of the Hippo pathway comprises two upstream kinases (mammalian Sterile 20-like protein kinase I, MST1 and MST2, for short) and Salvador I (also known as SAV1 or WW45), as well as large tumor suppressor homolog 1 and 2 (LATS1 and LATS2), and YAP1 (Yes-associated protein) [16, 17].

Hence, the aim of this study is to reflect the variation in the number of OGSCs and explore the expression changes of the core components of the Hippo pathway (MST1, LATS2, YAP1) during physiological aging and pathological aging of OGSCs in the mice OSE layers.

We located ovarian cells positive for mouse vasa homologue (MVH) and OCT4 protein, which are expressed exclusively in germ cells and pluripotent stem cells, respectively [24, 25]. The results confirmed that MVH and OCT4 were concurrently co-expressed in different stages of mouse ovarian cortex. Briefly, OGSCs were observed in the OSE layers, and the number of OGSCs gradually decreased from 7D to 20M.

Our recent results revealed that the expression of the core components of the Hippo pathway change during mouse follicular development [23]. In this study, MST1 and LATS2 were most highly expressed in the ovarian cortices of 7D mice, but YAP1 was more abundant in 2M than in 7D mice and was absent in 20M mice. Whereas the primordial follicles start developing unceasingly with age, follicular growth is accompanied by oocyte maturation and granular cell proliferation. MST1 and LATS2 were down-regulated during follicle formation and depletion with age, but the expression level of YAP1 was up-regulated at all time points until 10M. MST1, LATS2, YAP1 and MVH were specifically co-expressed in the mouse ovarian cortex, and the levels of MST1 and LATS2 also decrease among 7D, 2M and 20M mice, unlike the expression of YAP1. Moreover, the change in pYAP1 expression is similar to that of YAP1, but the ratio of pYAP1/YAP1 decreased significantly among 7D, 2M and 20M mice. Furthermore, the results show that Hippo signaling genes are active in different stages of mouse ovarian cortex, and OGSCs had a positive correlation with the level of MST1 and LATS2 and were contrary to the changes in YAP1 expression.

Likewise, MVH and OCT4 were co-expressed and these levels were remarkably decreased in the ovarian cortex of TPT- and CY/Bus-treated mice. Moreover, there is almost no difference in the effect of these two on the presence and quantity of OGSCs. In addition, the expression of OCT4 in the mouse ovarian cortex of both the TPT- and CY/Bus-treated mice was higher than that in 20M mice, suggesting that there are some putative stem cells in the ovarian cortex of mice undergoing pathological ovarian aging. Given these results, it has been speculated that OGSC proliferation suppression may be the key cause of follicular depletion by TPT and CY/Bus treatment and may also be the core cause of pathological ovarian aging caused by chemical treatment in cancer patients. We assessed the chemotherapy-induced ovarian follicular loss in mice, arguing that Hippo signaling factors are important for the development of OGSCs. MST1, LATS2 and YAP1 were significantly detected in the ovarian cortex of the TPT- and CY/Bus-treated mice and the control group, respectively. In addition, the proteins of pYAP1 were decreased in the TPT- and CY/Bus -treated group, as shown using western blotting, and the ratio of pYAP1/YAP1 increased significantly.

Now we isolate OGSCs from 7D mice ovaries by Magnetic-Activated Cell Sorting (MACS) technique. As shown in results, MVH/OCT4 was co-expressed in OGSCs. Moreover, MST1, LATS2 and YAP1 were distinctly localized in OGSCs. Analysis of Hippo signal pathway showed that OGSCs expressed MST1, LATS2 and YAP1 genes.

In conclusion, this study showed that Hippo signaling genes are expressed in OGSCs, and the number of OGSCs correlates with the dynamic changes of Hippo signaling. These findings confirmed that the Hippo signaling pathway may be involved in dictating the development schedule of OGSCs. At the present, we plan to use OGSC line as a useful tool to study Hippo signaling molecules and the effect on OGSCs transfected by lentivirus to mice ovaries. These future studies could be used for further research aimed at the autologous treatment of ovarian infertility and degenerative diseases.

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Conflict of Interest

All authors declare no conflict of interest.

References