主論文の要旨

Tamoxifen Activates Dormant Primordial Follicles in Mouse Ovaries

〔タモキシフェンはマウス卵巣の休眠原始卵胞を活性化する〕

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【Introduction】

Ovarian folliculogenesis starts with the recruitment of dormant primordial follicles into the growing follicle pool to form a primary, secondary, preantral follicle, and ultimately a preovulatory antral follicle. Most primordial follicles remain in a quiescent state, where dormant oocytes are arrested at the prophase of meiosis I, providing a reserve for continuous reproductive success. The activation or loss of primordial follicles is responsible for the irreversible decline in reproductive capacity. Recently, some reports have shown that environmental factors, such as angiogenesis, hypoxia, and mechanical stress, could affect the maintenance of the dormant state of oocytes.

Previous studies have shown that 17β-estradiol (E_2) inhibits oocyte cyst breakdown and primordial follicle formation *in vitro* and *in vivo*. Moreover, loss of estrogen receptor β (ESR2), the predominant estrogen receptor in the ovary, leads to primordial follicle activation (PFA). These results suggest that E_2 also controls PFA. In fact, our previous research showed that 17β-estradiol (E_2), stefin A (STFA), and cathepsins control PFA and the growth of primordial follicles in mouse ovaries. In this study, we attempted to promote PFA in mouse ovaries by peritoneal administration of tamoxifen, a competitive inhibitor of E_2. Tamoxifen is a selective estrogen receptor modulator that prevents and treats estrogen receptor-positive breast cancer in pre-and postmenopausal women. Furthermore, we investigated the relationship between PFA during the estrous cycle and estradiol concentration in the serum and ovarian tissue to clarify the physiological mechanism of PFA.

【Methods and Results】

Serial sections of ovaries were stained with an anti-FOXO3a antibody. FOXO3a localizes in the nuclei of oocytes in dormant follicles, whereas it localizes to the cytosol of oocytes in activated follicles. In primordial follicles, there were both dormant and activated follicles in the ovaries (Fig. 1a–b). In primary and secondary follicles, FOXO3a was localized in the oocyte cytoplasm (Fig. 1c-d). The number of activated primordial follicles in each ovary during the four stages of the estrous cycle (proestrus, estrus, metestrus, and diestrus) was evaluated. There were no significant differences in the rate of activated primordial and primary follicles during the estrous cycle (Fig. 1e).

To confirm whether inhibition of the E_2 effect promotes PFA in vivo, we injected tamoxifen into the abdominal cavity of metestrus mice and found that the rate of activated primordial follicles in the metestrus was the lowest during the estrous cycle. Tamoxifen was injected at 0.025, 0.05 and 0.1 mg/g body weight. After 24 h from administration, the rate of activated primordial follicles in 0.1 mg/g body weight tamoxifen-treated ovaries were significantly increased (Fig. 2a). Tamoxifen was then administered to 10–20-week-old mice of each estrous cycle stage at 0.1 mg/g body weight. Tamoxifen administration

increased the rate of activated primordial follicles in all estrous cycle stages, and there were no significant differences between each estrous cycle stage (Fig. 2b).

We assessed the E_2 concentration in the serum and ovaries during the estrous cycle (Fig. 3). E_2 levels in the serum and ovaries fluctuated during the estrous cycle (Fig. 3a and 3b). In the ovary, E_2 concentration is significantly higher during proestrus than all other stages and decline during estrus, and then gradually rises again during metestrus and diestrus (Fig. 3b). This result is consistent with the trend in E_2 serum concentration (Fig. 3a). The E_2 concentration level in the serum of some mice was too low to be detected, and therefore classified as undetectable data during the statistical processing. These results indicated that there was no relationship between PFA and the change in E_2 concentration in the serum and ovaries during the estrous cycle (Fig. 1e, 2b, and 3).

To clarify whether the E_2 concentration in the local area of the ovary affects PFA, we evaluated the number of primordial follicles within 50 μm around the antral follicles. The results showed that the rate of activated primordial follicles was lower than that of the rest of the ovaries (Fig. 4a). After tamoxifen administration, there was no significant change in the rate of activated primordial follicles around antral follicles in all stages of the estrous cycle (Fig. 4b). These results showed that tamoxifen promoted PFA except for the primordial follicles around the antral follicles.

Our previous research showed that E_2 regulates the expression of stefin A (STFA) and controls the activation and growth of primordial follicles through cathepsin-mediated digestion of the extracellular matrix (ECM) around primordial follicles. STFA was localized in the oocytes and granulosa cells of primordial follicles in 12-week-old mouse ovaries (Fig. 5a and b). The expression levels of STFA in the primordial follicles varied. Most primordial follicles strongly expressed STFA (Fig. 5a), but their expression levels were low in some primordial follicles (Fig. 5b). We further studied the effect of tamoxifen on the expression of *stfa* in ovaries. Tamoxifen tended to decrease the expression of *stfa* in all estrous cycle stages (Fig. 5c).

To confirm ECM digestion around the primordial follicles, we observed collagen type IV in the ovaries of mice treated with tamoxifen (Fig. 5d and e). The rate of primordial follicles containing some surrounding digested collagen type IV was significantly increased in tamoxifen-treated ovaries, with no significant difference between the four stages of the estrous cycle (Fig. 5f). This result correlated with the increased rate of activated primordial follicles in ovaries treated with tamoxifen (Fig. 2b and 5f).

【Discussion】

In this study, we confirmed that tamoxifen administration promoted PFA, consistent with our findings in the ovarian tissue culture experiments; thus, E_2 also inhibited PFA in vivo. However, there was no correlation between the concentration of E_2 in the ovaries and PFA.

Tamoxifen promoted PFA in mouse ovaries, but not around antral follicles.

Our present study found that STFA controls the growth of primordial follicles through cathepsin-mediated digestion of ECM around primordial follicles under the regulation of E_2 in cultured ovaries. Our data showed that STFA was expressed in primordial follicles and tamoxifen tended to decrease the expression of *stfa*, especially in the ovaries of the proestrus cycle. Tamoxifen administration induced the degradation of collagen type IV around primordial follicles, leading to the activation of primordial follicles. These results suggest that E_2 regulates the expression of stefin A and controls the degradation of collagen type IV around primordial follicles, thereby repressing PFA *in vivo*.

【Conclusions】

Tamoxifen activates primordial follicles in vivo and E_2 is a critical physiological factor for regulating PFA by controlling the digestion of ECM around primordial follicles. Our results indicate the possibility that tamoxifen may be useful as a therapeutic agent for infertility.