審査の結果の要旨

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The present study explored antioxidative role of Sirtuins 3 (SIRT3) in primary human granulosa cells (GCs). SIRT3 is one of the seven mammalian sirtuins, which has gained growing attention for its connection with many biological processes, especially counteraction with reactive oxygen species (ROS). Human ovaries were used to detect location of SIRT3 expression in the ovary. Hydrogen peroxide (H_2O_2) and human chorionic gonadotropin (hCG) were incubated with GCs cell line, COV434, and primary human GCs to investigate the possible relationship between ROS and reproduction. SiRNA-induced endogenous SIRT3 depletion was performed to observe ROS fluorescence intensity and to uncover the relationship between folliculogenesis- and luteinization-related genes and SIRT3. Results are as following.

1. SIRT3 expression was first confirmed in human ovaries by immunohistochemistry. The protein expression of SIRT3 was predominantly detected in the cytoplasm of human GCs at various stages of follicles, from primordial to preovulory and active corpus luteum. Additionally, SIRT3 protein has also been detected in the oocytes.
2. H_2O_2 was incubated with COV434 cells and human GCs in various concentrations (250, 500 μM). Antioxidants, SOD1 and catalase and SIRT3 were remarkably increased under the condition of high levels of H_2O_2 at protein level by Western blot and at mRNA level by quantitative RT-PCR.
3. SIRT3 gene was knocked down by 100nM SIRT3 siRNA for 48 hours in human GCs. The siRNA mediated SIRT3 depletion induced strongly fluorescence intensity in SIRT3 siRNA group compared to the siRNA control group, but there was no significant cell death between the two groups after SIRT3 gene was knocked down.
4. hCG was added into the human GCs, and its role on promoting luteinization was verified in the present study by the elevated mRNA expression of steroidogenic acute regulatory protein (StAR). Besides, it down regulated the mRNA expression of the two antioxidants, catalase and SOD1, and SIRT3.
5. The optimum concentration of 100 nM SIRT3 siRNA was transfected to the human GCs, and the SIRT3 mRNA levels were effectively suppressed after 48 hours. SIRT3 gene knock down in human GCs caused significant decreased mRNA expression of both folliculogenesis-related molecules, which were P450arom and 17β-hydroxysteroid dehydrogenase 1, and luteinization-related molecules which were StAR, 3β-hydroxysteroid dehydrogenase 1 and the cholesterol side-chain cleavage enzyme. Besides, the depletion of SIRT3 resulted in significantly lower progesterone concentration secreted by human GCs in medium.

On conclusion, in the present study SIRT3 was expressed in the human ovaries and human GCs. SIRT3 can neutralize excessive level of ROS, like other two antioxidants, catalase and SOD1, to positively regulate the folliculogenesis- and luteinization-related molecules, as well as progesterone production in human GCs. the SIRT3-involved reproduction modulation might have an important clinical implication in maintaining ovarian homeostasis. Therefore, the author of the present study deserves the award of doctor’s degree.