［課程－２］

審査の結果の要旨

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In my studies, I showed evidence that androgen stimulated apoptosis, increased RAGE expression and AGE accumulation in PCOS (Polycystic Ovarian Syndrome) ovaries, contributing to its pathology. The data from these studies demonstrated that the process was mediated by endoplasmic reticulum stress (ER stress). TUDCA, a clinically available ER stress inhibitor, successfully inhibited testosterone-induced-apoptosis as well as testosterone-induced-RAGE expression.

In my first study, I demonstrated that testosterone induced apoptosis in PCOS ovaries via the ER stress transcription factor, CHOP and DR5. The results obtained from this study's experiments are as below:

1. Testosterone upregulated ER stress in human GLCs. Testosterone increased the expression of UPR factor CHOP, including other UPR genes in these cells.

2. Testosterone promoted DR5 expression and apoptosis in human GLCs

3. ER stress inhibitor, TUDCA decreased testosterone-induced-CHOP and DR5 expression measured by qPCR. Using flow cytometry, my results showed that apoptosis rate was reduced in GLCs treated with testosterone and TUDCA compared to testosterone alone. This suggested that testosterone-induced apoptosis was mediated by ER stress.

4. Knockdown of both CHOP and DR5 decreased the testosterone-induced-apoptosis measured with flow cytometry. In CHOP knockdown cells, testosterone-induced DR5 expression was reduced, suggesting that testosterone-induced- apoptosis was mediated by CHOP-DR5 axis.

5. Testosterone-induced-apoptosis mediated by CHOP-DR5 pathway was mediated by androgen receptor signaling.

6. mRNA expression of CHOP and DR5 were increased in GLCs collected from PCOS patients.

7. Apoptosis measured by TUNEL staining was found to be significantly increased in PCOS patients and PCOS mice model's ovaries compared to control.

8. Expression of DR5 and CHOP analyzed with immunohistochemical technique showed that the expression of DR5 and CHOP were increased in both human and mouse PCOS ovaries.

9. Treatment of TUDCA decreased apoptosis, CHOP and DR5 expression in ovaries of PCOS mice model.

In the second study, I showed that testosterone-induced RAGE (Receptor for AGEs) expression and AGEs (Advanced Glycation End product) accumulation in PCOS ovaries and this was mediated by ER stress. The *in-vivo* and *in-vitro* results obtained from this study's experiments are as below:

1. Testosterone induced RAGE mRNA of human GLCs. Pretreatment with an ER stress inhibitor, TUDCA decreased the testosterone-induced RAGE mRNA expression.

2. Testosterone increased the accumulation of AGEs in human culture GLCs and TUDCA reduced this effect.

3. Knockdown of CHOP decreased the testosterone-induced-RAGE and AGEs accumulation in human GLCs analyzed by qPCR and western blotting. These results suggested that testosterone-induced-RAGE expression was mediated by ER stress transcription factor, CHOP.

4. Testosterone-induced RAGE expression via androgen receptors.

5. RAGE mRNA expression in GLCs of PCOS patients was increased compared to control.

6. *In-vivo*, RAGE and AGEs protein expression were increased in ovaries belonging to PCOS patients and PCOS mice models.

7. The systemic administration of an ER stress inhibitor, TUDCA or a RAGE inhibitor, FPS ZM 1 both reduced the expression of RAGE and AGE in ovaries of PCOS mice model.

8. Simultaneously, these treatments also improved PCOS mice model's estrus cycle and ovarian morphology.

よって本論文は博士(医学)の学位請求論文として合格と認められる。