論文の内容の要旨

論文題目　THE ROLE OF ENDOPLASMIC RETICULM STRESS ACTIVATED BY ANDROGEN IN THE PATHOPHYSIOLOGY OF POLYCYSTIC OVARIAN SYNDROME. (高アンドロゲン状態により活性化される小胞体ストレスの多嚢胞性卵巣症候群の病態形成における役割の検討)

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PCOS or polycystic ovarian syndrome is the most common cause of anovulation infertility with 90-95% of women seeking infertility treatment (Teede H. et al. 2010). It is a clinical syndrome which includes a combination of a few biochemical hormonal changes and clinical symptoms (Azziz R et al. 2018). PCOS is characterized by an increased level of androgens, menstrual irregularity and the presence of multiple cystic lesion within an enlarge ovaries. Many PCOS patients also suffer from other accompanying metabolic disorders such as obesity, insulin resistance and glucose intolerances leading to an increased risk of type 2 diabetes mellitus and cardiovascular diseases (Diamantis-Kandarakis et al. 2012). Evidence has demonstrated the deleterious role of excess androgen in the pathophysiology of PCOS (Nisentblat V. et al. 2009, Bertolo MJ et al. 2019). The driving mechanisms by which androgen is involved in the pathophysiology of PCOS ovaries involves several folliculogenesis stages. Ovarian cycle in humans begins with the recruitment of a cohort of the primordial follicle into the growing phase. At this stage, the surrounding granulosa cells are arranged in a single layer of flattened cells. In the next stage, the granulosa cells become cuboidal in shape, now term as a primary follicle. The late-stage forms the antral follicles when the number of granulosa cells and the size of the follicles increases. One dominant antral follicle will eventually ovulate, while the rest of the cohort undergoes atresia (Gougeon A. et al. 1996). Initially, androgen helps to promote growth (Suman Rice et al. 2007). However, androgen switches to an anti-growth action at a later stage of folliculogenesis. The reason behind this switch remains enigmatic. However, ER stress has been found to be activated at the later stage of follicular development (Harada et al. 2015). ER or the endoplasmic reticulum is a dynamic and unique organelle within a cell. The primary role of the ER is to serve as a site for protein synthesis and transportation (Schwarsz DZ et al. 2016). Following protein synthesis in the ER, proteins are folded and modified with the aid of chaperones and folding enzymes, before becoming fully functional. Unfortunately, even with chaperone proteins and folding enzyme in place, the folding capacity within the ER is easily

susceptible to failure leading to an accumulation of unfolded protein, creating an 'ER stress'. To restore the normal function, inhibition of protein translation, degradation of unfolded proteins, increasing folding capacity and increasing the production of chaperones and folding enzymes must occur, a rescue effort collectively term as UPR (unfolded protein response) (Ron D. et al. 2007., Takahashi N et al. 2017).
The activation of UPR signaling cascade is initiated by at least three primary ER-localized protein sensors; IRE1α, PERK and ATF6. These sensors possess a domain that senses the stress. The sensors then transmit signals to their specific transcriptional or translation apparatus activating several downstream genes such as XBP1, ATF4 and CHOP in attempt restore ER homeostasis. UPR, initially functioned as a pro-survival response, switches to a pro-apoptosis role if ER stress continues. (Urra H. et al. 2013). One of the primary events accountable for this switch is the induction of a UPR pro-apoptotic gene, C/EBP homologous protein (CHOP), an activated downstream of ATF4. (Urra H et al. 2013, Hetz C. et al. 2012). Additionally, unresolved ER stress can also lead to cell apoptosis through the mediation of death receptor 5 (DR5). Being a transcriptional target of CHOP, DR5 plays a critical role in ER stress-led-apoptosis. There is strong evidence to show that DR5 controls apoptosis under ER stress condition and that the level of DR5 is regulated by PERK.
In recent years, emerging evidence has supported the notion that the accumulation of AGEs in PCOS, specifically intraovarian AGEs contributed to PCOS related anovulation and insulin resistance (Diamantis-Kandarakis et al. 2015) including altering steroidogenesis, thus folliculogenesis (Gard et al. 2016). The advanced glycation end product or AGEs is one of the pro-inflammatory molecules found to be elevated in PCOS (Pertynska-Marczewska M. et al. 2015). AGEs are product manufacture at the end of a Maillard reaction, in which the protein loses its structure and function. (Cho SJ et al. 2007). AGEs exert its detrimental effect via a receptor-independent pathway or receptor-dependent pathway by interacting with its cellular membrane receptor RAGE (receptor for AGE). Activated AGEs damages the cellular structure inducing inflammation and apoptosis (Garg D. et al. 2016). The accumulation of AGEs is linked to diabetic complication, atherosclerosis, Alzheimer's disease and renal disease. Numerous studies have also demonstrated the activation of ER stress by AGEs in various cell disease model including osteoarthritis (Yamabe S. et al. 2013), diabetic complication (Danilo C. et al. 2018), and aortic endothelial diseases (Adamopoulos C. et al. 2014). This suggested a possible cross-talk between AGE and ER stress in the pathology of several diseases (Inagi R et al. 2011). Thus, AGE-inhibiting agents were regarded as a potent ER stress modulator capable of restoring

protein homeostasis within the cell, thus halting apoptosis (Piperi C. et al. 2012). Based on these findings, we first hypothesized that hyperandrogenism activates ER stress and the activated ER stress leads to apoptosis via CHOP-DR5 apparatus. Second, we postulate that hyperandrogenism also increases AGEs-RAGE level in PCOS ovaries and this is mediated by ER stress-UPR pathway.

Our qPCR analysis showed that in cultured human GLCs (granulosa lutein cells) exposed to testosterone of 50μg/m for 24 hours, activated various UPR genes expression which were the XBP1, HSP A5, ATF4 and CHOP. The activated UPR gene denotes the activation of ER stress. Testosterone pretreatment increased the expression of DR 5, as well as RAGE in GLCs. Consistent with this, pretreatment with an ER stress inhibitor, TUDCA abrogated the stimulatory effect of testosterone on the expression of these UPR genes, CHOP, DR5 including AGEs-RAGE. To assess cell apoptosis, we analyzed testosterone-induced-apoptosis using flow cytometry. We found that testosterone increased the rate of apoptosis and TUDCA (tarsoursodexoycholic acid), an ER stress inhibitor, abrogated this phenomenon.

Using RNA interference, we knockdown CHOP's endogenous expression. The silencing of CHOP gene reduced the mRNA expression of testosterone-induced- DR5 and RAGE and this was confirmed with immunoblotting suggesting that CHOP regulated testosterone-induced-DR5 and RAGE expression.

Simultaneously, GLCs pretreated with testosterone exposed to flutamide, an androgen antagonist, abrogated the up-regulation effect of testosterone on the CHOP and DR 5 mRNA as well as RAGE expression. Following these findings, we knockdown AR gene using siRNA and we demonstrated a decrease in the level of DR5, CHOP, and RAGE mRNA expression. These results imply that the mechanism by which testosterone-induced CHOP and DR5 mRNA expression, as well as RAGE, may have been via an androgen receptor.

To examine the involvement of DR5, CHOP and RAGE in the pathology of PCOS, we collected GLCs from PCOS and non-PCOS patients during their IVF procedures. The mRNA expression analyzed by qPCR showed an increased in mRNA expression of DR5, CHOP and RAGE in PCOS patients compared to control.

Consistent with the results obtained from human GLCs, immunohistochemical staining of PCOS patients' ovaries and DHEAS (Dehydroepiandrosterone)-induced PCOS model mice showed an increased reactivity of DR5 and CHOP, along with RAGE and AGEs in granulosa cells of antral follicles. Oral administration of TUDCA to the PCOS model mice reduced the immunoreactivity of DR5, CHOP with a commitment reduction of RAGE expression and AGE accumulation the granulosa cell of antral

follicles.

In addition, we have also examined the role of an anti-RAGE activity in the pathology of PCOS. The administration of RAGE inhibitors such as FPS-ZM1 to PCOS mice model similarly reduced the immunoreactivity of RAGE and AGEs. Both the treatment of TUDCA and FPS-ZM 1 to PCOS model mice improved PCOS estrus cycle in mice models. The number of atretic follicles, usually increased in PCOS ovaries, were also decreased in both treatment groups.

In summary, we showed that testosterone activated ER stress in granulosa cells of antral follicles and induced apoptosis via induction of DR5, which is mediated by the UPR factor CHOP. Expression of DR5 and CHOP is upregulated in granulosa cells of antral follicles in PCOS and contributed to apoptosis in these cells. In this part of our findings, suggested that ER stress activated by hyperandrogenism in PCOS contributed to growth arrest of antral follicles by promoting apoptosis of granulosa cells via induction of DR5. Simultaneously, testosterone increased the expression of RAGE and accumulation of AGEs in granulosa cells in a similar manner of activating ER stress. Treatment of PCOS mice with a RAGE inhibitor or an ER stress inhibitor reduces RAGE expression and AGEs accumulation in granulosa cells, improves estrous cycles and reduces atretic antral follicles. Finally, this suggests that hyperandrogenism in PCOS enhanced the accumulation of AGEs in the ovary via activation of ER stress. Targeting the ER stress system may serve as a novel approach for PCOS. A clinically safe and available ER stress inhibitor agent, TUDCA may be one of the strategies for future therapeutic use.