Editorial

Molecular Mechanisms of Obstetrics and Gynecologic Diseases

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Obstetric and gynecologic disease refers to a collection of benign and malignant disorders arising from female reproductive organs/ tissues, including ovary-fallopian tube, uterocervical system, and the placental-amniotic-uterus unit. Impacting directly or indirectly human reproduction, these diseases affect the health of both the current and the next generation. A common feature of the involved organs is their normal changes during menstrual and reproductive cycles, and along aging process, which are subject to the tight regulation of sex hormones, mostly estrogen and progesterone. This special issue of *Current Molecular Medicine* contains nine original articles covering the research fronts concerning several common obstetric and gynecologic diseases including epithelial ovarian cancers, premature ovarian failures, endometrial cancers, and endometriosis. In addition, there are two papers describe studies on the placental function and ERK1/2 signal pathways in human amnion epithelial cells. These diseases have relatively high incidence rates among women at certain ages, impose serious threat to women's lives and/or living quality. Better understanding on their molecular mechanism(s) is required for the more effective management of these diseases. Here we briefly review these articles organized by organs and relevant diseases.

OVARIAN BIOLOGY AND OVARIAN CANCERS

Epithelial ovarian cancer (EOC) accounts for most of the deaths caused by gynecologic cancers. The late diagnosis, lack of more effective drug, and high tendency of recurrence are challenging issues in the campaign against this deadly disease. It has been known that in human development, Sertoli cells in the male fetus produce Müllerian inhibiting substance (MIS). MIS receptors are expressed in human müllerian-derived cells and their activation by MIS leads to cell apoptosis and regression of müllerian structures in the male embryos. Thus, Müllerian inhibiting substance has been studied for its potential application as a cáncer therapeutic reagent. In the first article of this special issue, Eati Basal and colleagues report their new findings along this direction. They determined the expression of MIS receptors in epithelial ovarian cancers and assessed the relationship between expression patterns and clinical outcomes, and explored the MIS-dependent pathways in cell culture models. The authors performed tissue microarray analysis, though no clear correlation was found between MIS receptors and patient survival. On the other side, MIS-dependent Smad phosphorylation was detected in the primary cultures established from ascites harvested at surgery, and treatment of cell cultures with MIS led to reduced cell viability. The authors concluded that in the majority of EOC, the MIS-dependent signaling remains intact and EOC cells possess appropriate response to MIS. These findings warrant additional research efforts on the downstream targets and effects of MIS-dependent signaling pathways, and to explore which existing therapy could synergize with MIS treatment.

There has been an urgent need for new treatment modalities against drug-resistant ovarian cancers. Epigenetic inhibitors such as histone deacetylase (HDAC) inhibitors were known to be effective for treating lymphomas for many years. Many HDAC inhibitors display strong cytostatic and apoptotic activities in cells from hematologic malignancies. However, results from several clinical trials have not been satisfactory, and the potential of these reagents for treating solid tumors remains questionable. Ya-Li Wang *et al*. examined the effects of oxamflatin, a pan-activity HDAC inhibitor containing the aromatic sulfonamide and hydroxamic acid groups, in ovarian cancer cells. They demonstrated that oxamflatin at nM concentrations was able to induce significant morphological changes, a decreased cell viability, and a reduced DNA synthesis, in two ovarian cancer cell lines. In addition, significant changes in the expression levels of cell cycle regulators such as c-Myc, CDK4, E2F1, and the phosphorylation levels of Rb protein, were observed following oxamflatin treatment, suggesting a strong cytostatic effect. More

1875-5666/16 $58.00+.00

studies are required to determine if oxamflatin along or in combination with other chemotherapy reagents could deliver anticancer effects against ovarian cancers *in vivo*.

Ovarian failure or aging is characterized by a progressive reduction of ovarian follicles and the oocyte quality that will ultimately lead to reproductive senescence. Exposure to excessive levels of reactive oxygen species (ROS) was thought to be part of the pathological mechanism of premature ovarian failure or aging. Peroxiredoxin 4 (Prdx4) reduces the ROS level and protects cells/tissues from oxidative damage. Yi Qian *et al*. previously reported that Prdx4 was abundantly expressed in mouse and human ovaries, and matured follicles express higher Peroxiredoxin 4 levels than immature follicles. Here the research group revealed that Prdx4 was highly expressed in the granulosa cells of mouse ovaries and the expression levels decreased when mice reached the aged stage. A similar pattern was also detected in human ovaries where Prdx4 was expressed lower in premenopausal women than in young women. Their results from *in vitro* experiments indicated that Prdx4 mRNA and protein levels were upregulated by relatively low concentrations of H_2O_2, but were inhibited rapidly when exposed to high concentrations of H_2O_2, and the changes were closely related to the changes in cell proliferation. These results suggest that oxidative stress and antioxidant may be deeply involved in the follicular development and ovarian aging.

ENDOMETRIAL BIOLOGY, ENDOMETRIAL CARCINOMA AND ENDOMETRIOSIS

Endometrial carcinoma is the most frequent malignancy found in women's reproductive tract. Its strong association with obesity has been long recognized, but the molecular basis for this connection is not understood. PI3K/AKT activation promotes tumors growth and tuberous sclerosis complex 2 (TSC-2) and p27 opposes such effects. Little is known about the involvement of p27 in the development of endometrial cancer from an obesity background. Using a panel of endometrial cancer cell lines and PI3K inhibitors, Adrienne McCampbell and her collaborators observed that p27 was involved in the efficacy of interventions that inhibit endometrial cell growth. Moreover, p27 was moderately-to-severely reduced in endometrial complex atypical hyperplasia in obese women, and endometrial hyperplasia in obese rats. In obese Eker rats, an energy balance intervention led to reduced weight, increased adiponectin and lowered leptin, altered leptin:adiponectin ratio, and reduced circulating insulin levels. Increased p27 expression, nuclear translocation, and a significant decrease of hyperplasia incidence was observed following caloric restriction. The authors propose that p27 levels and localization could be a useful biomarker, and a possible determinant of risk for EC arising in the setting of obesity. These findings revealed an important pathological mechanism that may explain the high risk of cancer caused by obesity in reproductive as well as other systems.

Human endometrium provides a matrix for embryo implantation and blastocyst/placenta development. The glandular epithelial cell and the fibroblast-like, stromal cell are the two major cellular components of endometrium. Although the epithelial cells with their glandular differentiation represents a characteristic histological feature that is often used for determination of endometrium phase, or diagnosis of endometriosis and cancers, the significance of stromal cells should not be overlooked. Indeed, abnormalities in stromal cells as well as stroma-epithelium interactions have been found in benign and malignant diseases. Endometriosis, a disease associated with severe pain and infertility, affects approximately 10% of women at reproductive age. The dependency of endometriosis on estrogen has been well recognized, but the molecular mechanism along the estrogenic pathway has not been fully understood. Hong Pan *et al*. characterized the effect of 17β-estradiol (E2) on human endometrial stromal cell invasion and the involvement of c-fos and matrix metalloproteinase-9 (MMP-9) in this biological function. They found that E2 promoted not only endometrial stromal cell invasion, but also c-fos and MMP-9 expression in endometrial stromal cells. Moreover, treatment with estrogen receptor inhibitor ICI 182780, or siRNA-mediated c-fos or MMP-9 knockdown, was able to block the effect of E2 on stromal cell invasion. Importantly, siRNA-mediated c-fos knockdown partially blocked the effect of E2 on MMP-9 expression. The authors concluded that 17β-E2-induced stromal cell invasion may be dependent on c-fos-mediated MMP-9 expression. These findings enrich our knowledge on the pathogenic mechanisms of endometriosis and may provide a potential treatment modality for better management of endometriosis.

Progesterone resistance and changes in the progesterone regulation of gene expression has been observed in the endometrium of women suffering endometriosis. cAMP-Response Element-Binding 3-like protein 1 (CREB3L1) is a known target gene of progesterone, but its function has not been studied in the context of uterine biology and endometriosis. Following validation on progesterone regulation of Creb3l1 in the uteri of wild-type and progesterone receptor knockout mice, Jong Il Ahn *et al*. observed that CREB3L1 expression was significantly increased in the secretory phase of human endometrium compared to the proliferative phase. CREB3L1 expression was also significantly decreased in the endometrium of women with endometriosis. By transfecting CREB3L1 siRNA into cultured human endometrial stromal cells these authors showed that CREB3L1 was required for the decidualization process. Interestingly, phosphorylation of ERK1/2, a factor known to be critical for decidualization, was reduced in CREB3L1-silenced stromal cells. Since human endometrial stromal cells isolated from endometriotic women had impaired decidualization and that the dysregulation of progesterone pathway has been found in patients with infertility and endometriosis, the authors suggest that CREB3L1 could be required for decidualization, and may be involved in the pathogenesis of endometriosis.

Previous studies by Shannon Hawkins and colleagues on differentially expressed miRNAs and their target genes in endometriomas indicated that 5-hydroxymethylation of cytosine in genomic DNA may contribute to the pathogenesis of endometriosis. The whole genome expression analysis of endometriomas suggested dysregulation of the ten-eleven translocation genes (TET1, TET2, and TET3) which encoded enzymes converting 5-methylcytosine to 5-hydroxymethylcytosine (5-hmC). In this special issue they describe new findings from a follow-up study. They validated the expression of TET genes in ectopic and eutopic endometrium and in primary cultures of human endometrial stromal fibroblasts during *in vitro* decidualization, and quantified 5-hmC levels in patients with endometriosis. Human endometrial stromal fibroblast cultures were derived from eutopic endometrium of women with (HESF-ENDO) or without (HESF-CONTROL) endometriosis. A decreased expression of TET1, TET2, and TET3 was observed in endometriosis compared to control eutopic endometrium. Interestingly, the global 5-hmC level was higher in ectopic endometrium than control eutopic endometrium, whereas genomic DNA from blood of women with endometriosis contained significantly lower 5-hmC than women without endometriosis. During *in vitro* decidualization, control endometrial stromal fibroblasts showed decreased expression of TET3, but decidualized HESF-ENDO showed no significant change in the expression of TET1, TET2, or TET3. The authors concluded that TET genes are downregulated in ectopic endometrium and in HESF-ENDO, and suggested that TET genes may play a role in endometriosis. The increased global 5-hmC levels may represent a characteristic epigenetic alteration in endometriotic tissues. Since DNA methylation changes are pharmacologically reversible, the TET proteins or 5-hmC may serve as therapeutic targets for the treatment of endometriosis.

GHRH/GHRH-R REGULATION OF PLACENTAL FUNCTION

Placental malfunction is considered as a critical factor associated with pregnancy disorders such as early pregnancy loss, intrauterine growth restriction (IUGR) and preeclampsia. Growth hormone-releasing hormone (GHRH) represents one of many peptide hormones placenta synthesizes. Previosu studies have shown that extrahypothalamic GHRH exerts a broad spectrum of activities, including that on the regulation of placental trophoblast proliferation and differentiation. Ai-Xia Liu *et al*. examined the effects of GHRH pathway on placental cell function as well as the mechanisms using choriocarcinoma JEG-3 cells as a study model. Using real-time PCR and Western blotting methods, the authors demonstrated that the expression of GHRH-R in placental villous tissues and JEG-3 cells was significantly lower in villous tissues of early pregnancy loss than in normal controls. Moreover, GHRH antagonist JMR-132 inhibited cell viability and induced apoptosis through activation of caspase-3, p38, and p53, and inhibition of phosphorylation of Akt. In addition, ER stress markers including GRP78, ubiquitinated proteins and phospho-eIF2 were increased upon JMR-132 treatment. Knockdown of endogenous GHRH-R abolished the JMR-132-induced cleavage of caspase-3 and activation of p38. The authors concluded that expression levels of GHRH-R were closely related to the placental cell function, and GHRH/GHRH-R axis and the downstream pathways may contribute to the regulation of placental development.

ERK1/2 SIGNAL PATHWAY IN HUMAN AMNION EPITHELIUM CELLS

Normal fetal growth and development requires an appropriate quantity and quality of amniotic fluid. Oligohydramnios, a disorder caused by too small an amniotic fluid volume (AFV), is known to be associated with an increased risk of perinatal morbidity and mortality. Current clinical treatments of oligohydramnios with trans-abdominal amniocentesis, intravenous drip isosmotic solution and maternal hydration are not very effective. It was known that Salvia miltiorrhiza, a Chinese herbal drug, had some effects for treating oligohydramnios. Aquaporins are water-selective channel proteins that facilitate fluid movement across cell membranes to maintain water homeostasis in tissues and cells. There were data indicating that regulation of AFV is related to AQPs expression in placenta and fetal membrane. Based on these observations, Qi Shen *et al*. examined if Salvia miltiorrhiza may regulate aquaporin 3 expression in the human amnion epithelial cells (hAECs) from patients with normal amniotic fluid volume or isolated oligohydramnios. It is found that Salvia miltiorrhiza significantly up-regulated aquaporin 3 expression via the ERK1/2 signal transduction pathway. These results shed new lights on the mechanisms and may point to a potential therapeutic target for improved treatment for isolated oligohydramnios.

In summary, this special issue covers several common gynecologic and obstetric diseases and pathways with potential impact on human reproduction as well as the maternal and perinatal health. Despite great research efforts, our understanding on cellular and molecular mechanism underlying ovarian cancer, endometriosis and placenta deficiency has not reach a level sufficient to support the rational design of effective diagnostic and treatment approaches. By providing new information from research field, we hope that this collection will inspire and facilitate the studying efforts and ultimately, led to better management of these diseases.

ACKNOWLEDGEMENTS

The Guest Editors would like to thank the Editor-in-Chief Dr. David Li for giving us this opportunity to edit this special issue. We are honored and grateful to have all the support from contributing investigators and dedicated reviewers to bring together these valuable manuscripts under the topic of this issue. It has been a productive and pleasant experience working with the Director Publications Dr. Samina Khan. The Guest Editors deeply appreciate the strong institutional supports from Mercer University School of Medicine and The Second Affiliated Hospital of Wenzhou Medical University on this scholarly service.

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