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Review article

Multi-system reproductive metabolic disorder: significance for the pathogenesis and therapy of polycystic ovary syndrome (PCOS)

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ABSTRACT

Polycystic ovary syndrome (PCOS), a multisystem disease, is a major reason for female infertility around the world. It is no longer considered simply as a disease of ovary. Now researchers growing awareness of the multisystem features of this disease. PCOS has a higher relationship with metabolic disturbance and hypothalamic-pituitary-ovarian axis (HPOA) function disorders. This syndrome results in hyperandrogenemia (HA), hyperinsulinemia/insulin resistance (IR), increased estrone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) ratio imbalance, infertility, cardiovascular diseases, endometrial dysfunction, obesity, and including a litany of other health issues. Furthermore, PCOS has been garnered in recent times. Interventions like metformin, orlistat, hormonal contraceptives, GLP1 agonists, and VitD have been applied to ameliorate or reverse the pathological characterization of PCOS. Moreover, drug-combined therapy of PCOS is superior to single drug administration. This review will focus on the recent progress in pathogenesis and therapy of PCOS.

1. Introduction

Polycystic ovary syndrome (PCOS) is a multifactorial disease of the reproductive system, which is an enormous problem not only for the clinicians, but also for the scientific researchers [1]. A large number of non-maturating and atretic follicles, multiple tiny cystic follicular cyst, luteinized inner theca, stromal abnormal hyperplasia, thickening of ovarian cortex and ovarian abnormal hyperplasia were main and classical changes of PCOS morphology, which indicated the cessation of follicle genesis and development. The size of ovary more increased obviously than normal size (volume $> 10\,cm^3$ or > 12 antral follicles and at least one with 2–9 mm in diameter). Meanwhile, the histological result showed that a peripheral ring of at least eight small follicles (610 mm diameter) by ultrasound scan [2]. Meanwhile, the pathogenesis of PCOS was far from completely clear and may relate to hyperandrogenemia (HA), hyperinsulinemia/insulin resistance (IR), an imbalance ratio of luteinizing hormone (LH) to follicle-stimulating hormone (FSH), metabolic aberrant, inflammation, advanced glycation end products (AGEs) and endoplasmic reticulum stress/unfold protein response (UPR) [3–7]. The most common consequence is hepatic steatosis and/or cardiovascular disease that lead to a serious threat to human's life [8].

Significantly, early start therapy is not needed to a clear diagnosis for PCOS. Initiate treatment may down-regulate the risk of potential long-term complications even without a definitive diagnosis. There are usually diagnosed later, as a recommended option, before supplying expectant therapy and frequent follow-up of clinical symptom. Every woman with PCOS should be individualized to the demands, clinical

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feature and other parameters in the therapy scheme. Aims of therapy are to make a good quality of life and ensure long-term fitness and health [9]. Many of therapeutic strategies including metformin, hormonal contraceptives, orlistat, pioglitazone and vitamin D (VitD) are first-line treatment for PCOS, but often require to be paired with other therapeutic strategies to further ameliorate PCOS clinical symptoms [10–14]. Researchers and clinicians should be comprehended that PCOS is correlated with significant endocrine and metabolic comorbidity and deliberate for these issues properly.

Here, our main purpose is to review current literature on the pathogenesis of PCOS and the interaction between these pathophysiological changes. Also, emerging researches, significantly novel hypotheses and recent advances to exhibit a picture of the therapeutic trends in the therapy of women with PCOS were evaluated in detail below.

1.1. The main pathologic changes of PCOS

The dysregulation of liver and ovary is a major reason for PCOS, which results in an excess of androgen, insulin, estrone, LH and lipid. In this review, reference is mainly composed of the participation of endocrinic and biochemical disorders. In their description of PCOS pathogenesis, Michael and his colleagues emphasized that hyperandrogenemia (HA) is a leading cause, and disease throughout the course of PCOS. It has been also indicated that HA is not an isolated factor to plays an important role in PCOS procession [15]. In addition, an imbalance ratio of LH to FSH, increased estrone, lipid accumulation, hyperinsulinemia and IR have significant effects on PCOS, which are organized non-linearly. And they can form a network with HA through a series of protein interactions [15,16].

1.2. The effect of hyperandrogenemia (HA) on PCOS development

Mostly, the polycystic ovary syndrome (PCOS) was characterized by accumulation of excessive testosterone in plasma of the patients without hyperthecosis, adrenal hyperplasia or other endocrine or ovary disease. Thus, hyperandrogenemia (HA) played a significant role both in the course of basic research and clinical therapy for PCOS patients [17].

HA could restrain follicular development and maturity to induce atresia of developed follicular, causing persistent anovulation. Furthermore, accumulation of excessive testosterone could convert into estrone in adipose tissues to induce an increased ratio of estrone to estradiol, which not only affected follicular development but also upregulated the ratio of luteinizing hormone (LH) to follicle-stimulating hormone (FSH), causing ovulatory dysfunction [18,19]. Additionally, excessive androgen may join in the development and occurrence of the typical upregulated frequency and amplitude of LH and GnRH pulse secretion induced by impairing negative feedback, attributing to HPOA defection [20]. HA upregulates the function of ring finger protein 6 (RNF6)-mediated K48 site-specific androgen receptor (AR) polyubiquitination, which induce the down-regulation of growth differentiation factor 9 (GDF9) and kit (Kit) expression, resulting in antral follicle growth arrest [21]. AR has a positive correlation with HA in PCOS patients [22], which can obviously promote maturation precursor of the sterol regulatory element-binding protein (SREBP) to induce lipogenesis, mediated by activating and upregulating the expression of SREBP cleavage activating protein (SCAP) [23].

HA could increase the amount of type IIb skeletal muscle fibers with low insulin sensitivity, decrease the sensitivities and expression levels of glucose transporter protein-4, inhibit the degradation of insulin by liver. And the excessive free fatty acids could inhibit insulin-stimulated glucose transport in skeletal muscle in HA. These important pathophysiological functions of HA may be associated with hyperinsulinemia and insulin resistance [24]. Meanwhile, accumulation of excessive testosterone could redistribute extremital fat to abdominal fat, which is one of the potential mechanisms of insulin resistance [25]. Combination of the above, a vicious cycle had been created: androgen strongly stimulated the production of insulin, but androgen was increasingly stimulated by insulin.

One hand, the researchers indicated the excessive androgen can upregulate the level of anti-mullerian hormone (AMH) which could inhibit folliculogenesis and ovulation by inhibiting ovarian gene expression, steroidogenesis and FSH effect in GCs from PCOS women [26,27]. On the other hand, sex hormone-binding globulin (SHBG), a biomarker of PCOS, is secreted and synthesized by hepatic cells into blood where it binds androgen with high affinity to attenuate its biological activity. However, the expression level of SHBG is obviously decreased in PCOS, which may be a significant factor in the development and occurrence of PCOS [28].

These data demonstrated that HA in patients of PCOS were associated with persistent anovulation and that androgen affected not only integral endocrine and metabolic response but also the function of hypothalamus-pituitary-gonad axis. Androgen therefore played a central and significant role in regulating the occurrence and development of PCOS.

1.3. Hyperinsulinemia can facilitate the progression of PCOS

Insulin taken part in the development of the human body during physiological conditions, and it could be overly activated in pathological conditions such as PCOS. Increased hormonal readiness of insulin was accompanied by up regulation in HA in PCOS patients. But far more importantly, insulin could directly activate the cytochrome of P450c17 in thecal cells, which was associated with the synthesis of androgen [29].

David Cadagan et al. showed that the levels of CYP17 was significantly upregulated by the synergistic effect of LH and insulin, which could increase the overall thecal steroidogenic activity to stimulate the synthesis of androgen [30]. Thus, HA, and the closely related hyperinsulinemia, are an inextricable part of PCOS. In granulosa cell of PCOS patients and normal, insulin plays an important role in production of E2 though the aromatase, as insulin may be direct or induced by receptors of insulin-like growth factors, resulting in the production of E2 as effective or more effective than FSH in doing so [31]. Additionally, an accumulation of insulin can enhance the frequency and amplitude of GnRH and LH pulse secretion in PCOS, both in dose or time dependent way [32].

1.4. The role of insulin resistance (IR) in PCOS

When hepatic cells are insulin resistant, hyperinsulinemia will dominantly downregulate the production of insulin like growth binding protein (IGFBP1), thus upregulating the levels of IGF-II in PCOS-IR [33]. The increasing IGF-II suppresses folliculogenesis by enhancing the levels of androgen [34]. Furthermore, the observation that IR stimulation of testosterone production, unlike IGF-II pathway, requires inhibition of SHBG indicates that there may be a divergence in the IR signal distal to insulin, with one branch regulating IGF-II and another branch regulating SHBG production [35]. These results indicate that IR can induce HA [36]. In recent study, some researchers have come up with the finding that lower expression of insulin receptor-β (INSR-β) was observed in PCOS-IR group luteinized granulosa cells as compared to PCOS-NIR group luteinized granulosa cells. Then, the decrease of INSR-β not only increases apoptosis but also influences the downstream insulin signaling pathway, which up-regulates pIRS(ser 307), pP38 MAPK, ERK1/2, PPARγ and down-regulates PI3K, pAkt, PKC in PCOS-IR [37]. Increase in protein expression of pP38 MAPK and ERK upregulate thioredoxin-interacting protein to amplify the levels of reactive oxygen species (ROS) that is responsible for the lower cell survival rate [38,39]. Furthermore, significant activation of pP38 MAPK, a mediator for gonadotropin releasing hormone-stimulated luteinizing hormone b

promoter activity, could promote granulosa cells to be premature luteinization in PCOS [40]. The PPARγ enhances the gene expression of SREBP1c, CPT-1, FAS and ACC-1, and up-regulate the levels of cholesterol, fatty acids and other lipids in PCOS-IR [37]. Furthermore, ACC-1 play a significant role in denovo lipogenesis (DNL) that up-regulates fatty acid synthesis and down-regulates the fatty acid transporter CPT-1, thus increasing the levels of ACC-1 leading to decrease β-oxidation [37,41]. Taken together these results indicate that IR could promote lipid accumulation.

Furthermore, the levels of LH receptor (LHR) and FSH receptor (FSHR) is significantly increasing in PCOS-IR patients compared to PCOS-NIR and normal person. The result indicates that IR may potentiate the pathophysiology of gonadotrophic hormones in PCOS [37]. Interestedly, IR mediates ROS production by leukocytes at the pathophysiology of metabolic syndrome [42] and it also activates pro-inflammatory transcription factor nuclear kB (NF-kB) [43,44] and leukocyte-endothelium interactions [45].

1.5. The ratio of luteinizing hormone (LH) to follicle-stimulating hormone (FSH) is dysregulated in PCOS

There are four types of HPOA function disorders, including accelerated GnRH pulsatile activity, increased secretion of pituitary LH, theca-stromal cell hyperactivity and hypofunction of the FSH-granulosa cell axis [46].

In the recent study increase in LH/FSH ration is one of the most typical symptoms of PCOS [47]. The levels of FSH receptor (FSHR) and LH receptor (LHR) is significantly increased in PCOS [37]. The observation that polymorphic variants of FSHR and LHR in PCOS have a stronger relation with its clinical symptoms including excessive gonadotrophic hormones levels, the presence of hyperinsulinemia and HA thus leading to a series of endocrine disorder [48]. These pathophysiological changes imply that PCOS is a dysfunction of the hypothalamic-pituitary-ovarian or adrenal axis. FSHR and LHR are the gonadotropin receptors which play an important role in folliculogenesis and ovulation respectively.

In theca cell, higher level of LH interacts with LHR to activate adenylate cyclase. Subsequently, activated adenylate cyclase facilitates protein kinase A, ultimately increasing the levels of P450cc, CYP17A1 and 3β-HSD and StAR [49,50]. Furthermore, the interaction of LH and LHR can also activate PI3K to promote the phosphorylation of Akt, which will induce increased activity of CYP17A1 and the proliferation of theca cell [51–53]. The theca cell proliferation and the upregulated steroidogenic machinery increase significantly the levels of androgen, which could facilitate the formation of HA. In granulosa cells, the interaction between FSH and FSHR is increased, up-regulating PI3K/Akt/mTORC1 signaling pathway to increase the levels of CYP19A1 [50].

1.6. The consequence of obesity and metabolic disorders and its potential mechanisms

Obesity was one of the important aberrations in PCOS [54]. The study of PCOS patients, the majority showed an obesity and the appearance of male features. Their fats were characteristically accumulated in the abdominal wall and the visceral mesentery, which was more sensitive to catecholamine than insulin. Meanwhile, this type of body fat distribution could induce IR, hyperinsulinemia and abnormal glucose tolerance [55]. Recently, it was reported that high circulating FFA levels have been shown to increase the production of all androgens in normal women [56].

Adiponectin, a 244-amino acid protein with an important function of increasing liver insulin sensitivity [57], is decreased in PCOS patients compared to normal girl [58]. It can upregulate the levels of SHBG to reduce the content of androgen in HepG2 cell, which is attributed to that adiponectin activates AMPK to reduce the mRNA and protein levels of acetyl-coenzyme A (CoA) carboxylase (ACC) and to enhance the levels of carnitine palmitoyltransferase I (CPTI) and peroxisomal acyl-CoA oxidase (ACOX). These changes of enzymes mediated by adiponectin decrease free FA and triglycerides (TGs) levels, resulting in turn upregulated HNF-4 [59] which upregulates the production of SHBG [36].

1.7. Inflammatory cytokine mechanism of action in PCOS: downstream regulated protein and signaling pathway

Recently, it was reported that low-grade chronic inflammation state plays a significant role in the pathophysiology of PCOS [43,44,60], and that this can induce an increase in inflammatory cytokine production including tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-β) [61], which can facilitate cell apoptosis [62] and chronic anovulation [63].

Furthermore, increased TNF-α inhibits the mRNA and protein levels of HNF-4α to activate nuclear factor kappa B (NF-kB), which ultimately downregulate the production of SHBG in HepG2 cells [64–66]. Meanwhile, higher level of IL-β can also activate NF-kB to inhibit SHBG. And the potential molecular mechanism is attributed to decrease in levels of HNF-4α by the MAPK kinase (MEK)-1/2 and c-Jun N-terminal kinase (JNK) MAPK signaling pathways through the activated c-Jun transcription factors [64–66]. The downregulated SHBG can leads to HA [36].

On the other side, the increase of TNF-α could promote the phosphorylation of PIMT at an ERK2 target site to inhibit the expression of GLUT4, MEF2A and HDAC5, which could downregulate glucose uptake to induce IR [67]. Furthermore, accumulating IL-6 and TNF-α can induce endothelial dysfunction via reduced vasodilation and decreased nitric oxide (NO) synthesis [68]. NO-mediated signaling attenuation in blood vessels and platelets has been demonstrated in PCOS women, which may lead to a common complication that premature atherogenesis [69].

Meanwhile, Peroxisome proliferator-activated receptor (PPAR)-γ, a kind of nuclear hormone receptors (NRs) activated by ligand, belongs to the steroid receptor superfamily, which play a central role in controlling a unique network of downstream genes (IL-6 and TNF-α) to promote the occurrence and development of metabolic diseases and obesity [70–72]. In the ovary, the expression of PPAR-γ is primarily located in GCs. However, the mRNA level of PPAR-γ is decreased in PCOS women compared to non-PCOS women [70], which implies that the disorder of PPAR-γ is an intrinsic and initial reason for PCOS via the signaling of inflammatory cytokine.

The importance of PCOS study is obvious, and for ‘fertility storage condition’ to be proper functioned, one of the conditions that should reasonably be met is the multiple follicles. Inflammation is considered as an ocean waves, and to ebb it over, or detecting that woman body in the inflammatory state is unsuitable in pregnancy, the ovarian follicle is not apoptosis or necrocytosis but saved as cysts, for next ovulation, when appropriate situation will return. This is a potential cause that the multiple gestation is a common clinical feature of women with PCOS.

1.8. Endoplasmic reticulum stress/unfold protein response in PCOS physiology: key player in hyperlipidemia, hyperglycemia, IR, obesity and metabolic syndrome

The UPR, an extremely conserved signaling pathway activated by endoplasmic reticulum stress, interacts with three endoplasmic reticulum transmembrane receptors such as inositol requiring enzyme 1 α (IRE1α), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6α) and the master chaperone, glucose-regulated protein 78 kDa (GRP78, also known as BiP) to regulate protein synthesis, folding, and processing (as shown in Fig. 1) [73]. The major highly conserved downstream receptor of this pathway is IRE1α that bind to the endoplasmic reticulum chaperone BiP. The BiP are responsible for IRE1α activation via dimerization and autophosphorylation when the

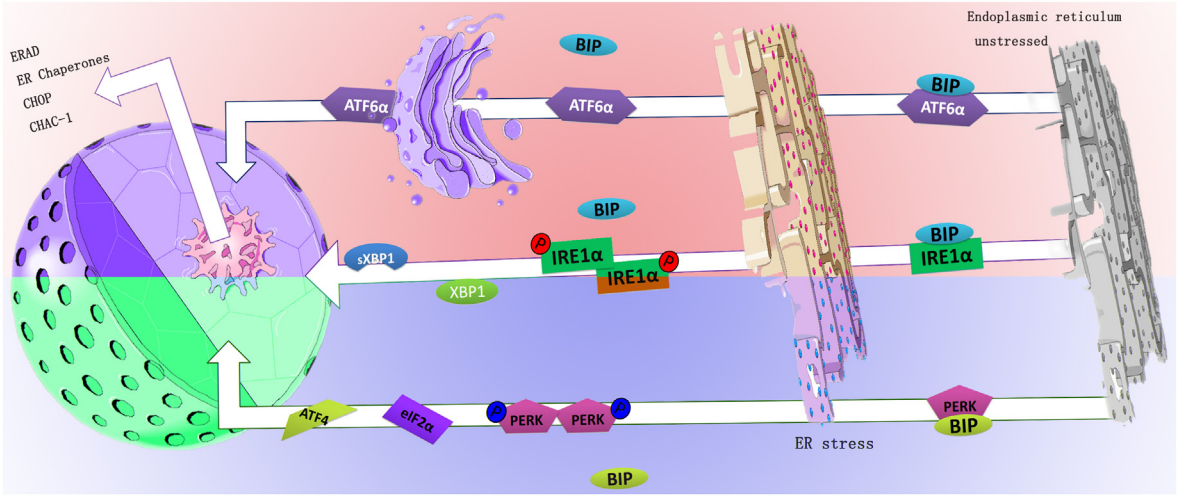

Fig. 1. The role of unfolded protein response (UPR) in PCOS.
Generally, BiP, the endoplasmic reticulum (ER) chaperone, is bound to these receptors such as PERK, ATF6α and IRE1α when ER is unstressed. In the ER stressed state, The BiP disassociate from PERK, ATF6α and IRE1α. Upon these receptors dissociation from BiP, PERK is activated by autophosphorylation and dimerization. The activated PERK induces phosphorylated eIF2α. Subsequently, phosphorylation of eIF2α promote the translation of ATF4. ATF6α translocate to the Golgi apparatus, which activate ATF6α by proteolytic cleavage to a 50-kDa transcription factor. IRE1α forms a dimer leading to its activation by autophosphorylation. The activation of IRE1α increase the spliced XBP1 that translocates to the nucleus. These factors translocated to the nucleus, XBP1s, ATF4, and ATF6α, activate lots of genes such as Chop protein, Chac-1 protein, ER chaperones, some genes related to ERAD, and ultimately promoting the development and occurrence of PCOS.

accumulation of unfolded proteins induces endoplasmic reticulum stress. The activated IRE1α up-regulates X-box binding protein (XBP1) which transcriptional regulates the endoplasmic reticulum chaperones and endoplasmic reticulum associated degradation (ERAD) [74]. The auto-phosphorylated PERK facilitates the phosphorylation of eukaryotic initiation factor (eIF2α) which is crucial for translation initiation and therefore protein synthesis [73]. Activation of eIF2α increase activating transcription factor 4 (ATF4), which promote the production of molecular chaperones and activate ERAD [75]. Furthermore, activated ATF4 promote the activation of C/EBP homologous protein (CHOP) that mediate GADD34 to dephosphorylate eIF2α, and ultimately making protein translation to resume [75,76]. Upon dissociation from BiP, the ATF6α translocates to the Golgi apparatus and therefore be activated. Subsequently, the activated ATF6α translocates to the nucleus and up-regulates endoplasmic reticulum chaperones, and ultimately promotes ERAD [77]. In recent year, Celia Bañuls not only evaluated mRNA expression of Bip and XBP1 but also protein levels of Bip and ATF6, which indicated that UPR was extremely up-regulated in the presence of PCOS subjects [78].

PCOS is intimately linked to hyperlipidemia, hyperglycemia, IR, obesity and, ultimately, metabolic syndrome. While the metabolic syndrome is a highly general condition known to potentiate the long-term evaluation of cardiovascular disease risk [79]. Previous studies have indicated that leukocytes from women with PCOS display the features of oxidative stress and mitochondrial impairment, while IR can exacerbate these dysregulations [7]. The raise of endoplasmic reticulum stress, inflammation and ROS play key roles in the interactions between leukocytes and the endothelium, thereby increasing the development of PCOS and vascular dysfunction [78].

1.9. The promotion of PCOS occurred by advanced glycation end products

Advanced glycation end products (AGEs), one of emerging pro-inflammatory molecules, can induce inflammation and oxidative stress by combining with receptor for AGEs (RAGE) which is widely distributed in many tissues, such as vascular system [80]. The formation of AGEs is increased by HA, hyperglycemia, IR, obesity, metabolic syndrome, hypoxia and ROS [3,81]. Interestedly, Diamanti-Kandarakis and his colleagues found that the level of RAGE in GCs is higher than theca cells [82]. Furthermore, high level of AGEs can potentially trigger insulin signaling alteration and interfere with GLUT4 membrane translocation (especially in GCs [81]), resulting in aggravation and increased illness [83]. A previous study indicates that the level of AGEs is increased in PCOS patients [3], which induce ovarian chronic inflammatory condition [82,84] and early ovarian aging [85].

In adipose tissue, the activation of CML-RAGE axis and the accumulation of CML (one of the AGEs) mediated by RAGE promote obesity and adipokines disorder, further suggesting a link among adiposity, AGEs and obesity [86]. Moreover, AGEs-RAGE axis activates inflammatory transcription factor (NF-κB) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, leading to product chemokines, inflammatory cytokines, adhesion molecules [87,88], which causes a positive feedback loop to exacerbate the inflammatory processes [89]. Additionally, many studies indicated that the levels of AGEs had a positive correlation among androgen [3], insulin [3] and AMH [90].

1.10. Genetic mutation and epigenetic modification of PCOS

Interestedly, with the deeply penetration in genetic predisposition of PCOS, it is demonstrated that there is a great association between numerous genes and pathways in PCOS. The patients with genes mutations, such as deletions, inversions, single nucleotide polymorphisms (SNPs), additions, are the possible causes for occurrence and development of PCOS. A series of such causative genes have been genotyped by whole-genome sequencing (GWAS) and polymerase chain reaction.

Some of important genes including 11-beta-hydroxysteroid dehydrogenase type 1 (HSD11B1) and hexose-6-phosphate dehydrogenase (H6PD) are significant genetic locus at chromosome lq32-41 [91] and lp36.2 [92], respectively. HSD11B1 gene product is association with the IR in the granulosa cells of PCOS [93]. The H6PD has been previously detected in PCOS, which indicates its pathologic role associated with obesity and adrenal activity [92,94]. Serotonin system polymorphisms have been reported in PCOS women. The gene polymorphisms of 5HTR1A rs6295, 5HTR1B rs13212041, and SLC6A4 5HTTLPR in PCOS have been shown to relate with glucose-stimulated insulin secretion. A study based on PCOS patients revealed that the SLC6A4 5HTTLPR gene is linked to PCOS occurrences and development [95]. Aberrant

signaling pathway of LH may be involved in augmenting ovarian androgen production in these women with PCOS and leading to anovulation [96]. The association between luteinizing hormone beta-subunit (LHβ) and LH/choriogonadotrophin receptor (LHCGR) variations and the predisposition has been traced [97]. The levels of LH is upregulated in PCOS, and genetic mutations of the LHβ and LHCGR have been detected in these woman with PCOS [97]. The polymorphism of follicle-stimulating hormone B polypeptide (FSHB) gene has been linked to the etiology of PCOS based on its association with LH level [98]. Furthermore, the variation of FSH receptors (FSHR) gene is a PCOS risk factor, which disturb FSH signaling to promote the occurrences and development of PCOS [99].

Monocyte chemoattractant protein-1 (MCP-1), an important chemokine in the inflammatory response, plays a vital role in recruiting monocytes to sites of injury and infection, whose polymorphism is linked to the risk of PCOS by affecting transcriptional activity [100]. Recently, it was reported that the variants in AMH is detected in PCOS, which significantly reduce the activity of this signaling that AMH downregulate androgen biosynthesis by decreasing CYP17 activity in theca cells [101]. The presence of shorter and longer (CAG)n repeats in the AR gene sequence, located in the Xq11-q12, is a risk factor for PCOS development [102]. Another factor for this predisposition is SREBP1 (Sterol regulatory element binding protein-1) gene, which is involved in PCOS development [103]. Recently, it was reported that the effect of cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) gene mutation on metabolic phenotypes was detected in women with PCOS. The intragenic rs733618 in CTLA4 gene was demonstrated. An emerging data suggests that under the downregulated expression of CTLA4, the body of PCOS patients may be relatively easier to keep low level of inflammatory conditions and enhance the quality of the fat, which are the primary clinical features of PCOS patients. Moreover, it is sufficiently suggested that CTLA4 may play a significant role in the metabolic systems mediatizing IR and lipid accumulation by influencing the activation of T-cell [104].

The association of Hippo signaling pathway gene (MOB1A, MOB1B, WWTR1, and YAP1) polymorphisms with the occurrence and development of PCOS, which has been reported in previous study. Normal stromal cells of ovarian expressed YAP at a very low level, while the down-regulation of YAP aromatase (CYP19A1) mediated by FSH and may be attributed to a potential mechanism for HA [105]. In PCOS patients, the density and number of ovarian cellular is more than in the ovary of normal women [106]. Maas and colleagues have indicated that these genes expression levels of Hippo signaling pathway are enhanced in PCOS patients when compared to normal women. This enhancement potentially induces un-controlled apoptosis and proliferative dysregulation. Therefore, dysfunction of the Hippo signaling pathway in women with PCOS is a possible influencing factor for cellular overgrowth [107]. Aberrant of the Hippo signaling pathway is potentially one of the underlying pathophysiological changes for the clinical PCOS features, leading to theca cells hyperplasia, enlarged ovaries with numerous small follicles and thickened ovarian cortices. Hence, they are associated to PCOS obviously. Genes coding for these proteins, and other key proteins in Hippo signaling pathway, potentially play a key role in PCOS [107]. The VitD receptor gene polymorphisms, *Bsm*I and TaqI, were closely linked to PCOS. Furthermore, VitD levels have a closed relation with the HA [108]. So, it is worthy of investigation if VitD receptor gene might be acting as a therapeutic target for HA in these women with PCOS. The genetic changes of PCOS are as follows in Table 1.

However, the potential role of epigenetics in the development and occurrences of PCOS are being investigated, such as histone acetylation, DNA methylation and other changes in chromatin structures. Post-transcription processing of proteins is significant for protein expression and functions, which like a switch to act the “on” or “off”. Recently, it was actually reported in many clinical classifications of PCOS and is linked to the disorder and aberrant transcriptional modifications of genes. A research based on PCOS or normal women demonstrated that hypomethylated or hypermethylated genes may disturb expression of these genes and facilitate biosynthesis of steroid hormones such as androgen, which could explain, at least partially, the underlying mechanisms of HA in PCOS [109]. In recent, A research based on 17 PCOS women and 14 controls demonstrated that PCOS is closely connected with disorder expression of skeletal muscle gene promoter with especial modification in muscle DNA methylation, which is the first research to identify that these women with PCOS have aberrant transcriptional modifications in skeletal muscle that can partially account for metabolic disturbance seen in these women [110].

1.11. Current idea for clinic therapy of PCOS

Importantly, lifestyle modifications, especially in weight loss, are the first-line treatment of PCOS, which can ameliorate response to ovulation induction and fertility therapy and maintain a regular menstrual cycle [111]. Multiple forms of fasting, intermittent fasting and periodic fasting, can change the circulating levels of insulin growth factor-1 (IGF-1), IGFBP1, glucose and insulin levels, which may have therapeutical effects to alleviate HA, female sterility, ovulation disorders in patients with PCOS(B. [112]). Furthermore, a series of significant nutraceuticals (such as inositol, lipoic acid and N-Acetylcysteine) has an important role in the metabolism of glucose and lipids, which can act in synergy with many drugs of insulin sensitization and/or nutraceuticals to adequately alleviate IR [113].

Drugs selection of PCOS contains metformin, orlistat, hormonal contraceptives, GLP1 agonists, and VitD. Metformin, a first-line medicine of insulin resistance or type-2 diabetes mellitus, is recommended in PCOS women, especially in obesity. It can impede the development and occurrences of PCOS mediated by decreasing these factors including insulin and androgens. The mechanism is mainly attributed to upregulate the levels of IGFBP (insulin-like growth factor-binding protein) and SHBG [11]. The combination of metformin and pioglitazone can ameliorate reproductive and metabolic factors including HA and hyperinsulinemia. Furthermore, the expression of FSHR and LHR mRNA can be upregulated by the combine treatment [13]. The combination of metformin and flutamide can decrease levels of plasma androgen indices, triglycerides, Apolipoprotein B lipoproteins 100 (ApoB100) and Apolipoprotein B lipoproteins 48 (ApoB48). This combination treatment also exhibits many capacities that include improvement of ovarian primary as well as preovulatory follicle frequency. Meanwhile, the treatment of metformin and flutamide not only up-regulates the mRNA expression of estrogen receptor α (ER-α) but also down-regulates AR in intestine, which further activate insulin signaling pathway such as AKT, insulin receptor as well as MAPK1 [114].

Orlistat, an FDA-approved drug, works by restraining fat hydrolysis into absorbable free fatty acids and upregulating monoglyceride in fecal fat excretion, which has better pharmacological function on decreasing body mass index than metformin in PCOS women with obesity and lipids accumulation [12]. On the other side, Orlistat is better tolerance and has lesser side effects than metformin [14].

Oral Contraceptives (OCs): a daily dose (20–35 micrograms) of ethinyl-estradiol can effectively inhibit androgen production in ovary via decrease the stimulation of HPOA, which has no effects on sexual behavior as happened for ultra-low estrogen concentration (15 micrograms). Drospirenone, a novel progesterone, have different and significant characteristics in anti-mineralocorticoid and anti-androgenic function, which has been increasingly applied to combination with estrogens in PCOS patients [115].

Diane-35, contains 2-mg cyproterone acetate (CPA) and 35-μg ethinylestradiol (EE), can ameliorate the pathogenic effect of HA and mitigate clinical symptoms of HA including seborrhea, acne and hirsutism. It is more effective in decreasing androgen when combined with orlistat other than Diane-35 alone [14]. Other studies also indicate that the combination therapy of metformin and oral contraceptives avert

Table 1
List of mutation gene involved in polycystic ovary syndrome (PCOS).

Genes	Full name	Function	Location
HSD11B1	11-Beta-hydroxysteroid dehydrogenase type 1	Association with the IR in the granulosa cells of PCOS	lq32–41
H6PD	Hexose-6-phosphate dehydrogenase	The mutation of H6PD may contribute to the PCOS phenotype by influencing obesity, insulin resistance and hyperandrogenism.	lp36.2
5HTR1A	5-Hydroxytryptamine receptor 1A	Mutation in the promoter of this gene has been associated with menstrual cycle-dependent periodic fevers	5q12.3
5HTR1B	5-Hydroxytryptamine receptor 1B	The encoded protein may be involved in several neuropsychiatric disorders and therefore is often a target of antidepressant and other psychotherapeutic drugs.	6q14.1
SLC6A4	Solute carrier family 6 member 4	SLC6A4 polymorphism influences insulin secretion in PCOS patients	17q11.2
LHβ	Luteinizing hormone beta-subunit	It is involved in augmenting ovarian androgen production in these women with PCOS and leading to anovulation	19q13.33
LHCGR	LH/choriogonadotrophin receptor	Mutations in this gene result in disorders of androgen production in patients with PCOS	2p16.3
FSHB	Follicle-stimulating hormone B	This gene encodes the beta subunit of follicle-stimulating hormone. In conjunction with luteinizing hormone, follicle-stimulating hormone induces egg and sperm production.	11p14.1
FSHR	Follicle-stimulating hormone receptors	This gene encodes the receptor of FSH, which functions in gonad development.	2p16.3
MCP-1	Monocyte chemoattractant protein-1	A pivotal chemokine in the inflammatory response, which induces IR and influences the way ovaries produce and release eggs	17q12
AMH	Anti-mullerian hormone	An important hormone that inhibits folliculogenesis and ovulation by inhibiting ovarian gene expression, steroidogenesis and FSH effect	19p13.3
SREBP1	Sterol regulatory element binding protein-1	Mutation of this gene can induce lipid accumulation in PCOS patients	17p11.2
CTLA4	Cytotoxic T-lymphocyte-associated antigen 4	CTLA4 may play a significant role in the metabolic systems mediatizing IR and lipid accumulation by influencing the activation of T-cell	2q33.2
MOB1A	MOB kinase activator 1A	The protein encoded by this gene is a component of the Hippo signaling pathway, which controls organ size and tumor growth by enhancing apoptosis.	2p13.1
MOB1B	MOB kinase activator 1B	A protein kinase essential for spindle pole body duplication and mitotic checkpoint regulation.	4q13.3
WWTR1	WW domain containing transcription regulator 1	TAZ is a widely used alternative name for the transcriptional coactivator with PDZ-binding motif (WWTR1) conflicting with the official symbol for tafazzin (TAZ).	3q25.1
YAP1	Yes associated protein 1	This gene encodes a downstream nuclear effector of the Hippo signaling pathway which is involved in theca cells hyperplasia, enlarged ovaries with numerous small follicles and thickened ovarian cortices.	11q22.1
VDR	Vitamin D (VitD) receptor	This gene encodes vitamin D receptor, which is a member associated with the clinical hyperandrogenism.	12q13.11

over-weight and further suppress HA. In addition, the efficacy and safety of liraglutide (glucagon-like peptide-1 (GLP-1) receptor agonists)-metformin on decreasing waist circumference and losing weight in PCOS patients have obtained [116]. Furthermore, this combination can significantly enhance pregnancy rate (PR) and embryo transfers (ET) in women with PCOS [117].

It had been also demonstrated that treatment of megadose VitD for 12 weeks to PCOS patients with IR got well curative effects on the levels of total testosterone, SHBG, free androgen index, serum hs-CRP and plasma total antioxidant capacity compared with low-dose VitD and control groups [10].

The confirmatory data on the role of laparoscopic ovarian drilling (LOD) in women with PCOS were published in the year of 1994 [118]. In an Egypt prospective multicenter study by Salah, 120 anovulatory women with PCOS who diagnosed by Rotterdam criteria were detected with pregnancy rates and ovulation rates within 1 year of follow-up [119]. In this study, the total number of 60 anovulatory women with PCOS underwent office microlaparoscopic ovarian drilling (OMLOD), and another 60 PCOS women underwent LOD, respectively [119]. They found that the ovarian morphology and function normalized by OMLOD is similar to after conventional LOD in PCOS patients. However, the intraoperative and postoperative pain scores of OMLOD is better than LOD [119].

Assisted reproductive technologies (ART) have been shown to increase the pregnancy rate in women with PCOS such as *in vitro* fertilization (IVF), embryo transfer (ET) and Intracytoplasmic sperm injection (ICSI, account for 99.5% of ART) [71,72]. However, it is worth noting that PCOS women undergoing IVF had lower FSH, the oocyte retrieval was higher, the fertilization rate slightly lower compared with controls [120,121] and clinical abortion rate in PCOS women seems to be higher than in healthy controls [120], which may be induced by myo-inositol (MI) deficiency [122]. The potential mechanism is attributed to MI, which is effective in both PCOS and non-PCOS women in saving gonadotropins during IVF ([123]).

2. Conclusions and perspectives

In this review, we have summarized the physiopathologic mechanism and therapeutic strategies of PCOS because it is authentically complicated, significant individual differences and has been and remains unclarified until now, which is exhibited in the schematic representation of Fig. 2. Plentiful gynecological researches demonstrate that PCOS, as a one of multiple etiology disease, seems to have close association with dysregulated steroid state, endocrine dyscrasia, inflammatory, neuroendocrine disease, afflicting females of reproductive age. Meanwhile, unhealthy living habits, epigenetics and genetics are factors that induce the occurrences and development of PCOS.

Molecular biology provides startling opportunities on PCOS, but it is important that researchists clarify a better understanding of the interactions among these potential mechanisms or characteristics including HA, hyperinsulinemia, HPOA function disorders, IR, metabolic aberrant, inflammation, AGEs and UPR. Due to the multiple etiology of PCOS, the multi-drug of so-called “cocktail therapy” will promote more systemic therapy for the disease.

Therefore, ongoing and future researches are required to elaborate the relationships among fundamental cause, trigger and clinical treatments of PCOS that could be developed novel treatment ideas with more scientific value and clinical value. Meanwhile, if we only regard PCOS as an infertility disease that we will lose the right path on which precision treatment of PCOS. Nowadays, an increasing number of researchers suggest that PCOS is a reproductive and endocrinologic disease with metabolic aberrant. Finally, the epigenetic concept has been widely applied in PCOS development and occurrences, which should contribute to us to explore novel treatment program that may assist us to diagnose and treat PCOS at early stages.

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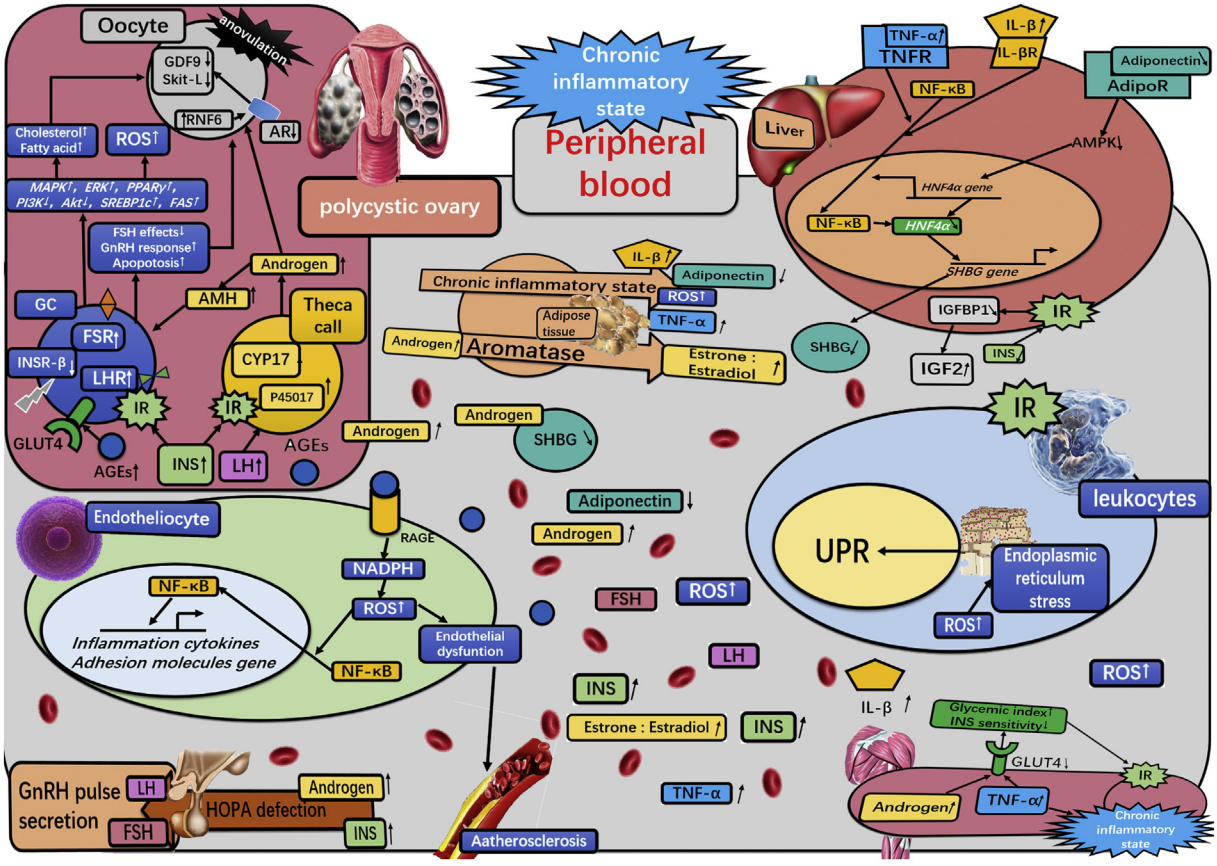

Fig. 2. The potential mechanisms of PCOS.
Women with PCOS are under chronic inflammatory state in a long term, and may have increasingly risk of PCOS mediated by upregulated androgen, insulin, LH, FSH, AGEs, IGF2, ROS, inflammatory cytokines, and downregulation of adiponectin and SHBG. These pathophysiologic change will lead to the dysregulation of hepatocyte, myocyte, adipocyte, leukocyte, endotheliocyte, theca cell, granulosa cell and ovocyte, which induce a series of typically clinical manifestations such as HA, IR, polycystic ovaries, metabolic disturbance, atherosclerosis and anovulation in PCOS patients.

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Conflicts of interest: None.

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