

FOXO3 is a potential biomarker and therapeutic target for premature ovarian insufficiency (Review)

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Received August 17, 2022; Accepted November 29, 2022

DOI: 10.3892/mmr.2022.12921

Abstract. Premature ovarian insufficiency (POI) is a common clinical disease of the reproductive system in which patients lose normal gonadal function prior to the age of 40. Common pathogenic factors include iatrogenic injury, genetics, inflammation, autoimmune, environmental and psychological factors. Patients with POI experience decreased estrogen secretion levels, ovulation disorder and infertility. POI appears frequently in clinical practice and the burden of the disease is heavy; however, the detailed pathological mechanism requires further experimental evaluation. Furthermore, there is a lack of effective treatment options. Certain causes of the pathogenesis of POI can be explained by epigenetic changes. Front fork transcription factor 3 (FOXO3) is a member of the forkhead box family of transcription factors. FOXO3 was initially considered to affect insulin/insulin growth factor signal transduction. However, the target gene range of FOXO3 includes

numerous genes that affect metabolism and protein stability and are associated with aging. There is an association between decreased FOXO3 expression levels and POI. In the present review article, the role of FOXO3 in POI was evaluated, which emphasized the importance of this protein in the investigation of this disease. Moreover, the present review evaluated the evidence for the potential targets and biomarkers of FOXO3 that may be used in the treatment and diagnosis of POI.

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1. Introduction

Premature ovarian insufficiency (POI) is a key cause of menstrual disorder and infertility. It is defined as a non-physiological menstrual cessation and primary or secondary infertility that occurs prior to the age of 40, accompanied by systemic genital atrophy, increased gonadotropin levels and decreased estrogen levels (1). POI typically includes follicular failure or apoptosis caused by oocyte atresia and follicular dysfunction (2). Current research indicates that POI is caused by numerous factors, such as iatrogenic injury, genetic, immune, environmental and psychological factors that may trigger the onset of this condition (3). However, >50% of patients present with unknown etiology of POI, which is known as idiopathic POI. To the best of our knowledge, there is currently no effective treatment for POI, which seriously affects the physical and mental health of patients with this disease.

Although >50% of clinical POI cases are idiopathic, 20–25% of POI patients still have a family genetic history (4,5). These genetic changes may include chromosomal imbalance

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Abbreviations: FOXO3, front fork transcription factor 3; POI, premature ovarian insufficiency; WES, whole exome sequencing; Kip1, kinase interacting protein 1; SIRT, sirtuin; OTUD1, OTU domain containing protein 1; CXCL, CXC motif chemokine ligand; CD, cluster of differentiation; MHC, major histocompatibility complex; SLE, systemic lupus erythematosus; GC, glucocorticoid; CBX, Carbenoxolone; MHCI, Major histocompatibility complex class I; MHCII, Major histocompatibility complex class II; SIRT, sirtuin

Key words: premature ovarian insufficiency, FOXO3, autoimmunity, inflammation, heredity, environment, psychology, iatrogenic damage

(such as X chromosomes or autosome imbalance) and point mutations (6).

Recent study have reported that front fork transcription factor 3 (FOXO3) is the primary regulator and an effective inhibitor of primordial follicle activation; loss of FOXO3 function in mice is due to overall follicle activation (7). Screening genetic samples from patients with premature ovarian failure has demonstrated six mutations in the FOXO3 coding region (8). These missense mutations may result in abnormal oocyte apoptosis and primordial follicle activation, which leads to depletion of early follicles in the ovary (8). The mechanism by which FOXO3 affects the progression of POI requires further study. FOXO3 directly affects development of oocytes and POI via other factors. To the best of our knowledge, no literature review has fully elucidated the relationship between FOXO3 and the pathogenesis of POI, such as iatrogenic injury, genetics, inflammation and autoimmunity, as well as environmental and psychological factors. Therefore, it is imperative to summarize the latest research on the association between FOXO3 and POI to evaluate the role of this protein in the pathophysiology of POI. The present review article used POI, FOXO3, iatrogenic injury, heredity, inflammation, autoimmunity, psychology and environment as keywords to search relevant literature from electronic databases, such as PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), Springer (<https://link.springer.com/>), Wiley (<https://www.wiley.com/en-us/business>) and ScienceDirect (<https://www.sciencedirect.com>). The mechanism by which FOXO3 is reported to affect POI was systematically summarized to provide evidence for the use of this protein as a potential treatment target and biomarker for POI.

2. Role of FOXO3 in iatrogenic injury-induced POI

Previous study have reported that pelvic surgery, such as ovariectomy, is an important cause of POI (9). Following removal of one ovary, levels of hormones secreted by the ovaries decrease and expression levels of the follicle-stimulating hormone secreted by the pituitary gland increase to compensate for this loss; therefore, the probability of developing POI in the remaining ovary increases (9-11). Concomitantly, when patients with cancer receive radiotherapy and chemotherapy, normal cells may also undergo damage. Radiation therapy to the abdomen or pelvis simultaneously damages normal ovarian tissue and germ cells (12,13).

A previous study have reported that iatrogenic injury is key in the pathogenesis of POI (14,15). In mammalian ovaries, the FOXO3 protein regulates atresia and follicular growth by promotion of the induction of apoptosis in ovarian granulosa cells. The PI3K/AKT and FOXO signaling pathways restore ovarian function and induce follicle formation, which suggests that FOXO3 phosphorylation may increase, which changes the balance of oocyte growth and apoptosis, and avoids numerous egg development disorders following ovariectomy (14). Furthermore, the establishment of postmenopausal osteoporosis animal models by removal of both ovaries in rats has been reported to demonstrate that inhibition of the expression of FOXO3a regulates the FOXO3a/Wnt2/ β -catenin signaling pathway and effectively improves bone structure following ovariectomy (15-17). Increased porosity increases

blood calcium content and decreases the bone density of the femur and vertebrae (15). FOXO3 also serves an important role in radiotherapy and chemotherapy treatment of patients with cancer. Previous study (18) have reported that melatonin decreases cisplatin-induced follicle loss by the prevention of phosphorylation of members of the PTEN/AKT/FOXO3a signaling pathway. Inhibition of FOXO3a phosphorylation increases the binding affinity of FOXO3a with the p27 [kinase interacting protein 1 (Kip1)] promoter, which affects the dormant state of primordial follicles, induces excessive activation of dormant primordial follicles and leads to loss of ovarian reserve in mice (19). Radiation therapy is a common cancer treatment. The inhibition of ovarian FOXO3 expression has been significantly reversed in a γ -ray irradiation mouse model, leading to an increase in the reserve of primordial follicles; this may provide a novel treatment method for radiation-associated POI (20).

3. Role of FOXO3 in the development of POI induced by epigenetic changes

During the pathogenesis of POI, a series of extensive and complex biological processes occur, including DNA replication and repair, germ cell development, mRNA transport and translation and sex hormone regulation (21-24). These processes are often disrupted by both common and rare gene mutations, which constitute a complex genetic pathogenic mechanism for POI. As infertility is an important manifestation of POI, it is difficult to systematically collect family history. However, with the development of whole exome sequencing (WES), certain studies have reported the role of rare destructive coding variants in the ovary (25,26). By studying a single family and performing a comprehensive WES analysis of patients in multiple unrelated families, certain gene variants associated with POI have been identified, such as helicase for meiosis 1, eukaryotic translation initiation factor and basonuclin (27). Medical records of patients with POI indicate that 13% of cases involve changes in the number and structure of the X chromosomes (2). The more common chromosomal abnormalities include partial X chromosome deletion, inversion, balanced X chromosome ectopic, X monosomy and trisomy (28).

Numerous study have reported that genetic changes serve an indispensable role in the pathogenesis of POI (29,30). A previous study reported that aging of the reproductive system is closely related to DNA damage. DNA damage in ovarian granulosa cells is closely related to the development of POI (31). The depletion of DNA-damaged oocytes occurs by different cell death mechanisms, such as apoptosis, autophagy and necrosis, which are primarily mediated via the PTEN/PI3K/AKT/FOXO3 signaling pathway. The activation of this pathway cascade increases cytoplasmic transport of FOXO3a in the follicles, which in turn increases the pool size of growing follicles and rapidly depletes the number of dormant follicles. The latter cannot be activated into the growth phase, which leads to final maturation and ovulation failure (32-34). Experiments have reported the location of the FOXO3 protein in the nucleus and cytoplasm and provided evidence for genetic study to examine this pathway (35). Furthermore, the increase in FOXO3a phosphorylation increases the binding affinity of FOXO3a to the p27 (Kip1) promoter in primordial follicles,

which results in activation of primordial follicles, an increase in the number of granulosa cells, a decrease in the number of atretic follicles and an increase in serum E2 and progesterone levels. Therefore, repairing ovarian function may be a new strategy for the treatment of POI (36,37).

4. Role of FOXO3 in psychological and environmental factor-induced POI

A previous study reported that 10% of POI cases are caused by bisphenol A, phthalates and polycyclic aromatic hydrocarbons. These pollutants cause changes in the epigenetic modification that affect the growth of follicles, which results in decreased follicular activity (38).

Psychological factors are key in the pathogenesis of POI. Strong and long-term negative emotions, such as anxiety, depression, sadness and fear, affect ovarian function. These factors affect normal hormone (For example, testosterone, gonadotropin releasing hormone) secretion via the hypothalamic-pituitary-ovarian axis and lead to the occurrence of POI (39,40).

A study have reported that changes in psychological and environmental factors are key in the pathogenesis of POI (41). It has been reported that tetrachlorodibenzo-p-dioxin, polychlorinated biphenyls and polycyclic aromatic hydrocarbons, present in the environment, amplify the accumulation of nuclear p53 caused by benzo(a)pyrene or dibenzo(a,l)pyrene (42). This effect is associated with attenuation of polycyclic aromatic hydrocarbon-induced apoptosis and decreased levels of FOXO3 phosphorylation at threonine 32 (43). This process may promote the translocation of the FOXO3a-p53 complex from the nucleus to the cytoplasm. Therefore, the dephosphorylation effect of FOXO3 has been previously studied (44). In addition, it is reported that FOXO3 is also related to environmental pollutants (such as cadmium and arsenic), which affect ovarian health, indicating that this protein may be an important factor leading to the development of POI caused by environmental factors (45). Increased phosphorylation of FOXO3a can alleviate adverse psychological states. A previous study reported that d-fenfluramine reduces nuclear FOXO1 and FOXO3a levels of these proteins, and enhances their phosphorylation, which thereby achieves a therapeutic effect (46). Previous results have indicated that the PI3K/AKT/FOXO3a signaling pathway has an antidepressant-like effect on depression caused by chronic stress (47-49). FOXO1 and FOXO3a can affect the processes related to anxiety and depression, which provides a molecular framework for potential therapeutic targets.

Oxidative stress is a common pathogenic factor in reproductive system disease. A previous study reported that oxidative stress may be a potential pathogenesis for POI induced by *Tripterygium wilfordii* polyglycosides (50). Subsequent studies have reported that SIRT (Sirtuins)-mediated signal transduction can reduce oxidative stress and apoptotic damage, which are required for development of POI (51,52). A study of clinical data reported that the levels of oxidative stress markers, such as nitric oxide synthase, myeloperoxidase and reactive oxygen species (ROS), are elevated in patients with POI. The upregulation of the expression levels of the nuclear factor erythroid 2-related factor 2/heme oxygenase pathway proteins improves ovarian function of patients with POI by the

inhibition of oxidative stress, which suggests that the latter is associated with development of this disease (53).

A study have reported that oxidative stress is key in the pathogenesis of POI (54). FOXO3 may serve a key role in the development of oxidative stress and serves as a signaling molecule associated with autophagy, which inhibits oxidative stress by binding to cytoplasmic STAT3 (55). The internal regulatory mechanism of ROS-mediated autophagy is regulated by the ROS-FOXO3-light chain 3/Bcl-2 interacting protein 3 autophagic signaling pathway (56). Previous studies have reported that SIRT1 has a dual effect on the function of FOXO3; it increases the ability of FOXO3 to induce cell cycle arrest and resist oxidative stress and concomitantly inhibits the ability of FOXO3 to induce cell death (57). SIRT3 deacetylates FOXO3 to protect mitochondria from oxidative stress. It also regulates mitochondrial quality, ATP production and clearance of defective mitochondria, while ensuring the quantity and quality of the mitochondria and maintaining mitochondrial reserve capacity for oxidative damage (58). Taken together, this evidence indicates that FOXO3 may be a potential target for treatment or the delay of ovarian aging and POI-associated diseases.

5. Role of FOXO3 in inflammation-induced POI

Inflammatory aging refers to the chronic and low-grade pro-inflammatory state that occurs with age and is a new concept in the field of aging research (59). Increased levels of inflammatory cytokines and decreased levels of anti-inflammatory cytokines serve a key role in the development of POI (60). Recent studies have reported that TNF- α and IL-6 may serve a role in ovarian function; therefore, controlling the development of inflammation and aging may be a method to treat POI (61,62). The expression levels of TNF- α , IL family proteins and inflammatory proteins in plasma along with other inflammatory markers can be used to monitor ovarian function and treat POI (62).

Previous study reported that inflammation is key in the pathogenesis of POI (63). FOXO3 may serve an important role in the development of inflammation. In the autophagic process of inflammation, the constitutively active form of FOXO3 induces autophagy, which indicates that this protein serves as a downstream target of the PI3K pathway to inhibit autophagy (64). Under inflammatory conditions, the inducible OTU domain containing protein 1 (OTUD1) serves as an immune checkpoint and the FOXO3 signal is necessary for the induction of OTUD1 following antigenic stimulation (65). A previous study reported that FOXO3 is significantly inactivated/phosphorylated in fibroblast-like synovial cells in rheumatoid synovitis (66). Furthermore, mRNA analysis has indicated that the inactivation of FOXO3 is important for the sustained pro-inflammatory interferon response to TNF- α , CXC motif chemokine ligand (CXCL) 9, CXCL10, CXCL11 and TNF superfamily member 18 (67). In terms of the mechanism of action, the inactivation of FOXO3 is caused by downregulation of phosphoinositide-3-kinase interacting protein 1, which is induced by TNF- α (68). Treatment of 16HBE cells with FOXO3 small interfering (si)RNA can increase IL-8 and decrease chemokine ligand 20 expression levels (69). FOXO3 can also promote translation

via N6-methyladenosine modifications of mRNA molecules. The increase in FOXO3 mRNA expression levels under steady-state conditions contributes to its role as a negative regulator of antiviral immunity and stabilizes the host's antiviral effect and immune function, which in turn prevents inflammation (70).

6. Role of FOXO3 in immunity-induced POI

According to a previous report, 5-30% of patients with POI suffer autoimmune diseases (71). It has been reported that during autoimmune ovarian inflammation, the immune antigens target the ovary; therefore, other autoimmune diseases are associated with POI (72). Furthermore, changes in cellular immunity involving macrophages and dendritic cells, changes in the ratio of cluster of differentiation (CD)4 β /CD8 β , as well as inappropriate expression of major histocompatibility complex (MHC) class II antigens by granular cells have been reported to be associated with development of POI (73). Moreover, weakened autoimmunity is the primary mechanism for the development of POI-associated autoimmune diseases (74). Thyroid disease, autoimmune polyglandular syndrome, systemic lupus erythematosus (SLE) and endometriosis have all been reported to negatively affect the physiology of the ovary (74).

Numerous studies have reported that immunological changes are key in the pathogenesis of POI (75-80). The expression of FOXO3 is important for the maintenance of the differentiation of regulatory T cells that are necessary for immune tolerance, which highlights the role of FOXO3 in controlling immune homeostasis (75). FOXO3 negatively regulates the cytotoxicity of CD8 $^{+}$ T and natural killer cells to tumor cells, thereby serving as a driving force for cancer development (76). Furthermore, the gene expression levels of typical M1 markers, such as CD80 and CD86, in siFOXO3-transfected macrophages are decreased and the activation of FOXO3 results in increased expression of CD86, major histocompatibility complex class I (MHCI) and MHCII, which indicates that the FOXO3 transcription factor regulates the role of IL-10 (77). Moreover, in common autoimmune diseases, FOXO3 participates in complex regulatory processes. FOXO3 imbalance serves as a sign of thyroid cancer (78). The specific mechanism is as follows: The expression of FOXO3 target genes terminate induction of DNA damage inducing protein 45 α and the Bcl-2 interacting cell death mediator, which leads to programmed cell death (78). FOXO3a has been reported as a molecule that is downregulated in SLE. FOXO3a serves a key role in glucocorticoid (GC) treatment of SLE by inhibiting the inflammatory response (79). Further studies have reported that upregulation of FOXO3a expression by GC depends on the inhibition of FOXO3a phosphorylation mediated by PI3K/AKT and the blockade of FOXO3a in the nucleus (80-82). FOXO3a is essential for the GC-mediated inhibition of the NF- κ B activity, which may involve its interaction with NF- κ B p65 protein (83). Overall, these data indicate that FOXO3 serves an important role in body immunity. Targeting FOXO3 may provide novel therapeutic strategies for numerous diseases.

7. Conclusion

The disease development of POI is related to molecular genetics. Several genetic changes have an effect on the pathogenesis of

POI, including oocyte-specific transcription factors (folliculogenesis specific basic helix-loop-helix transcription factor and newborn ovary homeobox-encoding) and other transcription factors that affect follicle formation (Wilms tumor 1, forkhead box L2 and nuclear receptor subfamily 5 group A member 1) (83-85). However, the regulatory role of other genes in the development of POI is still unclear. Therefore, additional attention should be paid in the future to their functional mechanisms, which may provide ideas for the design of novel treatment methods for patients with POI.

FOXO3 is expressed in numerous tissues in the human body; however, its expression levels, functions and targets are specific. The function of FOXO3 includes regulation of key biological processes, such as cell death and survival, substrate metabolism and protein conversion (7,8). FOXO3 has also been reported to be a type of longevity gene, which regulates the aging process and is related to the aging of the ovary in POI (86,87). Numerous studies have reported that expression levels of FOXO3 in patients with polycystic ovary syndrome significantly increase and that FOXO3 may serve a key role in the development of POI (88-90). Furthermore, autoimmune factors are key causes of POI. FOXO3 serves an indispensable regulatory role in the progression of various autoimmune diseases. FOXO3 also serves an important role in the development of follicular cells and iatrogenic damage (35). In summary, these results indicated that FOXO3 is a potential therapeutic target and biomarker for POI and may be a key mediator that affects the occurrence and development of this condition (Fig. 1).

FOXO3 exerts various effects through numerous molecular signaling pathways in the progression of POI from iatrogenic injury. Through regulation of phosphorylation of the PI3K/AKT signaling pathway and reverse regulation of the downstream Wnt2/ β -catenin signaling pathway, these pathways are involved in induction of apoptosis of follicle cells in different medical injury models. These results suggested that FOXO3 is linked with complex apoptotic mechanisms. Therefore, the progression of POI may be inhibited by prevention of FOXO3-induced decrease of the follicular reserve. FOXO3 interferes with primordial follicle dormancy by its effects on certain epigenetic processes, such as phosphorylation and acetylation (14). This is a double-edged sword. In infertile patients who require cryopreservation of follicles for future artificial insemination, strengthening the activation of FOXO3 increases the success rate of fertilization (19,34). In patients with POI with excessive loss of ovarian follicular reserve, enhanced FOXO3 activation further accelerates disease progression (33). A number of environmental pollutants inhibit the cell cycle by the induction of cell cycle arrest (42). FOXO3a participates in regulation of the cell cycle by changing the phosphorylation levels of p53 in cells, activation of oxidative stress and induction of the DNA damage response (43); FOXO3a also affects regulation of follicle dormancy. Furthermore, under stress conditions caused by psychological factors, FOXO3 can slow down the development of depression and play a role in alleviating POI (46) (Fig. 2).

FOXO3 inhibitors may serve an important auxiliary role in the treatment of POI. In certain studies, medium flux fluorescence polarization analysis was used to screen drugs. These studies reported that carbenoxolone (CBX), a derivative of glycyrrhetic acid, is a potential FOXO3 inhibitor (91). Pharmacological correlation with FOXO3 inhibition was

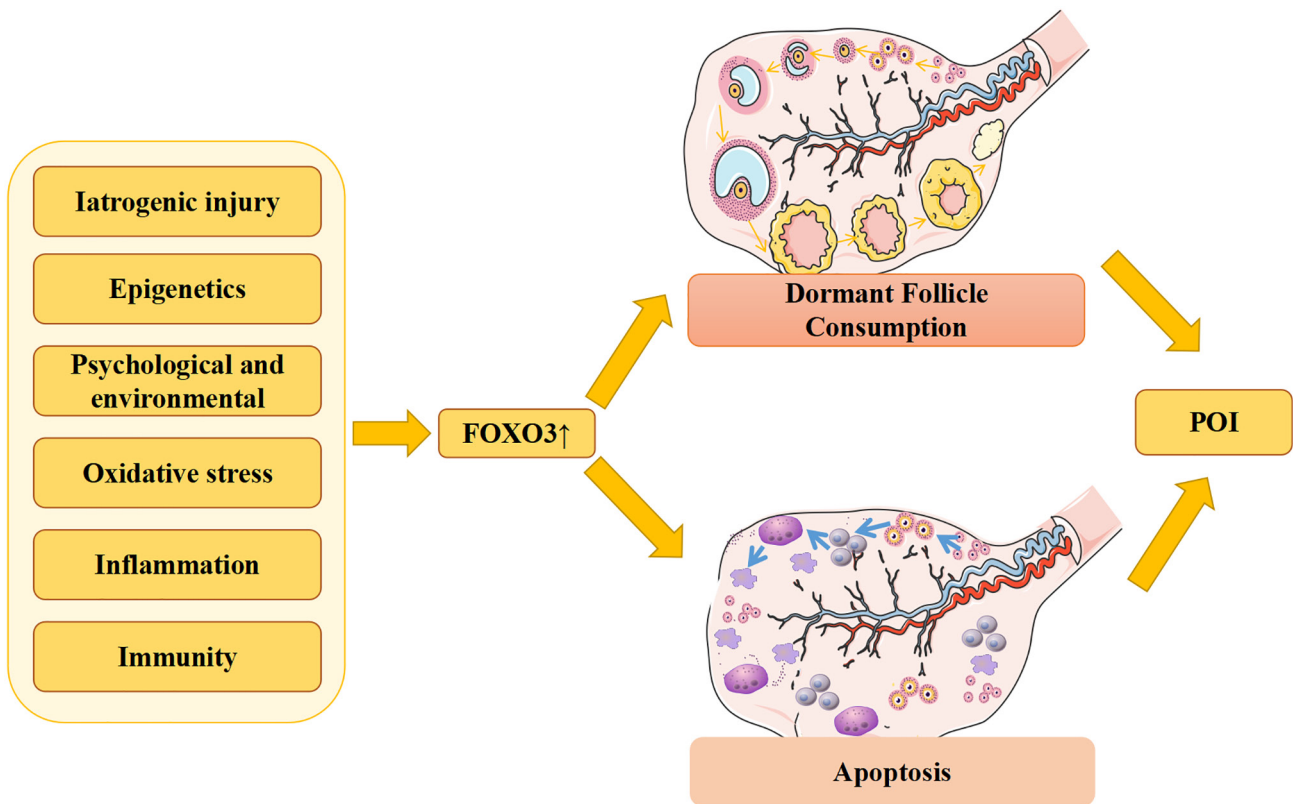


Figure 1. Development of POI is associated with numerous factors. Common causative factors include iatrogenic injury and epigenetic factors. The role of FOXO3 is associated with iatrogenic injury, epigenetics, inflammation, autoimmunity, environmental and psychological factors. The figure was constructed using Procreate 5.2 (Savage Software Group Pty. Ltd.) and Microsoft PowerPoint 2010 (Microsoft Corporation). POI, premature ovarian insufficiency.

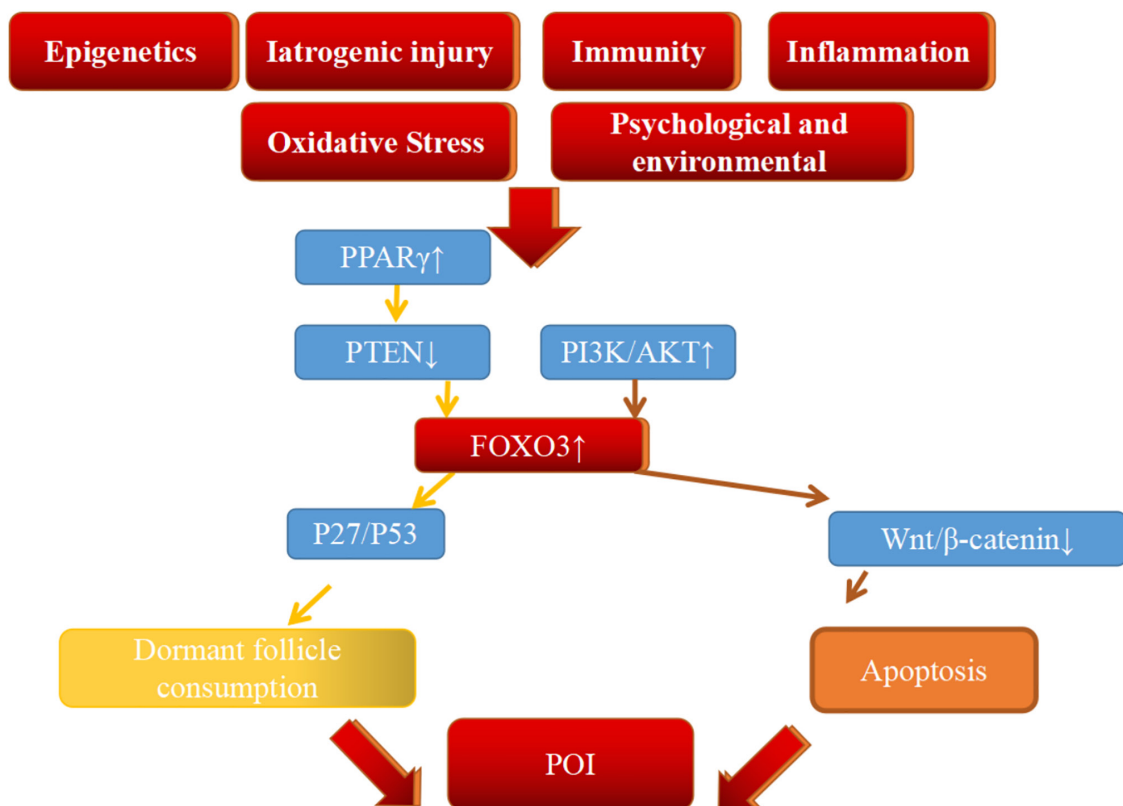


Figure 2. FOXO3 serves a role in the progression of POI through numerous molecular signaling pathways. FOXO3 affects POI by participation in the induction of apoptosis or the depletion of dormant follicles via the regulation of $\text{PPAR}\gamma$, PTEN, PI3K/AKT and other signaling pathways. The figure was constructed using Procreate 5.2 (Savage Software Group Pty. Ltd.) and Microsoft PowerPoint 2010 (Microsoft Corporation). PPAR, peroxisome proliferator activated receptor; POI, premature ovarian insufficiency.

reported in CBX treatment study (92). However, similar drugs are still in the laboratory research stage and are some distance from entering the clinical stage, which suggests more attention to the progress of drug research is required.

Nevertheless, several questions are worthy of further study and attention. Firstly, FOXO3 serves a complex regulatory role in the pathogenesis of POI; therefore, other FOX family member proteins with the same promoter target sequence may have similar effects. Secondly, the role of FOXO3 is primarily mediated by certain signaling pathways; however, the role of mRNA in the regulation of FOXO3 needs to be further evaluated. Thirdly, given the complex role of FOXO3, crosstalk with other transcription factors should be considered. The role of FOXO3 in the development of POI requires further assessment. Finally, certain differences exist with regard to FOXO3 gene polymorphisms. Therefore, the effect of different ethnicities/races should be considered in a clinical data analysis.

Acknowledgements

Not applicable.

Funding

The present study was supported by The Natural Sciences Foundation of Hunan Province (grant no. 2021JJ30593), The Scientific Research Key Funding Project of the Ministry of Education of China Hunan Foundation (grant no. 19A428) and The Postgraduate Research and Innovation Project of China Hunan Foundation (grant no. CX2020961).

Availability of data and materials

Not applicable.

Author's contributions

SL and XW contributed to the design of the study. XM and LP wrote the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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