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RESEARCH ARTICLE

THE EMERGING ROLES OF AUTOPHAGY-RELATED MICRORNAS IN PCOS

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Abstract

Autophagy is a conserved catabolic process that involves the destruction and recycling of damaged bio macromolecules or organelles via lysosomal-dependent pathways and is essential for maintaining cell homeostasis. As a result, aberrant autophagy is linked to a variety of illnesses, including PCOS, neurological diseases, and cancer. Autophagy is involved in endocrine and anovulation disorders. However, the association between hyperandrogenism and autophagy in PCOS patients' human granulosa cells remains unknown. MicroRNAs (miRNAs) are small non-coding RNA molecules that control gene expression at the post-transcriptional stage by silencing specific mRNA. MiRNAs offer significant regulatory potential for various essential biological processes, including autophagy. As a result, studying autophagy-related miRNAs expression in PCOS will provide insights into the disease and lead to the discovery of novel therapeutic targets in PCOS. We summarize current knowledge of miRNA dysregulation during autophagy in PCOS, with a focus on the link between autophagy and miRNAs, and address their role in PCOS treatment and diagnosis.

Introduction:-

PCOS is recognized as a diverse condition in women of reproductive age [1]. In 1935, Stein and Leventhal defined PCOS as a heterogeneous disorder with a slew of signs and symptoms, including (i) androgen excess (AE) (clinically manifested as hirsutism and/or acne), (ii) polycystic ovarian morphology, and (iii) ovulatory dysfunction (oligo-ovulation/anovulation) [2, 3]. PCOS is the most common metabolic and endocrine condition, with a global prevalence of 6-20% in reproductive-aged women [4-6]. According to all known epidemiological statistics, the prevalence of PCOS in India ranges from 3.7% to 22.5% [7]. Women with PCOS are more likely to have disorders connected with metabolic and reproductive abnormalities. As a result, they are more likely to develop type 2 diabetes mellitus (T2DM), obesity, endometrial cancer, infertility, mood and eating disorders, and cardiovascular disease [8].

Autophagy, or self-eating of cellular components, is required to reprocess damaged cell organelles in order to obtain fresh building blocks for cellular homeostasis [9]. Autophagy, which regulates cellular bioenergetics and eliminates harmful protein agglomerates, is required for cell survival during stress conditions (nutrient shortage) [10].

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Autophagy is typically accompanied by cell death, either through modifying autophagic elements for cell death or by causing apoptotic cell death [11]. Kim et al. [12] investigated the role of autophagy in regulating cellular metabolism in mice using an autophagy-related (Atg) gene deletion research. Features associated with metabolic disorders (obesity and T2DM) such as dyslipidemia, oxidative stress, hyperglycemia, and hyperinsulinemia contribute to interrupted and compromised autophagy, which leads to cell death, cardiomyopathy, and cardiac dysfunction [13,14]. Obesity-related difficulties are linked to autophagy, as autophagy regulates cellular bio-power under starvation, demonstrating autophagy's critical function in obesogenesis [15]. Autophagy may have a role in avoiding beta-cell death in the pancreas in T2DM [16]. Autophagy plays a role in abnormal folliculogenesis in the PCOS ovary. Autophagy produces follicular atresia by causing granulosa cell death in the pre-antral follicle [17]. Autophagy is linked to anovulation in PCOS. The underlying cause of anovulation linked with PCOS is abnormal folliculogenesis in the early stages. Autophagy is important in metabolic disorders associated with PCOS [18]. Autophagy regulates metabolic response in a variety of metabolic organs, including adipose tissue [19], the ovary [20], skeletal muscles [21], the liver [22], and the heart [23].

In light of these issues, the goal of this review is to describe past research on the link between miRNAs and autophagy in PCOS. A better knowledge of the underlying linkages underlying this complex and heterogeneous condition would enhance PCOS diagnosis and results.

PCOS Pathogenesis

The cause of PCOS is multifaceted, with individual variances such as aberrant ovarian steroid secretion, hyperinsulinemia, elevated luteinizing hormone, and other factors that lead to complimentary or synergistic effects and influence the disease's progression [24]. As a result, the precise cause of PCOS remains unknown. PCOS is thought to be a multi-gene-mediated disease because to its polymorphism [25, 26]. For example, PCOS has previously been linked to the insulin receptor (INSR) gene with ethnic disparities [27, 28], and a family history of PCOS could be a risk factor [29]. Visceral obesity, pro-inflammatory variables, hyperinsulinemia, and IR have also been linked to the development of PCOS [30].

Patients with polycystic ovarian syndrome who have hyperinsulinemia or IR are not affected by obesity, body fat distribution, or androgen levels, and their risk of impaired glucose tolerance and type 2 diabetes mellitus is higher than in healthy people [26]. The constant release of inflammatory factors from adipose tissue may be linked to the elevated androgen level and development of IR in PCOS patients [31, 32]. A large number of studies have shown that inflammation plays a role in the pathogenesis of PCOS [33], and that increased inflammatory markers such as C reactive protein (CRP), ferritin, tumor necrosis factor (TNF) alpha, interleukin-6 (IL-6) and interleukin-18 (IL-18) are associated with the occurrence of PCOS [34]. Increased amounts of plasminogen activator inhibitor-1 (PAI-1) and free fatty acid influence serine phosphorylation, resulting in IR. PCOS patients have high levels of ferritin and transferrin of hemoglobin, as well as a decrease in anti-inflammatory cytokines and anti-oxidant factors, resulting in chronic inflammation [35, 36]. As a result, obesity may enhance oxidative stress in adipose tissue, activate inflammatory signaling, and ultimately aggravate the chronic inflammatory state and IR in PCOS patients [37].

Activation of Autophagy

Autophagy is involved in follicle development, atresia, and differentiation. Autophagy dysfunction can result in cell death, influencing oocyte development and quality. Several studies have suggested that androgen and AR play essential roles in autophagy activation.

A recent study found that autophagy is activated in PCOS, and that the autophagy-related network involving EGFR, ERBB2, FOXO1, MAPK1, NFKB1, IGF1, TP53, and MAPK9 is to blame [38]. Furthermore, autophagosome buildup may cause GC apoptosis. Excess testosterone can greatly increase the expression of autophagy-related genes ATG5, ATG7, BECLIN1, and the ratio of autophagy marker protein light chain 3B II/I (LC3 II/I) in PCOS patients [39].

However, whether or not autophagy is initiated is debatable. According to a study by Kobayashi et al, the aggregation of p62 and ubiquitin, an aggregated protein and impaired autophagy indicators, are present in the theca cell layer in PCOS, indicating autophagy impairment [40]. They believe that deregulation of late-stage autophagy in theca cells causes androgen synthesis and fibrosis via the reactive oxygen species (ROS)/p38 and JNK signaling pathways [40].

PCOS

From the formation of oocytes to the growth of follicles and their degradation in the ovary, adequate functional autophagy is required [41]. Under normal conditions, follicular cells require autophagy to maintain oocyte formation, follicular expansion and differentiation, follicular atresia, and the reproductive cycle [42-44]. Female infertility is caused by poor oocyte quality, which is caused by faulty autophagy in ovarian follicular cells [45]. As a result, we now understand the involvement of autophagy in PCOS etiology.

EGFR signaling pathway

Beclin1 tyrosine phosphorylation (pY229, pY233, and pY352) can be induced by EGFR tyrosine kinase, creating Beclin1 homodimers and limiting autophagy. Beclin1 tyrosine phosphorylation causes the breakdown of the Beclin1-VPS34 complex, which inhibits autophagy. Rubicon and Bcl-2 then attach to Beclin1 and function as autophagy inhibitors [46]. EGFR promotes the mechanistic target of rapamycin complex 1 (mTORC1) pathway and can operate as an autophagy inhibitor [47]. Active mTORC1 inhibits Beclin1-VPS34 activity, inactivates ULK1, and suppresses autophagy [48]. MTORC2 causes AKT1 to be stabilized and activated [49]. Autophagy is activated by EGFR/RAS/RAF/MEK/ERK signaling, which causes the serine phosphorylation of Beclin1. These data imply that EGFR signaling and autophagy are interconnected.

ERBB2

Drug resistance occurs when the tyrosine kinase receptor ERBB2 is expressed in cancer cells. Autophagy, or "self-eating" inside the cell, is a medication resistance mechanism in cancer cells. Although ERBB2 activation has been demonstrated to promote autophagy in breast cancer cells, the underlying processes remain unknown. We found that ERBB2 enhances autophagy by increasing protein levels of the autophagy gene ATG12 (autophagy-related 12), which contributes to breast cancer cell resistance to chemotherapy or ERBB2-targeted antibody therapies and also discovered that ATG12 expression in ERBB2-positive breast cancers was associated with a poor patient survival outcome. Finally, lapatinib is an EGFR protein family tyrosine kinase inhibitor that stimulates autophagy in cells holding only EGFR but inhibits autophagy in cells containing only ERBB2. This shows that ERBB2 enhances autophagy by upregulating ATG12 [50].

FOX1

FoxO1, a transcription factor, regulates gene expression and participates in gluconeogenesis, low-density lipoprotein synthesis, oxidative stress, and cell death [51]. Furthermore, numerous investigations have shown that FoxO1 is involved in the etiology of PCOS. Changes in hormone levels, TNF alpha, and GLUT4 in PCOS patients may influence FoxO1 signaling on glucose transport, leading to IR [52]. Furthermore, alterations in FoxO1-mediated signaling may promote the emergence of low-grade chronic inflammation in the body, leading to PCOS hyperandrogenism.

NF-κB signaling

Autophagy may be a pathway through which NF-B signaling components are specifically destroyed, resulting in either the cessation of the initial NF-B activation or, conversely, the chronic stimulation of this process. NF-B signaling, in turn, can regulate autophagy via a variety of pathways that vary depending on the cellular environment and stimulus: Members of the NF-B family can either activate or inhibit signaling pathways that induce autophagy, and they also regulate the transcription of a subset of pro-autophagic-regulating genes. Furthermore, NF-B signaling components can enhance autophagy independently of NF-B by stimulating autophagy-activating signaling pathways or triggering the transactivation of a subset of autophagy-related genes [53].

Interrelation between miRNAs and autophagy

According to research, miRNAs are important regulators of the autophagy process, taking part in numerous phases of autophagy, including the upstream pathways that initiate autophagy, the succeeding developmental stages, and the latter stage of destruction. Meanwhile, autophagy is important for miRNA synthesis and maintenance.

miRNAs regulate the major cascades of autophagy

Autophagy is comprised of at least six sequential events: induction, vesicle nucleation (or phagophore formation), vesicle elongation and autophagosome formation, ATG protein and lipid retrieval, autophagosome fusion with a lysosome/endosome and autolysosome formation, and autolysosome cargo degradation. The ULK complex, which consists of ULK1/2 [ATG13, FIP200, and ATG101], is activated by mTORC1 inhibition and commences the autophagy program. The class III PI3K complex, which consists of Beclin-1 [54], class III PI3K (i.e., Vps34) [p150

(i.e., Vps15), ATG14L, and some regulatory factors, is required for vesicle nucleation. Two ubiquitin-like protein conjugation systems form two essential complexes (the LC3-IIPE complex and the ATG5-ATG12-ATG16L1 complex) that are required for vesicle elongation. The transmembrane proteins VMP1 and ATG9 are also involved in the development of nascent autophagosomes [55].

miRNAs are involved in the induction and regulation of autophagy

Growth signals, energy status (the quantity of glucose and amino acids), genotoxic stress, hypoxia stress, ER stress, and ROS all activate signaling pathways that initiate or suppress autophagy cascades [56]. AMPK-mTORC1 is at the center of autophagy regulation, combining various inputs and pathways into a signal for the autophagy beginning site, the ULK complex. Furthermore, ER stress and ROS influence autophagy without relying on the AMPK-mTORC1 pathway [57].

Roles of autophagy-related miRNAs in PCOS

MiRNAs are tiny non-coding RNAs (22 nucleotides in length) that bind complementarily to the 3′-untranslated region (3′UTR) or coding sequence (CDS) of target mRNAs to control gene expression at the post-transcriptional level. More data suggests that miRNAs influence target molecules directly and are linked to the epigenetic machinery. Anomaly in miRNA expression is now connected to a number of human disorders, including PCOS, and has the potential to be exploited as a diagnostic biomarker [58]. Only a few miRNAs have been thoroughly studied thus far, leaving many more possible possibilities to be examined [Table 1].

Table 1:- Autophagy-associated miRNAs in PCOS.

Effect on autophagy	Name	Dysregulation	Autophagy-related target	Ref.
	hsa-miR-92b-3p, hsa-miR-20b-5p	-	PCOS dysfunction	59
Inhibition	let-7e	Upregulation	P21 pathway	60
Inhibition	miR-126-5p miR-29a-5p	Down regulation	Suppression of insulin growth factor 1 (IGF-1R), fibroblast growth factor (FGF) and Wnt family member 1 (Wnt1)	61, 62

Conclusion:-

We summarized and addressed how miRNAs regulate autophagy in this review, as well as their functions in PCOS. Targeting autophagy may thus establish a link between ovarian physiology, hyperandrogenism, insulin resistance, obesity, and PCOS-related endocrine abnormalities. However, there is a dearth of precise knowledge in this area, and the underlying mechanism has to be investigated further.

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