cGAS-STING pathway participates in endometriosis by up-regulating autophagy:Review.

sennan zhu[1], qiuyu chen[1], Xinyi Du[1], Fengming Xu[1], Zongwen Liang[1], yu zhao[1], and qiong zhang[1]

[1]Affiliation not available

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Abstract

Double-stranded DNA (dsDNA) sensor cyclic-GMP-AMP synthetase (cGAS) and its downstream interferon gene stimulator (STING) act as important immune monitoring media have become a research hotspot. cGAS-STING pathway may play a key role in the occurrence and development of endometriosis by regulating autophagy. The combination of inhibitors targeting cGAS-STING with estrogen or surgery may become a therapeutic approach for endometriosis, and we present a review about the mechanism and physiological function of cGAS-STING pathway, typical autophagy and STING-dependent atypical autophagy, autophagy expression in endometriosis, and a new idea that cGAS-STING may be involved in endometriosis by regulating autophagy.

Introduction

Endometriosis, a common gynecologic disease affecting approximately is a common gynecologic disease affecting 8%-10% of fertile women, is characterized by the presence, transfer, invasion, and cultivation of growing endometrial tissue outside of the uterine cavity. (1, 2)However, the etiology and pathogenesis are not clear: the implantation theory of Sampson, the coelomic metaplasia theory of Mayer, and the theory of induction are the three classic theories that have tried to identify the certain pathophysiology of endometriosis, but they have failed to establish it.(3, 4) One of the focuses in the study of endometriosis is the mechanisms of establishment and survival of endometrial cells outside the uterus. Recent studies revealed that autophagy might play a role in the survival of endometrial cells and the maintenance of endometrial lesions.(5)

Autophagy is a cell cycle system, which exists in almost all types of eukaryotes. The system consists of various proteins whose function is to transport intracellular cargo to lysosomes to form autophagosomes with degraded contents.(6) As a basic biological activity, autophagy is closely related to cell proliferation and apoptosis, which are ubiquitous in the process of dynamic endometrial refreshing. And the autophagy level may influence the functions and state of endometrial cells in some way.(7)

It has also been suggested that oxidative stress, an imbalance between reactive oxygen species (ROS) and antioxidant, may be a potential factor involved in the pathophysiology of endometriosis.(8-10)

As we all know, excess production of ROS may lead to mitochondrial dysfunction.(11) What's more, mitochondrial dysfunction results in the leakage of mitochondrial DNA (mtDNA) into the cytoplasm .(12) mtDNA in the cytoplasm can activate cGAS, which can induce the polymerization of GTP and ATP into cyclic GMP-AMP (cGAMP). And STING located on the endoplasmic reticulum (ER) surface can be activated by cGAMP which acts as a second messenger.(13, 14)

Studies have shown that activated STING shifts to the ERGIC to trigger autophagosome, which plays a key role in STING dependent autophagy.(15) the level of autophagy that defined by Giulia and her colleagues

was increased in ovarian endometriosis which result in the promotion of invasion and metastasis abilities in human endometrial stromal cells.(16, 17)The above evidence reveals that cGAS-STING pathway, which was activated by oxidative stress, can up-regulate the migration and invasion ability of ectopic endometrial cells by inducing autophagy. In this review,we highlight the mechanism of oxidative stress in endometriosis, cGAS-STING pathway, the role of STING dependant autophagy in endometriosis and latent clinical utility of cGAS-STING in endometriosis.

Oxidative stress in endometriosis

the mechanism of oxidative stress in endometriosis

Oxidative stress (OS) is known as a key role in endometriosis pathophysiology (Fig 1).(9) Previous studies have observed that the concentration of OS biomarkers increased, and serum total antioxidant capacity (TAC) and FF decreased in patients with endometriosis.(18) Because of the cyclic bleeding in endometriosis tissue, the release of hemoglobin during hemolysis can lead to high levels of heme accumulation.(2) Moreover, Giulia Allavena et al. have revealed the significant increase of oxidative stress-related (heme oxygenase-1) proteins.(5) It's not hard to understand that accumulated heme is degraded into biliverdin, carbon monoxide and iron by heme oxygenase-1.(19, 20) Excessive iron is involved in the Fenton reaction, which can enhance the toxicity of nitrogen and oxygen by producing various reactive oxygen species(ROS) and triggering cell oxidative damage.(21) On the other hand, the activity of superoxide dismutase(SOD), an OS enzyme which converts superoxide into hydrogen and oxygen peroxide, is decreased in the plasma of patients with endometriosis, suggesting a declined antioxidant capacity in these patients.(22) Overall, the expression of antioxidant enzymes and pro-oxide enzymes in endometrium changes in endometriosis. These studies indicated that OS may be a vital role in metabolism in endometriosis progression

2、oxidative stress and mtDNA damage

It has been suggested that mitochondria are an immediate target of ROS and mitochondrial DNA (mtDNA) suffers more mutations than nuclear DNA in the presence of high ROS.(23, 24) ROS can indirectly cause mtDNA damage by reducing the proofreading ability of Pol γ.(11, 12, 23).And mtDNA damaged can lead to the leakage of mtDNA in the cytoplasm by aggravating mitochondrial dysfunction.(25) In summary, mtDNA may leak into cytoplasm in ectopic endometrial cells because of oxidative stress. Recent studies have focused on the cGAS -STING,a pathway widely associated with chronic and immune diseases,which can be activated by abnormal cytoplasmic DNA. cGAS-STING pathway may also be a potential mechanism for the development of endometriosis.

cGAS-STING pathway

cGAS-STING pathway is a cytoplasmic double-stranded DNA (dsDNA) sensor, enabling the body to respond to infection, inflammation and cancer through innate immunity.(26) An important ligand of cGAS-STING is mtDNA which can be released into the cytosol under mitochondrial dysfunction.(27, 28) Cyclic GMP–AMP synthase (cGAS; also known as MB21D1), as a cytosolic dsDNA sensor,undergoes a conformational change to an active state after interacting with the dsDNA in a sequence-independent way.(29, 30) Then activated cGAS catalyzes the polymerization of ATP and GTP to produce cGAMP. cGAMP binds to and activates STING, which will transport to Golgi from ER and forms tetramer through higher-order oligomerization.(31, 32) Transporting STING can bind directly to TANK binding kinase 1(TBK1) or IκB kinase(IKK)complexes and be phosphorylated by them.(33, 34)And they respectively activate the transcription factors IRF3 and NF-κB. These transcription factors induce the expression of type I IFN and cytokines in autocrine and paracrine ways.(35, 36)

The cGAS-STING pathway can provide protection or resistance to infection; however, improper or excessive activation may lead to severe inflammatory pathologies, including autoimmunity.(37) Recently, most of the researches on cGAS-STING have focused on tumor immunity. For example, DNA sensing through STING promoted the tolerant immunomodulatory expression of indoleamine 2,3-dioxygenase (IDO), which intensified tumor growth in Lewis lung cancer mouse tumor metastasis model.(38) It is revealed that the

cGAS-STING pathway, as an important part of the host's innate immunity, may play an important role in anti-cancer therapy. What's more,it is the downstream signal of cGAS, especially IFN, which connects innate immunity and adaptive immunity.(39, 40) Simultaneously, cGAS-STING is indispensable in autoimmune diseases. Interferon type I (IFN-I) activated by cGAS-STING axis plays a key role in the pathogenesis of systemic lupus erythematosus (SLE). Therefore, blocking cGAS axis is a promising target for the treatment of SLE.(41, 42)

Recent evidence suggests that immunology is involved in the pathogenesis of endometriosis.(43) Moreover, endometriosis is a recognized chronic inflammatory hormone-dependent disease.(44) However, the mechanism of endometriosis is still unclear. cGAS-STING pathway plays an important role in inflammation and immunity.(45)As for the mechanism of cGAS-STING involved in endometriosis, autophagy is one possibility.

Autophagy

Autophagy is a highly conserved process of self-degradation and energy dynamic cycle in the process of eukaryotic cell proliferation, differentiation and maturation. It provides energy and basic materials for the steady state and survival of cells by degrading the protein misfolded or aggregated in cytoplasm, damaged organelles or macromolecules.(46, 47)There are three types of autophagy: macroautophagy, chaperone-mediated autophagy(CMA) and microautophagy, all of which end up in the transport of cytoplasmic material to lysosomes for degradation and recycling.(48, 49) Macroautophagy ,an intrinsic and lysosome dependent clearance procedure, involves the formation of double membrane vesicles in cytosols, selective or nonselective isolation of cytosolic components in autophagosomes (autophagic vesicles), and transport of cargo to lysosomes (autolysosomes) for degradation.(50) Macroautophagy allows the removal and recycling of proteins, redundant or damaged organelles (such as mitochondria, endoplasmic reticulum, peroxisome, etc.) and abnormal protein aggregates.(51) CMA is a self- degradation process, which drives selected soluble proteins into lysosomes for cell quality control .(52) CMA is responsible for the direct transfer of lysosomes and the elimination of protein proton sets containing unique pentapeptide sequence, the KFERQ motif and nearly one-third of soluble proteins are considered as potential CMA substrates .(53) Microautophagy was once thought to be a nonselective autophagy pathway, in which the surrounding cargo is directly and randomly engulfed by the invagination of lysosomal membrane. Besides, it can selectively recognize specific proteins, known as EMI. Moreover, starvation and oxidative stress can also induce microautophagy.(52, 54)

Macroautophagy is recognized as the most widely studied and characteristic autophagy pathway. In macroautophagy induction, the key step is to activate the unc51-like kinase (ULK) complex, which recruits and modifies downstream Atg proteins to start macroautophagy.(55) There are two major receptors that sense intracellular or extracellular stress and activate the ULK complex and downstream signals. One is the mammalian target of rapamycin (mTOR),the inhibition of which will rapidly lead to ATG13 dephosphorylation and translocation of ULK complex to the nucleation site of the membrane in the absence of nutrition, thus promoting the initiation of autophagy.(56-59)And the other one receptor is 5'-AMP-activated protein kinase (AMPK). Upon glucose deprivation, AMPK could be activated and inhibit the activity of mTORC1.In addition, AMPK can also phosphorylate PI3KC3 and Beclin 1 to activate PI3KC3 complex I.(60, 61)

The core mechanism of classical and best-characterized autophagy includes the following steps: initiation; prolongation and nucleation of the isolation membrane; cargo replenishment and maturation of autophagy bodies; transport of autophagy bodies; docking and fusion of autophagic lysosomes with late endosomes or lysosomes during autophagic lysosome recombination cycle; and lysosome regeneration.(62) Different stimulants activate AMPK and prevent PI3K from phosphorylating its downstream target Akt, thus inhibiting mTOR and reducing the phosphorylation of ATG13,which interacts with ULK1, FIP200, and ATG101 to form ULK1 complex, which promotes autophagy induction. The activity of Beclin1-ATG14-VPS34-VPS15-PI3K core complex was enhanced by activating ulk1 complex to phosphorylate Beclin1. PI3K complex phosphorylates PI to form PI3P, which is necessary for recruiting PI3P binding protein atg18 and its partner ATG2. These proteins are involved in the ATG9 cycle. ARG4b cleaved 22 C-terminal residues of LC3 precursor (prolc3) to produce LC3-I. After ATG3 (E2 like enzyme) interacts with ATG16L1 complex (E3 ligase) and ATG7, LC3 binds with PE to form LC3-PE (LC3-II). LC3-II is specifically located in the in-

ner and outer membranes of autophagosomes and remains on mature autophagosomes until they fuse with lysosomes. Autophagosomes fuse directly with lysosomes, or fuse with endosomes first, and transport to lysosomes afterwards to form autophagic lysosomes.(2, 62-64)

In recent years, the research of autophagy in various diseases has been deepened gradually, such as psychiatric diseases, cutaneous melanoma, and so on.(65, 66) Simultaneously, autophagy plays an important role in the pathogenesis and progression of endometriosis.(5) It has been revealed that the expression of LC3-II in endometriosis of the fallopian tube and ovary was significantly higher than that of secretory endometrium.(67) In addition,compared with eutopic endometrium of sick or healthy women, the autophagy defined by Giulia and her colleagues was up-regulated (increased levels of lipid LC3-II protein and LC3-II / lc3-I ratio).(16) It has been proved that inhibition of autophagy can effectively inhibit the proliferation and colony formation of endometrial cells, and inhibit the migration and invasion of endometrial cells.(68) These evidences show that the up-regulation of autophagy is indeed one of the etiology of endometriosis and indicate that cGAS-STING may be involved in the occurrence and development of endometriosis by regulating autophagy. However, the specific mechanism is still unclear.

cGAS-STING regulates autophagy in endometriosis

The role of autophagy induced by cGAS-STING in some diseases has been studied. For example, cGAS-STING may be a therapeutic target for hepatocellular carcinoma by inducing autophagy.(69) Similarly, the role of STING-mediated autophagy in neuritis suggests a new therapeutic target.(70) Furthermore, STING plays an antiviral role during viral infections by inducing autophagic events.(71)Therefore, autophagy regulated by cGAS-STING may provide a new possibility for the study of endometriosis.

In recent years, studies on the specific mechanism between cGAS-STING and autophagy are increasing and deepening. Gui et al. have shown that induced autophagy is the original function of cGAS-STING pathway.(15) It is well known that LC3 is a key marker of autophagy, and it is transferred to the growing autophagosome by LC3 lipidation.(72) It has been demonstrated that STING containing ERGIC is the membrane source of LC3 lipidation, which is a key step in autophagy biogenesis.(15) This evidence suggests that the activation of STING is essential for autophagy induction.

There are two pathways involved in STING-dependent autophagy. One of them is that STING serves as a potential autophagy receptor, which directly interacts with LC3 through its LIR motif to mediate autophagy and autophagy degradation, thus regulating the innate immune response. Another pathway is that STING induces ATG5-dependent atypical autophagy. (70) The mtDNA in the cytoplasm mentioned above stimulates cGAS to produce cGAMP, which can activate and combine with STING. Then the cGAMP-STING complex recruits SAR1 and SEC24C to form COP-II complex, which will migrate from ER to ERGIC. ERGIC is the membrane source of LC3 lipidation, which depends on WIPI2.And LC3 positive membrane encapsulated dsDNA, bacteria or virus to form autophagosomes. Autophagosomes transfer to lysosomes and are digested by various enzymes.(71)

Interestingly, it has been reported that autophagy in the ectopic endometrium of patients with ovarian endometriosis is up-regulated.(5) The expression of LC3II and Beclin-1 in ectopic endometrium increased significantly.(73, 74) Liu et al. have shown that autophagy promotes the invasion and migration of human ectopic endometrial cells. (17)

The process that cGAS-STING is involved in the development of endometriosis by up-regulating the level of autophagy in endometriosis includes several points (Fig 2):(a) Oxidative stress in ectopic endometrial cells results in mtDNA damage and leakage into the cytoplasm;(b) Abnormal mtDNA in cytoplasm activates cGAS-STING pathway;(c)cGAMP-STING complex induces atypical autophagy pathway which depends on ATG5 and WIPI2; (d)Up-regulation of autophagy promotes migration and invasion of ectopic endometrial cells.

Targeting The cGAS-STING Pathway For Treatment

Considering that cGAS-STING pathway plays a key role in the development of endometriosis by up-

regulating autophagy, negative regulation of this signaling pathway is a promising pathway. Inhibition of cGAS, cGAMP, and STING can block the activation of cGAS-STING pathway.

It is revealed that some antimalarial drugs, such as suramin, inhibit cGAS by blocking the interaction between DNA and cGAS.(75)And suppressive oligonucleotides (ODNs) containing repetitive TTAGGG motifs in mammalian telomeres effectively inhibit the activation of cGAS by intracellular DNA, and have been proved to be useful in the treatment of autoimmune diseases including SLE.(76)Recently,a study has also shown that cGAS antagonist A151 can improve brain injury after ischemic stroke by inhibiting cGAS-STING pathway.(77) Interestingly, aspirin can block the activity of cGAS by acetylating the three lysine residues of cGAS.(78)As for STING, the cyclopeptide astin C can be used as a small molecule inhibitor of STING.(79)And H-151 can block palmitoylation of STING to inhibit it.(80) For cGAMP, novel small molecules such as RU320521 or G150 can inhibit cGAMP synthesis by occupying the enzymatic pocket of species-specific cGAS competitively.(81, 82) All of these agents are potential candidates for the treatment of endometriosis by targeting cGAS-STING pathway.

Concluded Remarks And Future Perspects

Although there are many researches on cGAS-STING pathway in cancer and immune diseases, the researches and applications of this pathway in gynecological diseases are still rare.

It is well known that cGAS-STING pathway plays an important role in inflammatory and immune diseases, and its role in autophagy has also attracted attention in recent years. Some studies have even suggested that regulating autophagy is the original function of cGAS-STING pathway. Under the background that autophagy has been proved to be up-regulated in endometriosis and promotes the migration and invasion of ectopic endometrial cells, cGAS-STING pathway may be involved in the pathological mechanism of up regulation of autophagy. Oxidative stress in endometriosis causes mitochondrial dysfunction in ectopic endometrial cells, which leads to mitochondrial DNA leakage into the cytoplasm. mtDNA abnormally activates cGAS-STING pathway, which binds cGAMP and up-regulates autophagy, thus promoting the migration and invasion of ectopic endometrial cells. If the role of cGAS-STING pathway in the development of endometriosis can be further studied, new ideas for the treatment of endometriosis will be provided. The specific inhibitors targeting cGAS-STING pathway may be the candidate drugs for the treatment of endometriosis.

Furthermore,endometriosis is also recognized as a estrogen-dependent chronic inflammatory disease. It may be a new idea to combine cGAS-STING pathway targeted drugs with hormone therapy or anti-inflammatory therapy. It is a challenge to master the indications accurately in that way. On the other hand, for patients with endometriosis who need surgical treatment,the expression of cGAS or STING may be a prognostic criterion.

In addition, in order to target the cGAS-STING pathway for therapeutic purposes,, drugs should be optimized to enhance the expected effects and prevent their harmful effects. For example, designing drugs that precisely target to inhibit cGAS-STING pathway and down-regulate autophagy, which result in the inhibiting the migration and invasion of endometrial cells, might be a promising and novel method for endometriosis treatment. However, over concentration of drugs on the down-regulation of autophagy may ignore IFN and NF-kB, which are the main downstream signals of this pathway. Therefore, some drugs that can stimulate and maintain the effects of IFN and NF-kB may play complementary roles.

Up to now, the researches on cGAS-STING pathway and the pathogenesis of endometriosis are mainly carried out in the laboratory, but there is still a lot of room for exploration in the clinical field. It still has a long way to go to furher investigate the relationship between the cGAS-STING pathway and the pathogenesis of endometriosis, and apply them to clinical practice.

Disclosure

The authors have no conflicts of interest to declare. All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in

agreement with the manuscript.

Contribution to Authorship

Zhang Q and Zhao Y identified this theme. Xinyi Du, Fengming Xu and Zongwen Liang earched for literature. Zhu SN and Chen QY wrote the manuscript draft. Zhang Q and Zhao Y revised the manuscript. All authors read and approved the final manuscript

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Legends for figures

Figure 1: oxidative stress in endometriosis

HO-1: heme oxygenase-1

SOD: superoxide dismutase

ROS: reactive oxygen species

mtDNA: mitochondrial DNA

1. Cycle bleeding in endometriosis results in heme accumulation;
2. Heme is decomposed under the action of HO-1 to produce Fe which produces excess ROS through Fenton reaction
3. The reduction of SOD leads to a decrease in antioxidant capacity
4. Excessive ROS and decreased antioxidant capacity together lead to severe oxidative stress, thereby causing mtDNA damage

Figure 2: cGAS-STING plays an important role in endometriosis by up-regulateing autophagy

mtDNA: mitochondrial DNA

cGAS: cyclic-GMP-AMP synthetase

GTP: guanosine triohosphte

ATP: adenosine triphosphate

cGAMP: cyclic-GMP-AMP

COP: coat protein

ER: Endoplasmic reticulum

ERGIC: Endoplasmic reticulum-Golgi intermediate

(a)mtDNA damage causes it to leak into the cytoplasm

(b)mtDNA in the cytoplasm activates the cGAS-sting pathway

(c)Atypical STING-dependent autophagy is activated

(d)Autophagy promotes the migration and invasion of ectopic endometrial cells