

Review Article

Update on the role of endoplasmic reticulum stress in asthma

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Abstract: Asthma has long attracted extensive attention because of its recurring symptoms of reversible airflow obstruction, airway hyperresponsiveness (AHR) and airway inflammation. Although accumulating evidence has enabled gradual increases in understanding of the pathogenesis of asthma, many questions regarding the mechanisms underlying asthma onset and progression remain unanswered. Recent advances delineating the potential functions of endoplasmic reticulum (ER) stress in meeting the need for an airway hypersensitivity response have revealed critical roles of unfolded protein response (UPR) pathways in asthma. In this review, we highlight the roles of ER stress and UPR activation in the etiology, pathogenesis and treatment of asthma and discuss whether the related mechanisms could be targets for therapeutic strategies.

Keywords: Asthma, endoplasmic reticulum stress, unfolded protein response, therapeutic strategies

Background

Asthma is a chronic airway inflammatory disease that can be subdivided into several phenotypes on the basis of clinical, physiological and inflammatory markers [1]. The main manifestations of asthma are shortness of breath, wheezing, and chest tightness, which are caused by variable airflow restriction. The symptoms of asthma attacks are reversible: they can resolve within a short time after treatment, or even spontaneously, and only a few of them are persistent [2]. Asthma attacks are often induced by certain factors, such as house dust mites (HDMs), tobacco smoke, chemical irritants, air pollution and viral infections [3]. Many patients have attacks with obvious biological rules that occur or worsen at 2~6 am every morning and generally occur in spring or winter [4]. Asthma is a major global disease that with considerable public health consequences, including high morbidity and very high rates of mortality in severe cases. The disease is reported to kill nearly 250,000 people worldwide each year [5].

The endoplasmic reticulum (ER), which is distributed throughout the cytosol, is a specialized organelle in eukaryotic cells. The ER forms an extensive network that has many connections with other organelles in the cell [6]. Ample connections between the ER and endocytic organelles are observed in many cell types, highlighting the prominent physiological roles of the ER. This organelle is mainly responsible for folding and modifying proteins synthesized in ribosomes and for transporting these proteins to Golgi bodies via vesicles. In addition, the ER also exhibits other functions; for example, it regulates lipid biosynthesis, calcium homeostasis and cellular stress [7]. ER stress involves accumulation of unfolded or misfolded proteins due to disruption of homeostasis in the ER. ER stress is caused by many factors, such as excessive protein processing loads, insufficient nutrition supply, viral infection, calcium imbalance, and reduction/oxidation (REDOX) imbalance. ER stress can trigger calcium ion imbalance, ER overloading, apoptotic pathway activation and other adverse reactions. When ER suffers from such imbalance, various pathways

are activated to restore the normal functioning of the organelle; however, these pathways also elicit side effects. The activity of these collective pathways is known as the unfolded protein response (UPR) [8]. ER stress is involved in the pathogenesis of various diseases; therefore, in recent years, it has been studied extensively in the context of diseases such as obesity, diabetes, neurodegenerative diseases, asthma and pulmonary fibrosis [9-12]. Although the contributions of ER stress to many diseases have been studied, the role of ER stress in asthma remains unclear. Therefore, in this review, we discuss the influence of ER stress on asthma from the perspectives of disease triggers, pathophysiological characteristics and treatment. Our aim is to deepen and expand the current understanding of the relationship between asthma and ER stress so that better therapeutic strategies can be developed in clinical settings to combat this widespread disease.

Asthma initiation and progression

The pathogenesis of asthma is complex and multifaceted on the cell, tissue and organ levels, involving an intricate regulatory network under the combined action of genetic and environmental factors (**Figure 1**). Asthma is a disease with genetic predisposition and strong family trends, as has been confirmed by numerous genome-wide association studies (GWASs) [13]. The major genetic determinants include IL-1RL1, ST2, IL-33R, IL-33, SMAD3, IL-2RB, GSDML and ORMDL3 [14, 15]. Among them, ORMDL3, which is specifically associated with the risk for childhood-onset asthma, is correlated with the degree of ER stress [16]. Asthma attacks are also caused by a variety of environmental factors, including smoking, allergies and fungal infections. Asthma is defined as a chronic airway inflammatory disease, and on the cellular level, it can be roughly divided into Th2 inflammation-related and non-Th2 inflammation-related asthma. The inflammation associated with Th2-type asthma is mainly eosinophilic inflammation, which can be further classified as allergic or nonallergic inflammation. Th2-mediated allergic eosinophilic inflammation is the classic inflammation type in asthma [17]. When exogenous allergens enter the body and are phagocytized by antigen-presenting cells, Th2 cells are activated to produ-

ce related Th2 cytokines (such as IL-4, IL-5 and IL-13). These cytokines can activate B cells to synthesize and secrete IgE, which can bind to mast cells, and induce the release of various active mediators that cause asthma-related symptoms. Th2 cells can also directly activate eosinophils, mast cells, and alveolar macrophages to secrete such mediators [18, 19]. In addition, Th2-mediated eosinophilic inflammation can result from the activation of type 2 innate lymphoid cells (ILC2 cells) by IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) in a T cell-independent manner [20]. Non-Th2 inflammation is mediated predominantly by Th1 and Th17 pathways. Th1/Th17 cells can secrete IL-17, ILC3 and other cytokines to activate alveolar macrophages and neutrophils, thus causing neutrophil inflammation. Suppression of Th2-type inflammation can upregulate Th17 immunity and increase the levels of Th1/Th17 cytokines; therefore, some neutrophilic asthma may be iatrogenic, occurring as a consequence of Th2-suppressing asthma therapies such as corticosteroids [21]. In addition to inflammation, there are two other important features of asthma: airway hyperresponsiveness (AHR) and airway remodeling. In AHR, the airway becomes highly sensitive to various stimuli, exhibiting an overly strong or premature contraction response when exposed to these stimuli. AHR is mainly caused by chronic airway inflammation and is significantly affected by genetic factors. Airway remodeling is an important pathological feature of asthma that mainly manifests as mucous metaplasia of airway epithelial cells, hyperplasia and hypertrophy of smooth muscle, and distant subepithelial deposition of collagen [22, 23].

ER stress and the UPR

Adaptive UPR

As mentioned above, pathophysiological states that increase the demand for protein folding or that disrupt normal folding processes result in accumulation of misfolded proteins in the ER, which leads to ER stress and activation of the UPR [7]. Three transmembrane proteins that mediate three major UPR pathways exist in the ER lumen: activating transcription factor 6 α (ATF6 α), protein kinase RNA-like ER kinase (PERK) and inositol-requiring protein 1 α (IRE1 α). ATF6 α contains a leucine zipper

ER stress in asthma

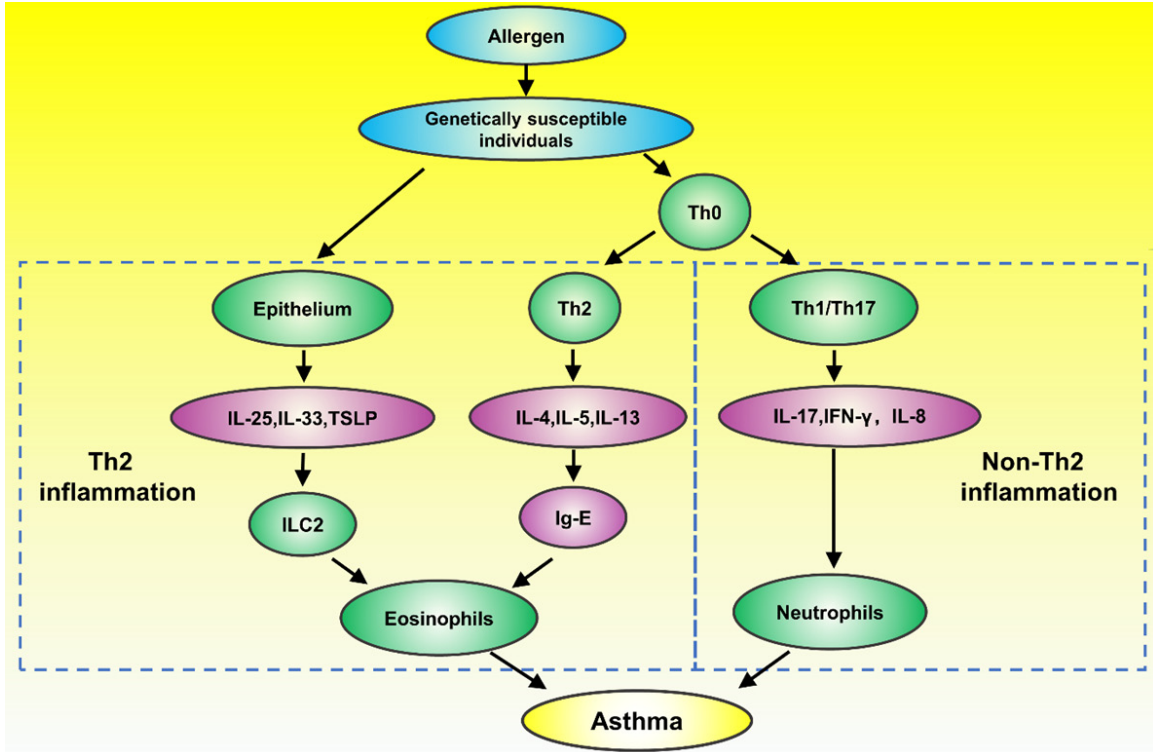


Figure 1. Overview of the pathogenesis of asthma. The combined influence of genetic and environmental factors mediates Th2 and non-Th2 types of inflammation, leading to the production of various cytokines by eosinophils, neutrophils, mast cells, etc. and ultimately causing AHR, mucus hypersecretion and airway remodeling. ILC2: type 2 innate lymphoid cells; TSLP: thymic stromal lymphopoietin; IFN- γ : interferon- γ .

domain that is activated by proteases, and PERK and IRE1 α contain similar cytoplasmic Ser/Thr kinase domains that are activated by autophosphorylation [24]. These three transmembrane proteins act as sensors and inhibit ER stress through physical interaction with immunoglobulin-binding protein (BiP)/GRP78, a chaperone of the heat shock protein family. Once ER stress is induced, BiP combines with misfolded proteins and disassociates from the three transmembrane proteins, and UPR pathways are triggered to restore protein folding homeostasis [25-27]. The first pathway is mediated by ATF6 α . Under stress conditions, ATF6 α is activated and transported into the Golgi, where its N-terminal cytosolic domain is cleaved by the proteases S1P and S2P. Then, cleaved ATF6 α enters the nucleus to induce the transcription of several UPR-related genes to promote protein folding. The second pathway is mediated by PERK; when PERK is activated, it phosphorylates eukaryotic translation initiation factor 2 α (eIF2 α), which can then upregulate the translation of ATF4 mRNA to reduce

initiation codon (AUG) recognition and translation, thereby reducing protein synthesis and the burden of ER damage. The third pathway is mediated by IRE1 α ; activated IRE1 α elicits the activity of an endoribonuclease that selectively cleaves a 26-nucleotide segment from X-box-binding protein 1 (XBP-1) mRNA to create transcriptionally active XBP-1s, which then enters the nucleus to activate UPR-associated genes and ER-associated degradation (ERAD) [25, 28-31].

Nonadaptive UPR

The initial purpose of UPR pathway activation is to prevent excessive accumulation of misfolded proteins, maintain ER homeostasis, and promote ER functional recovery (through the adaptive UPR). However, sustained or prolonged activation of the UPR pathway can lead to toxicity and side effects, causing cell inflammatory states and even apoptosis (through the nonadaptive UPR) [32]. Nuclear factor- κ B (NF- κ B) is a transcription factor that induces the expression of inflammatory response-related

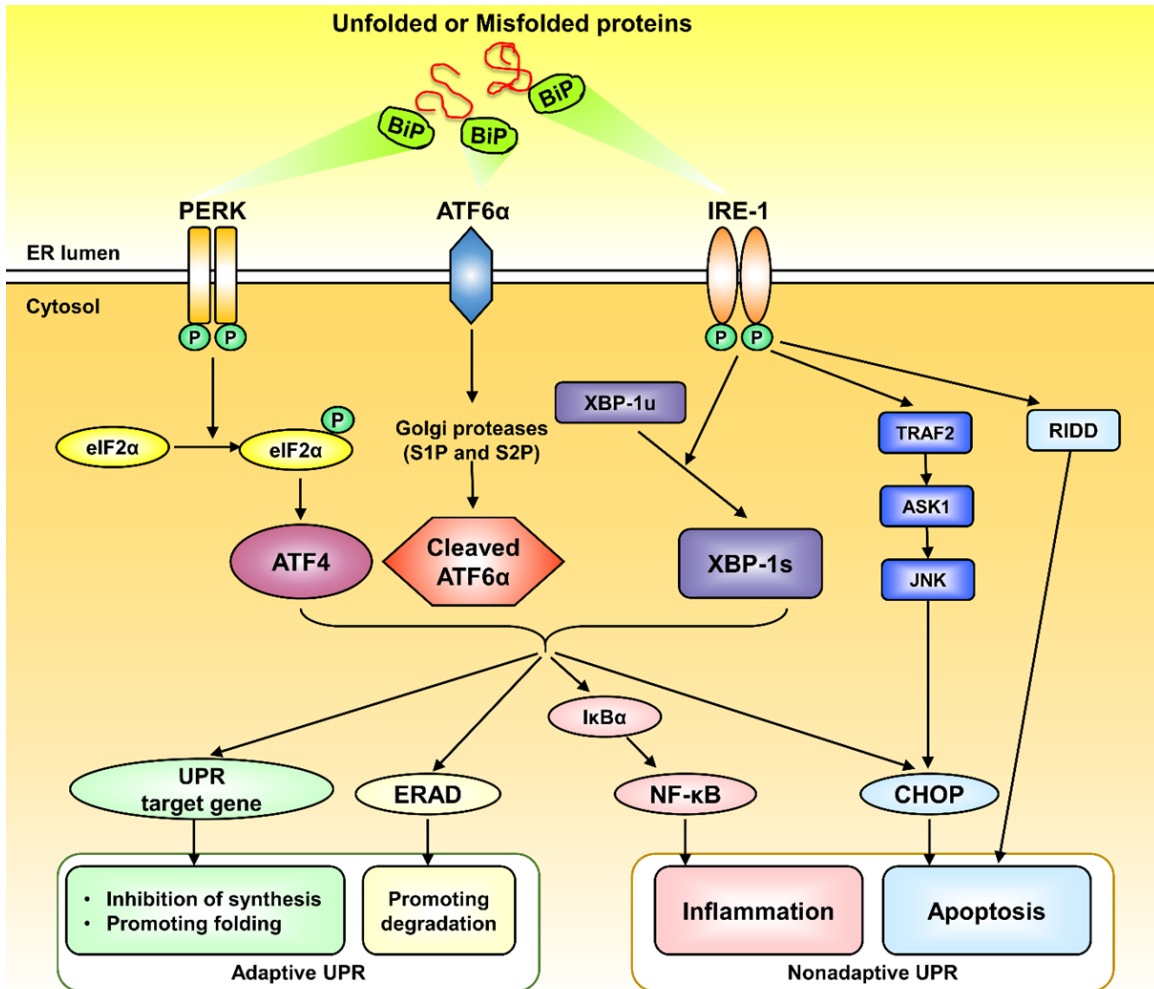


Figure 2. Classic UPR signaling pathways. Under ER stress, Bip dissociates from three ER stress sensors (ATF6, PERK, and IRE α) and binds to unfolded or misfolded proteins. The UPR pathway includes adaptive UPR and non-adaptive UPR. Adaptive UPR can reduce ER stress and restore homeostasis. Persistent ER stress can lead to non-adaptive UPR, causing stress-related damage such as inflammation and apoptosis. BiP: binding protein; ATF6 α : activating transcription factor 6 α ; PERK: protein kinase RNA-like ER kinase; IRE1 α : inositol-requiring protein 1 α ; eIF2 α : eukaryotic translation initiation factor 2 α ; ATF4: activating transcription factor 4; XBP-1: X-box-binding protein 1; ERAD: ER-associated degradation; NF- κ B: nuclear factor- κ B; CHOP: C/EBP homologous protein; TRAF2: tumor necrosis factor-related factor 2; ASK1: apoptotic signal regulation kinase 1; RIDD: regulated IRE1 dependent decay.

genes that encode cytokines, chemokines, and adhesion receptors [33]. According to previous studies, NF- κ B is closely associated with ER stress, and UPR pathway activation can activate NF- κ B in a variety of ways, thus causing relevant inflammatory responses [34, 35]. C/EBP homologous protein (CHOP), a major transcription factor regulating cell death under ER stress, can be activated by all three UPR pathways [36]. CHOP can upregulate the proapoptotic gene Bax/Bak and downregulate the antiapoptotic gene Bcl-2, resulting in mitochondrial-related dysfunction, caspase-12 and cas-

pase-9 activation, caspase cascade reaction initiation, and ultimately apoptosis [37, 38]. In addition, some other pathways can induce apoptosis. For instance, long-term ER stress causes continuous interaction between IRE1 α and tumor necrosis factor-related factor 2 (TRAF2), which activates the downstream apoptotic signal regulation kinase 1 (ASK1)-JNK signaling pathway, triggering apoptosis [39]. Furthermore, activation of IRE1 α can mediate mRNA breakdown through regulated IRE1-dependent decay (RIDD) [40]. The three UPR pathways are intricately interconnected with many

intersections, and they are often activated together (Figure 2).

ER stress and its role in the etiology of asthma

Asthma is a disease with complex characteristics and a polygenic inheritance tendency. The etiology of the disease is closely related to heredity and the environment. In individuals with certain genetic susceptibility, asthma can be triggered by allergens inhalations, smoking, and viral or fungal infection.

Genetic factors related to ER stress in asthma: ORMDL3

ORMDL3 is an ER transmembrane protein that contains 153 amino acids and is encoded by a gene on chromosome 17q21. The discovery of ORMDL3 has been important in the context of a variety of inflammatory diseases including asthma [41]. Studies have shown that genetic variation affecting the expression of the ORMDL3 protein is an important determinant of asthma susceptibility [42-44]. Mechanistically, abnormal ORMDL3 expression can lead to calcium outflow from the ER by inhibiting the sarco-ER calcium pump (SERCA); decreased calcium content in the ER is one of the major causes of the UPR, mainly through the PERK-eIF2 α pathway [45]. Pesticide exposure is often associated with asthma attacks; for example, benomyl, a common pesticide, can increase intracellular Ca²⁺ levels and trigger asthma. However, ORMDL3 knockout alleviates the effects of benomyl on intracellular Ca²⁺ and pro-inflammatory cytokines associated with the pathogenesis of asthma [46]. Genes play regulatory roles; rather than acting independently, genetic factors usually act in combination with environmental factors. Notably, ORMDL3 also mediates host-pathogen interactions. Increased ORMDL3 expression can regulate rhinovirus-induced ER stress and IFN- γ production [47], while ORMDL3 silencing can significantly reduce the expression of the rhinovirus receptor ICAM1. In addition, ORMDL3 silencing can reduce ER stress after IL-1 β stimulation and reduce the release of inflammatory factors such as IL-6 and IL-8 [16]. In an ER stress model induced by *Alternaria*, ORMDL3 has been found to drive the ATF6-mediated UPR pathway arm and to further activate the ERAD pathway [48]. However, some studies have revealed

inconsistent results; for example, it has been suggested that changes in ORMDL3 in airway epithelial cells do not play important roles in regulating immune responses and UPRs in the lungs [49]. Furthermore, Debeuf et al. suggested that wild-type, ORMDL3-deficient (Ormdl3^{-/-}) and ORMDL3-overexpressing (Ormdl3^{Tg/wt}) mice showed no differences in asthma characteristics after HDM sensitization [50]. In general, the effect of ORMDL3 on asthma has been widely recognized, but whether ORMDL3 plays a role through ER stress or other pathways needs to be further studied.

Asthma-associated environmental factors and ER stress

Dust mite: Many microorganisms can cause asthma attacks, including HDMS, fungi, virus, and others. Among these, HDMS, which is ubiquitous in the air, is one of the most important causes of asthma [51]. Studies have reported that allergic reactions to HDMS in early childhood are significant determinants of the subsequent development of asthma [52]. HDM stimulation can significantly upregulate ER stress-related markers in bronchial epithelial cells, mainly through the ATF-6 and IRE-1 pathways. The ATF-6 pathway can further activate the downstream CHOP pathway, inducing caspase-3 activation and apoptosis. In addition, the ER chaperones GRP78, GRP94, and ER-resident protein 57 (ERP57) are also markedly upregulated by HDMS [53]. ERP57 is an endoplasmic REDOX chaperone protein involved in the folding and secretion of glycoproteins, and is significantly upregulated in epithelial cells of asthmatic patients. However, in a mouse model of allergic asthma, inflammatory cell counts and airway resistance induced by HDM stimulation have been found to be significantly reduced in ERP57 knockout mice compared with wild-type mice [54]. In contrast to HDMS, *Dermaphagoides farinae* (Der f) mainly induce ER stress by activating IL-25; IL-25 activates the downstream CHOP pathway through PERK-eIF2 α arm, which further upregulates the expression of caspase3 and downregulates the expression of Bcl-2 to mediate apoptosis [55]. Some targeted treatment strategies have been developed for HDM-induced ER stress and asthma attacks. For example, tauroursodeoxycholic acid (TUDCA), a taurine-coupled form of ursodeoxycholic acid mainly used in the treat-

ment of cholestatic liver disease, effectively inhibits apoptosis partly by modulating the PERK-eIF2 α ER stress pathway and the Akt pathway [56, 57]. In the context of asthma, TUDCA markedly reduces HDM-mediated airway inflammation, mucus secretion, AHR, and airway remodeling by inhibiting ER stress [58]. In addition, given its inhibitory effect on ER stress, the effects of TUDCA on many other conditions, such as diabetes, retinopathy and neurological diseases, have also been studied [59, 60]. Platycodi Radix extract (PRE) has been identified as another possible drug for the treatment or prevention of HDM-related allergic airway inflammation that acts by inhibiting ER stress and its related reactive oxygen species (ROS) signaling pathway [61].

Fungi: Fungi are also present as allergens in indoor air, especially in dark, damp and poorly ventilated areas. Recently, studies have suggested that ER stress and associated molecules, including phosphoinositide 3-kinase- δ (PI3K- δ), may be vital for the development of fungal infection associated asthma [63]. Among fungi, *Alternaria* spp. and *Aspergillus fumigatus* (Af) have been identified as the most important risk factors for asthma mediated by ORMDL3 [64]. *Alternaria* spp. potently induce cellular stress and the UPR by activating ATF6-XBP1 signaling [48]. Studies have shown that PI3K- δ inhibitors can effectively inhibit ER stress and the inflammatory response in an Af-induced cortisol-resistant mouse model [65]. The overall effects of PI3K- δ inhibition on ER stress induced by fungi are achieved through reductions in inflammation-associated intra-ER hyperoxidation, disruption of protein disulfide isomerase (PDI) chaperone activity and stabilization of ER membrane fluidity and permeability [66]. In addition, PI3K- δ inhibition can improve Af-induced allergic inflammation by regulating the production of mitochondrial ROS (mtROS) and thereby modulating the NLRP3 inflammasome [67]. However, ER stress affects not only the human body but also the AbHacA gene of the fungus itself, which encodes the major UPR transcription regulator in *Alternaria* spp. Deletion of the AbHacA gene prevents induction of the UPR, resulting in a complete loss of virulence associated with cell wall defects [68].

Viruses: A growing number of reports have linked acute asthma attacks to respiratory viral

infections. For example, the influenza virus, one of the most common airway pathogens, can cause airway inflammation and asthma attacks by mediating ER stress and subsequent UPRs [69]. Mechanistically, eosinophils have emerged as important links between airway virus infection and allergic asthma exacerbation. When the airway is infected by a virus, eosinophils are activated to clear the virus from the respiratory tract. At the same time, endoplasmic reticulum stress occurs, causing the secretion of activated mediators that can induce asthma-related symptoms. However, this process requires the presence of prolyl isomerase. The eosinophils of prolyl isomerase knockout mice cannot activate the ER stress-induced UPR and fail to activate the intrinsic immune response, thus failing to clear viruses [70].

Smoking: In addition to microbial factors, cigarette smoke is an important trigger for asthma, especially in children whose parents smoke. ER stress plays a significant role in smoking-induced inflammation, apoptosis and autophagy. According to a gene set variation analysis of the bronchial epithelial cell transcriptome, current-smokers show enrichment of ER stress-associated genes compared with ex-smokers and nonsmokers [71]. In vitro, cigarette smoke extract (CSE) can significantly upregulate various ER stress markers (IER1 α , PERK, GRP78, eIF2 α , ATF4, CHOP) and induce related inflammatory responses, leading to upregulation of inflammatory markers (IL-6, IL-8, NF- κ B) [72, 73]. In addition, CSE can induce autophagy and apoptosis in alveolar epithelial cells through ER stress pathways. Smoking-mediated downregulation of PERK, GRP78, and eIF2 α in the UPR pathway upregulates CHOP and ATF4, promotes epithelial cell apoptosis and inhibits autophagy. However, there is a delicate balance among ER stress, apoptosis and autophagy induced by smoking, and different UPR pathways have disparate regulatory effects on apoptosis and autophagy. In addition, apoptosis and autophagy are regulated by mutual inhibition [73]. However, CSE can also increase the expression of HRD1, which, when overexpressed, can mediate ERAD to reduce ER stress-induced apoptosis as an adaptive protective measure [74]. Given that smoking can induce apoptosis through ER stress pathways, protective drugs targeting ER stress have become the focus of attention. Progranulin, a glycoprotein, has been reported to play a pro-

protective role in the context of smoking-induced apoptosis. CSE-induced alveolar epithelial cell apoptosis is significantly decreased in progranulin-overexpressing cells, and the activation levels of ER stress-associated markers are correlated with progranulin expression levels [75]. Another study has shown that H₂S inhibits lung tissue injury by reducing CSE-induced pulmonary ER stress in vivo and attenuates nicotine-induced ER stress-mediated bronchial epithelial cell apoptosis in vitro [76]. However, considering the toxicity of H₂S itself, it is difficult to translate its use into clinical application.

In general, asthma is associated with a variety of environmental factors that are closely related to ER stress. Targeting ER stress may provide a new direction for the future treatment of asthma attacks induced by such factors.

ER stress and its role in the pathophysiological characteristics of asthma

The pathophysiological characteristics of asthma include chronic airway inflammation, epithelial apoptosis, AHR and excessive mucus secretion, which eventually cause airway remodeling. During this process, ER stress plays an important role in regulating epithelial cell inflammation and apoptosis and affects mucus secretion, collagen deposition and smooth muscle hyperplasia.

ER stress-mediated airway inflammation and epithelial apoptosis

Chronic airway inflammation is the first major feature of asthma. The newly discovered function of the ER as a regulator of inflammation suggests potential strategies for the treatment of various inflammation-related diseases. NF- κ B, which is a transcription factor, regulates the expression of many genes involved in inflammation [77]. In the context of ER stress, various UPR pathways can reduce the expression of I κ B α , an inhibitory protein of NF- κ B, in different ways, thereby upregulating NF- κ B to mediate the inflammatory response [78, 79]. First, phosphorylated IRE1 can bind with TRAF2 to activate the JNK/AKT pathway, leading to phosphorylation of I κ B kinase (IKK). IKK activation leads to the cleavage of I κ B α , which eventually induces the activation of NF- κ B [80]. Second, PERK activation by autophosphorylation results in eIF2 α phosphorylation and ATF4

activation, which can inhibit the translation of various proteins, including I κ B α , thereby reducing I κ B α production and inducing NF- κ B transcription [81, 82]. Third, ATF6 is cleaved into active ATF6 α and ATF6 β in the Golgi, which induces the phosphorylation of Akt in a specific way and then inhibits the expression of I κ B α , leading to the activation of NF- κ B [83]. In addition to the three classic UPR pathways, BIP, a chaperone protein that mediates the initiation of the UPR, can directly leak into the cytoplasm to induce the activation of IKK and then downregulate I κ B α , leading to the activation of NF- κ B [84]. In addition, the sigma-1 receptor, an ER-resident protein, can restrict cytokine expression and the endonuclease activity of the ER stress sensor IRE-1 but does not inhibit the classic inflammatory signaling pathways [85]. ER stress plays an important role in regulating inflammation through NF- κ B and this phenomenon has also been verified to be involved in asthma. The results of one study in which samples obtained from airway epithelial brushing of individuals with asthma and normal subjects were analyzed by RNA sequencing showed that the expression of type 2 markers, IFN-stimulated genes (ISGs) and ER stress-related genes was significantly higher in the asthma group than in the normal group; in addition ER stress was obviously correlated with the type 2 inflammatory response and ISGs [86]. Researchers have also confirmed that the expression levels of ER stress markers (p-PERK, ATF4 and CHOP) are elevated in lung tissue in ovalbumin (OVA)-lipopolysaccharide (LPS)-OVA mouse models and that tunicamycin, used to induce ER stress, can further increase the expression levels of inflammatory cytokines, suggesting that PERK-ATF4-CHOP signaling is associated with airway inflammation in the context of neutrophilic asthma [87]. In addition, the ER stress blocker 4-PBA can significantly reduce the expression of NF- κ B, resulting in downregulation of the expression of Th2 cytokines (IL-4, IL-5, IL-13) and airway inflammatory response factors (IL-1 β , TNF- α , IFN- γ) as well as reductions in the populations of neutrophils and eosinophils [88].

CHOP, an ER stress marker and transcription factor, plays a core role in ER stress-induced apoptosis. CHOP can be activated by three classic UPR pathways: the PERK-eIF2 α -ATF4, ATF6-Golgi-cleaved ATF6, and IRE1-XBP1 path-

ways. In addition, IRE1 also activates the ASK1-JNK pathway through TRAF2 to mediate the activation of CHOP [89, 90]. After CHOP is activated, it can promote apoptosis through multiple other pathways. Activated CHOP can upregulate the expression of Bim and downregulate the expression of Bcl2, thereby inducing apoptosis by affecting the Bax/Bak-mediated permeability of the mitochondrial outer membrane. Increased permeability of the mitochondrial outer membrane can then lead to a caspase cascade to mediate apoptosis [91, 92]. Another major pathway of apoptosis mediated by CHOP is the ERO1 α -IP3R-calcium-CaMKII pathway. In this pathway, CHOP activates IP3R (a calcium release channel in the ER) via ERO1 α and then induces the release of calcium from the ER into the cytoplasm. The resulting increase in intracellular calcium ion content activates the calcium-sensing kinase CaMKII, inducing calcium-dependent apoptosis [93, 94]. CHOP can also induce apoptosis in other ways, such as through the DR4-DR5-caspase8 pathway [95].

During the past few years, our laboratory has focused on the role of CHOP in pulmonary diseases. We obtained the first evidence that pulmonary fibrosis alters CHOP expression and ER stress both in patients with idiopathic pulmonary fibrosis (IPF) and in animals with bleomycin-induced pulmonary fibrosis. Consistent with these observations, mice deficient in Chop are protected from bleomycin-induced lung injury and fibrosis. Specifically, loss of Chop significantly attenuates TGF- β production and M2 macrophage infiltration in the lungs following bleomycin induction. Mechanistic studies have revealed that Chop deficiency suppresses the M2 program in macrophages, which subsequently attenuates TGF- β secretion. Loss of Chop enhances the expression of SOCS1 and SOCS3, thereby inhibiting STAT6/PPAR γ signaling, which is essential for the macrophage M2 program [96]. Similarly, a study on an OVA-induced allergic airway inflammatory model has revealed that Chop regulates STAT6 phosphorylation, thereby enhancing the expression of mouse transcription factor EC (Tfec), which then transcribes IL-4 receptor α (IL-4R α) to promote the M2 program in macrophages [11]. Taken together, these data provide novel insights into the role of ER stress in modulating M2 macrophage polarization, which contributes to

the pathogenesis of fibrotic and asthmatic diseases.

ER stress-mediated hypersecretion of mucus

Increased airway mucus secretion, mucus retention, and mucus plug formation are other major characteristics of asthma. In mammals, 5 mucin-related genes mediate mucus formation; among them, MUC5AC and MUC5B are significantly highly expressed in airways and regulate mucus secretion in the respiratory system [97]. A genetic analysis of small airway epithelial cells brushed through fiberoptic bronchoscopy revealed a list of 73 MUC5AC-associated core genes with 9 categories, one of which included ER stress-related genes [98]. Recently, ER stress has begun to be regarded as a target for inhibition of mucus secretion. Researchers have explored the abilities of numerous molecules involved in ER stress to affect mucus secretion. For example, the expression of MUC5AC in an OVA-LPS-OVA-induced mouse asthma model has been found to be significantly downregulated by 4-PBA, an ER stress inhibitor [99]; astragalus polysaccharide (APS) has been found to significantly reduce ER stress marker levels and reduce mucus production [100]; kaempferol has been found to alleviate mucus hypersecretion by blocking bronchial epithelial ER stress through inhibition of IRE1 α -TRAF2-JNK pathway activation, thus reducing the expression of MUC5AC [101]; and knockout of anterior gradient homolog 2 (AGR2), a gene associated with airway and intestinal epithelial mucin production, has been found to reduce MUC5AC and MUC5B expression and mucus secretion in an OVA-LPS-OVA-induced mouse asthma model [102]. IRE1 β is a component of the UPR pathway; but unlike IRE1 α , it is expressed only in the gut and respiratory system. IRE1 β can upregulate the expression of AGR2 by mediating XBP-1 splicing, thereby increasing mucus secretion [103]. In addition to affecting mucus secretion through AGR2, XBP-1s can be activated by IRE-1 β and directly bind to the proximal region of the MUC5B promoter variant rs35705950, thereby inducing mucus hypersecretion [104]. In addition to the IRE1 pathway, the NF- κ B, ATF6, and CHOP signaling pathways also involved in the regulation of mucus secretion. Inhibition of ER stress by Lyn kinase leads to blockade of NF- κ B, thereby downregulating MUC5AC expres-

sion [105], and siRNA-mediated knockdown of XBP-1, CHOP, and ATF6 can decrease the mRNA expression and protein levels of MUC5AC and MUC5B [106]. Interestingly, ER stress can regulate the expression of MUC5AC, and MUC5AC can in turn exert a regulatory effect on ER stress. MUC5AC knockout in cells attenuates the increases in ER stress markers caused by LPS stimulation [107].

ER stress-mediated AHR and airway remodeling

In AHR an airway is highly sensitive to various stimulating factors, and airway smooth muscle contraction is hyperactive. The main causes of AHR are chronic inflammation, airway epithelial injury, apoptosis, and abnormal airway smooth muscle contraction [108]. As mentioned above, ER stress is involved in the development of airway inflammation and airway epithelial cell apoptosis. However, the contraction of airway smooth muscle is also regulated by ER stress [109]. Contraction of smooth muscle depends on two key factors: intracellular calcium concentrations and smooth muscle calcium sensitivity. ER stress can induce intracytoplasmic calcium imbalance by activating CHOP; this is one of the mechanisms by which ER stress mediates abnormal smooth muscle contraction [110]. Acetaldehyde stimulation significantly increases the levels of ER stress markers in airway smooth muscle cells, resulting in upregulation of the expression of NF- κ B, a key molecule regulating inflammation [111]. In addition, while PI3K δ is closely associated with fungal infection-mediated asthma attacks, as we have mentioned, it can also induce airway inflammation and AHR by activating NF- κ B signaling through ER-associated ROS and RIDD-RIG-I activation [112]. In general, ER stress participates in AHR by regulating the inflammatory response, apoptosis, and smooth muscle sensitivity.

The combined action of the above pathological features ultimately causes airway remodeling, or airway structural changes. The main characteristics of airway remodeling are subepithelial collagen deposition and fibrotic proliferation after epithelial injury. Studies have confirmed the close relationship between ER stress and pulmonary fibrosis. ER stress can induce pulmonary fibrosis by mediating apoptosis and the

epithelial-mesenchymal transition (EMT) in epithelial cells, the polarization of macrophages to M2-type cells and the activation of fibroblasts [24, 113]. The IRE1-XBP1 pathway can promote the EMT by mediating snail expression, thereby causing fiber proliferation [114]. N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) can reduce type II alveolar epithelial cell apoptosis and inhibit collagen deposition by suppressing the PERK/eIF2 α /CHOP pathway [115]. In conclusion, ER stress can mediate inflammation, apoptosis, mucus hypersecretion, smooth muscle contraction and collagen deposition through multiple pathways that together constitute the pathological mechanism of asthma.

Role of ER stress in the treatment of asthma

Traditional treatments for asthma include glucocorticoids combined with beta agonists or leukotriene modulators. However, a subset of asthma cases are steroid-resistant, especially Th2-mediated nonallergic eosinophilic asthma and non-Th2 asthma cases [116]. ER stress participates closely in the pathogenesis of steroid-resistant asthma by mediating the activation of the PI3K, MAPK and NF- κ B pathways [117]. The molecular mechanisms of ER stress and steroid-resistant asthma overlap, suggesting a therapeutic strategy for severe asthma [118]. Thus far, many compounds have been studied or used for ER stress-based asthma treatment. Suhuang antitussive capsules, which are adjuvant drugs commonly used in the field of pneumology, disrupt NLRP3 inflammatory activation by inhibiting ER stress in the context of asthma [119]. In addition, conjugated bile acids (CBAs) have been reported to be able to inhibit allergen-induced UPRs and airway allergic disease in mice by specifically binding to ATF6 α [120]. 4 μ 8c, an inhibitor of IRE1 α RNase, can reduce the secretion of IL-4, IL-5, and IL-13 proteins by activated CD4⁺ splenocytes in naive mice and reduce the secretion of IL-5 by established TH2 cells [121]. In addition, trimethylamine-N-oxide (TMAO) and 4-phenylbutyric acid can reduce UPR marker levels, airway inflammation, and remodeling in a dose-dependent manner [122]; gubenfangxiao decoction can significantly attenuate persistent airway inflammation in a respiratory syncytial virus (RSV)-OVA-induced asthma mouse model at least partially through inhibition of ER stress [123]; Ghrelin can inhibit ER stress by stimulat-

ER stress in asthma

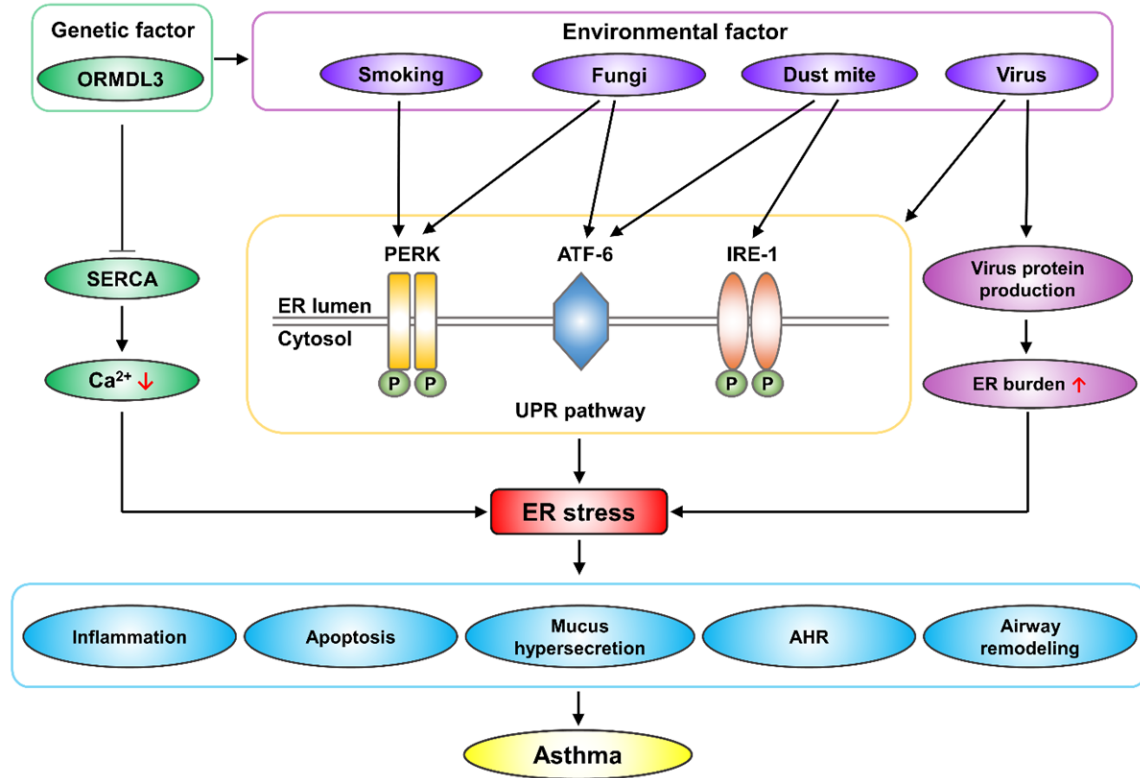


Figure 3. The relationship between ER stress and asthma. Under a genetic background dominated by high ORMDL3 expression, various environmental factors participate in inducing ER stress. ER stress further mediates inflammation, apoptosis, airway mucus hypersecretion, AHR and airway remodeling through different mechanisms. ER stress can be targeted as a new way to treat asthma. ER: endoplasmic reticulum; SERCA: Sarco-ER calcium pump; ATF6 α : activating transcription factor 6 α ; PERK: protein kinase RNA-like ER kinase; IRE1 α : inositol-requiring protein 1 α ; AHR: airway hyperresponsiveness.

ing the Akt signaling pathway, thereby reducing inflammatory responses and ameliorating asthma in mice [124]; and sevoflurane can restrain ER stress to recover the protein processing of aquaporins, thus relieving asthma [125]. Given the increasing evidence of the relationship between ER stress and asthma, we believe that targeting ER stress may be a new approach for steroid-resistant asthma treatment.

Conclusion

We reviewed recent studies on ER stress mainly from the perspectives of the etiology and pathogenesis of asthma. Both genetic and environmental factors mediate ER stress, which in turn regulates airway inflammation, apoptosis, mucus secretion, AHR and airway remodeling in different ways to ultimately cause asthma (Figure 3). The reviewed studies thoroughly demonstrate the close relationship

between ER stress and asthma. However, additional in-depth mechanisms are worth elucidating, and uncertainties remain, such as how to best translate the existing mechanistic research into clinical applications. In the future, targeting ER stress may be a new strategy for asthma treatment, especially in the context of steroid-resistant asthma.

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Disclosure of conflict of interest

None.

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