Mechanisms Targeting the Unfolded Protein Response in Asthma

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Running title: UPR in asthma

Authors' contribution: SG, AAZ and PS conceived the review. All authors contributed equally in writing the review and approved the final version.

Conflict-of-interest statement: The Authors have no conflict of interest.

Abbreviations:

4-PBA: bba4-phenyl butyric acid, **AEC**: Airway Epithelial Cell, **AGR2**: Anterior Gradient 2, **AHR**: Airway Hyper-Responsiveness, **APC**: Antigen-Presenting Cell, **ARG2**: Arginase 2, **ASL**: Airway Surface Liquid, **ASM**: Airway Smooth Muscle , **ATF4**: Transcription Factor 4, **ATF6**: Activating Transcription Factor 6, **BAX**: BCL2-Associated X Protein, **C/EBP**: CCAAT/enhancer-binding Protein, **CHOP**: CCAAT/enhancer-binding Protein (C/EBP) Homologous Protein, **COPD:** chronic obstructive pulmonary disease, **CS:** cigarette smoke, **DC**: Dendritic Cell, **eIF2α**: Eukaryotic Translation Initiation Factor 2α, **ER**: Endoplasmic Reticulum, **ERAD**: ER-associated Degradation, **ERp57**: ER Resident Protein 57, **FeNO:** fraction of exhaled nitric oxide, **GCM**: Goblet Cell Metaplasia, **GRP78**: 78-kDa Glucose-Regulated Protein, **GWAS**: Genome-wide Associated Studies, **HDM**: House Dust Mite, **HRV**: Human Rhinovirus, **IPF:** idiopathic pulmonary fibrosis, **IgE**: Immunoglobulin E, **IL13**: Interleukin, **IRDD**: IRE1-dependent Decay, **IRE1α**: Inositol–requiring Enzyme 1α, **Ire1β**: Inositol Requiring Enzyme-1beta, **JNK**: c-Jun NH2-termina Kinase, **miRNA**: microRNA, **MUC 5AC**: Mucin 5AC, **NF-kβ**: Nuclear Factor kappa B, **NO**: Nitric Oxide, **Nrf2**: Nuclear Factor Erythroid 2-related Factor 2,

ORMDL3: Orosomucoid like 3, **PDIA5**: Protein Disulfide Isomerase A5, **PERK**: Protein kinase RNA-like Endoplasmic Reticulum Kinase, **PP1**: Protein Phosphatase 1, **PPP1R15A**: Protein Phosphatase 1 Regulatory Subunit 15A, **RIP**: Regulated Intra-membrane Proteolysis, **ROS**: Reactive Oxygen Species, **SERCA**: Sarco-endoplasmic Reticulum Calcium-ATPase Pump, **SNP**: Single Nucleotide Polymorphism, **SP**: Site-1 and site 2 Protease, **Th2**: T helper type 2, **TNF**: Tumor Necrosis Factor, **TUDCA**: Taurohyodeoxycholic Acid, **UPR**: Unfolded Protein Response, **VEGF**: Vascular endothelial growth factor, **VEGF**: Vascular Endothelial Growth Factor, **XBP1**: X-box-binding Protein 1

ABSTRACT

Lung cells are constantly exposed to various internal and external stressors that disrupt protein homeostasis. To cope with these stimuli, cells evoke a highly conserved adaptive mechanism called the unfolded protein response (UPR). UPR stressors can impose greater protein secretory demands on the endoplasmic reticulum (ER) resulting in the development, differentiation, and survival of these cell types to meet these increasing functional needs. Dysregulation of the UPR leads to the development of the disease. The UPR and ER stress are involved in several human conditions such as chronic inflammation, neurodegeneration, metabolic syndrome, and cancer. Further, potent and specific compounds that target the UPR pathway are under development as future therapies. The focus of this review is to thoroughly describe the effects of both internal and external stressors on the ER in asthma. Further, we discuss how the UPR signaling pathway is activated in the lungs to overcome cellular damage. We also present an overview of the pathogenic mechanisms with a brief focus on potential strategies for pharmacological interventions.

Keywords: endoplasmic reticulum, ER stress, asthma, unfolded protein response.

THE UNFOLDED PROTEIN RESPONSE (UPR)

The "cellular stress response" is defined as the cellular changes in response to stressors such as environment, starvation, and/or cytotoxic compounds (1-3). Cellular damage activates compensating signaling pathways in all organelles to initiate the adaptive immune system, restore intracellular homeostasis, and promote cell survival (4). The focus of this review is to describe the effects of stress on the endoplasmic reticulum (ER) and the pathways activated in this vital and multi-functional organelle to overcome cellular damage in asthma (5-9).

ER stress can disturb the fine balance between cellular demand for protein biosynthesis and the ER's capability for protein folding. Various stressors can directly or indirectly induce instability in the ER microenvironment leading to ER stress. Some of these include glycosylation inhibitors (e.g. tunicamycin), hypoxia, glucose deprivation, calcium metabolism mediators, calcium ionophores (such as A23187), calcium pump inhibitors (e.g. thapsigargin), viral infections, and reducing agents (dithiothreitol and 2-mercaptoethanol) that cause changes in the oxidative state of the ER lumen and proper protein folding (6, 10-13). These stressors have a significant impact on the ER, leading to the accumulation of misfolded proteins in the ER lumen and the initiation of defensive mechanisms against the misfolded protein accumulation (14, 15). Under normal cell conditions, there is a basal level of improperly folded proteins. These proteins are returned to the cytosol and then are degraded through the cellular degradation system. However, any deficiency in the function of this degradation machinery leads to the accumulation of misfolded un-degraded proteins that are potentially toxic leading to ER stress (16-20).

ER stress triggers a cascade of cellular signaling pathways known as the unfolded protein response (UPR) (21-24). The UPR functions principally to restore intracellular homeostasis and

proteostasis through establishing proper protein folding in the ER and activation of other

pathways such as ER-associated degradation (ERAD) (25, 26) to remove unwanted proteins

(unfolded/mutated/misfolded), and to inhibit translation of new proteins in order to stabilize

intracellular homeostasis (5, 19, 27). Of note, the UPR system balances the loading of incoming

proteins to the ER and its protein folding capacity, allowing the cell to adapt more easily to the

specific demands and any subsequent imbalances in protein handling (28, 29). In most cases, ER

stress occurs due to pathological conditions within the cell, whereas the UPR system normally

operates in specialized secretory cells in a continuous and active mode (30). The UPR also plays

an important role in immune responses during the assembly of heavy chains and antibody

secretion (31). Recent studies indicate that the UPR is activated in cells under both acute and

prolonged stress and plays a key role in many diseases including diabetes, cancers, various

immune diseases, neurodegenerative disorders, cataracts, pulmonary fibrosis, cardiomyopathy,

atherosclerosis, ischemia, nephrotic syndrome, non-alcoholic steatosis, and psoriasis (32-34).

A fundamental function of the UPR is to alleviate the accumulation of unfolded

proteins in the ER and to re-establish quality control and secretory proteostasis during ER stress

(29, 35, 36). The UPR controls the expression of a variety of genes involved in protein folding,

secretion, and quality control. Thus, the UPR regulates ER function and other aspects of cellular

physiology, such as mitochondrial biology, lipid metabolism, and intracellular apoptotic

pathways.

Mitochondria, like the ER, have a specific quality control system called mitochondrial

UPR (UPR^{mt}), which is activated by several cellular disorders (28, 32, 37). Although the UPR

has been well-studied in the ER and the cytosol, its correlation with UPR^{mt} has not been clearly

determined. The UPR^{mt}, acting as a stress response pathway, may regulate the protein folding

capacity of the mitochondrial matrix by monitoring the load of folding proteins and signaling to the nucleus with subsequent upregulation of the respective chaperone-encoding genes, in normal physiology, as well as disease conditions. UPR^{mt} function results in mitochondrial-nuclear (mito-nuclear) communication that is an adaptive transcriptional response pathway. This signaling increases mitochondrial recovery to ensure that specific proteins are properly translated, folded, and degraded through chaperones, such as Hsp60 and mtHsp70, in the mitochondria. It can also promote proteolysis of unfolded proteins as well as glycolytic and detoxification of genes (38-41).

UPR arms in ER stress

The UPR system is controlled by three ER transmembrane proteins, acting as stress sensors, as well as 78-kDa glucose-regulated protein (GRP78). The transmembrane proteins include inositol–requiring enzyme 1α (IRE1α), protein kinase RNA-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6) (21, 42-44). Under physiological conditions, these sensors are inhibited by GRP78, which is bound to their luminal domains. In the event of unfolded protein accumulation, GRP78 dissociates from the ER to overcome the perturbation in protein folding due to its higher affinity for unfolded protein which is required for its release from IRE1 and PERK, and this leads to activation of the UPR signaling pathway (Figure 1).

GRP78

GRP78, is one of the significant characterized chaperones that is conserved from yeast to human with ATPase activity. GRP78 has two motifs: a signal peptide that targets GRP78 to the ER and a C-terminal KDEL that acts as a retention signal to the ER by changing its monomeric

and oligomeric form. In the monomeric and unmodified structure, GRP78 is associated with unfolded peptides in the ER lumen, and it is under the influence of post-translational modification, i.e., ADP-ribosylation, in the oligomeric form. GRP78 is low at basal levels but significantly increases during ER stress, calcium homeostasis, and active UPR processes (45, 46).

GRP78 is responsible for several cellular processes: it facilitates the translocation, assembly, and folding of newly synthesized nascent proteins. It prevents misfolding and aggregation of proteins in cells and removes unfolded/misfolded proteins in the ER-associated degradation (ERAD) pathway. It also regulates calcium homeostasis *via* the influence of *ER-mitochondrial calcium* crosstalk. In general, the most important role of GRP78 is to serve as a sensor, marker, and the master regulator of ER stress by its anti-apoptotic function as an ER chaperone and its ability to control the initiation of the UPR signalling pathway (43, 47, 48).

IRE1

IRE1 is a type 1 transmembrane protein with two enzymatic features in cytosolic domains; a serine/threonine kinase and endoribonuclease function. It has two unique homologs in humans: IRE1α and IRE1β (structurally more similar to murine IRE1 than human) (27). While IRE1α is expressed ubiquitously, IRE1β is only expressed in pulmonary, intestinal epithelial, and mucosal epithelial tissues. Among the UPR signaling pathways, IRE1α is a key sensor for regulating cells' fate. Deletion of IRE1α results in embryonic lethality although previous studies have shown that IRE1β knock-out mice are viable. Therefore, many recent investigations in UPR pathways have been focused on the IRE1α (49-51).

Dissociation of GRP78 triggers activation of IRE1, which itself can target the X-box-binding protein (XBP1) mRNA with its kinase and endoribonuclease activity. IRE1α promotes an atypical splicing of XBP1 mRNA near the ER membrane to produce XBP1s (splice). XBP1s is a transcription factor and localizes in the nucleus to regulate the expression of several genes that are involved in the ERAD pathway, expansion of the ER compartment, protein folding, trafficking, and secretory pathways. IRE1 also promotes cell survival, i.e., inhibition of ER stress-induced apoptosis, *via* inhibition of CCAAT/enhancer-binding protein (C/EBP) (CHOP) (24, 32). Hyperactivation of IRE1 can influence c-Jun NH2-termina kinase (JNK), NFκβ, and initiate the process known as IRE1-dependent decay (RIDD). This process decreases some mRNAs and microRNAs (miRNAs), including miR-17, miR-34a, miR-125b, and miR-96 (52, 53) to reduce ER protein loading.

PDIA6 is a subtype of protein disulfide isomerase in eukaryotic ER. Mammalian IRE-1 has three cysteines in its luminal domain including Cys109, Cys148, and Cys332. The PDIA6 targets Cys148, reducing the disulfide bond in the region; and consequently, increasing the inactivation of IRE-1 (or terminating) signaling. The PDIA6 acts independently on UPR signaling arms. However, during stress conditions and disrupted ER Ca^{+2} homeostasis, XBP1 splicing increases. Under these conditions, the Ca^{+2}/PDIA6 crosstalk increases the IRE-1 activity that will further support ER homeostasis (54-56).

PERK

PERK is activated after separation of GRP78; and then, it is auto-phosphorylated in the Ser/Thr kinase domain, which leads to phosphorylation of eukaryotic translation initiation factor

2α (eIF2α). This results in suppression of protein translation and a significant reduction in the number of proteins entering the ER (34).

Despite its potent pro-survival role, phosphorylated eIF2α can activate the translation of transcription factor 4 (ATF4). ATF4 acts as a repressor or activator of the transcription of several genes involved in key biological processes such as bone resorption, glutathione synthesis, amino acid metabolism (i.e., asparagine synthetase), resistance to oxidative stress, neovascularisation (VEGF), and ER chaperones. It can also promote DNA damage response, expression of CHOP (C/EBP homologous protein) known as growth arrest and DNA damage-inducible protein GADD34 (PPP1R15A), and pro-apoptotic BCL-2 family proteins. GADD34 dephosphorylates activated eIF2α via a protein phosphatase 1 (PP1)-interacting protein and prevents its ability to inhibit translational events. PERK can also induce G_1 cell cycle arrest that is mediated by the loss of cyclin D1 via the inhibition of its translation as well as increased degradation during ER stress (57).

ATF6

Activating transcription factor 6 (ATF6) is a type 2 transmembrane protein in the ER with transcription factor activity located in its cytosolic domain. In contrast to the activation of IRE1 and PERK during ER stress, ATF6 is transported to the Golgi compartment after dissociation from GRP78 and becomes activated by regulated intra-membrane proteolysis (RIP) with site-1 and site 2 (SP) protease. This leads to the translocation of the cytosolic domain (50 kD) to the nucleus as a transcription factor; and consequently, an increase in the expression of CHOP, ER chaperones, ER quality control, and protein folding machinery, ERAD components, and XBP1.

In addition to GRP78, protein disulfide isomerase A5 (PDIA5) and the cysteine oxidation status of ATF6 have major impacts on the activation of ATF6 (34, 58, 59).

Activation of the ATF6 signaling pathway is targeted via oxidoreductase ERp18, which is associated with ATF6 following ER stress. It also regulates the quality of ATF6 during trafficking from the ER to the Golgi and protects the cell against prolonged ER stress. ERp18 performs a regulatory role during UPR and induction of ATF6 via reducing disulfide within the ATF6 luminal domain by masking Golgi-localization sequences in the ATF6. During ER stress, ERp18 reduces ATF6 disulfide, leading to the release of BiP from ATF6. The reduced ATF6 is then packaged into COP II vesicles to be transported to the Golgi, then the reduced ATF6 is cleaved by S1P and S2P. Following this, activated ATF6 (ATF6 N-terminal domain) translocates to nucleus to alter gene expression which is required to restore ER homeostasis (60, 61).

ASTHMA

Asthma is a common respiratory disease that clinically manifests as wheezing, chest tightness, dyspnea, and cough (62, 63) (63-68). Approximately 5-10% of the general population is affected by asthma, and the incidence is higher among preschoolers (67). Asthma is increasingly recognized as a heterogeneous disease defined by reversible and periodic airway obstruction (i.e. swelling and narrowing of airways), mucus metaplasia/hyperplasia (i.e. increased mucus production), airway smooth muscle cell hypertrophy, and airway hyper-responsiveness (AHR). Severe asthma is approximately 5-10% of the asthma population, is difficult to manage, and is very distinct from milder forms of asthma.

Asthma can be categorized according to molecular phenotypes of type 2 and non-type 2 inflammation (69), may be allergic or non-allergic in origin (62, 66, 67, 70), and is associated

with both genetic and environmental factors (67, 70). Allergic asthma is the most common type of asthma where allergens induce eosinophilia and T helper type 2 (Th2 or type 2) inflammatory responses (62, 65). Exposure to environmental factors, such as house dust mite (HDM), pollen, animal dander, fungi, air pollution, and viral or bacterial infection are identified as asthma triggers (62, 71). Corticosteroid-resistance is a hallmark of some forms of severe asthma, where neutrophilic and non-type 2 inflammation can also predominate (62).

Histopathology of Asthma

Airway epithelial cells (AECs) form a physiological mucosal barrier (the airway epithelium) between the lungs and the external environment (64). The AECs consist of different cell types (e.g. club cells (Clara cells), mucus-producing cells, basal cells, etc.), form apical junction complexes and produce an airway surface liquid (ASL) layer (72). The epithelium can initiate and control various immune and inflammatory responses that are induced by allergens, various infectious agents, and pollutants (72, 73). Other resident airway cells crosstalk with the epithelium and play an integral part in the pathophysiology of asthma including airway smooth muscle (ASM) cells, submucosal mucus glands, resident immune cells, and the vasculature.

Various indoor and outdoor stimulants can alter epithelial cell fate by activation of specific receptor groups on the AEC surface which initiates downstream intracellular signal transduction cascades (64, 72, 74). Activation of AECs induces the secretion of manifold inflammatory cytokines and growth factors (64) relevant to asthma pathogenesis. Certain inflammatory cytokines such as interleukin-13 (IL13) can disrupt the integrity and functionality of airway cells, leading to impaired cell function, and adverse tissue remodeling (72). In addition to the activation of innate lymphoid cells, T cells, dendritic cells, and inflammatory cell

recruitment, adverse airway remodeling including AEC thickening, goblet and mucus cell metaplasia, basement membrane thickening, and ASM hypertrophy and hyperplasia all contribute to bronchoconstriction and AHR (69). Additionally, mucus gland hypertrophy and proliferation of airway blood vessels (induced by vascular endothelial growth factor (VEGF)) can lead to further airway narrowing (73).

Onset and Progression of Asthma

Asthma can be triggered by various environmental aeroallergens and stimuli that activate Th2 lymphocytes leading to the accumulation of mast cells, secretion of inflammatory mediators, and the infiltration of eosinophils, neutrophils, and macrophages (64, 71, 74). Pulmonary alveolar macrophages (AM) extend from alveolar space to the upper airways. AMs are activated in response to direct allergen exposure such as HDM. Activated AM phagocytosis and the secretion of various cytokines (e.g. tumor necrosis factor (TNF)), reactive oxygen species (ROS), and nitric oxide (NO) as part of the innate immune response (71, 75) in asthma.

Activated AECs and AMs release cytokines that stimulate resident airway dendritic cells (DCs), which are multifunctional immune cells that coordinate allergic responses in the lung. During exposure to foreign antigens, these antigen-presenting cells (APCs) activate T- and B-cells, thus connecting innate and adaptive immunity. The activation of T- and B-cells leads to antibody production by B lymphocytes (71) relevant to asthma pathogenesis.

AMs also block antigen presentation thereby modulating immune responses by inhibiting DCs, T-cell, and B-cell activity in healthy tissues (75). In allergic asthmatic patients, the phagocytic activity of AMs is significantly decreased compared to healthy subjects, while the

antigen presentation activity of DCs is significantly increased. This highlights the importance of AMs in the coordination and control of allergic responses in asthma (75).

Through activation of immune responses in airways of asthmatic patients, the transcription factor GATA3 and the alarmin IL-33 help native T-cells differentiate into Type 2 cells (Th2). The Th2 phenotype induces type 2 immune responses in patients with asthma (74). During this inflammatory process, eosinophil influx into the airways causes persistent airway inflammation, airflow obstruction, and AHR. Eosinophils activate and damage airway tissues by secreting high concentrations of pro-inflammatory cytokines, chemokines, growth factors, and peptides from their secretory granules (63). Mast cells are another immune cell that produces inflammatory mediators including immunoglobulin E (IgE) and acute-phase mediators such as cysteinyl leukotrienes (73, 74). Production of IgE promotes histamine secretion, an acute inflammatory mediator, that contributes to the clinical manifestation of asthma, such as mucus metaplasia and bronchospasm (74).

ER Stress and the Pathogenesis of Asthma

Induction of ER stress and activation of the UPR in airway epithelial cells (AECs) adversely affects asthma. Stimuli important in the development of allergic asthma include aeroallergens, oxidative stress, bacteria, pollutants, and viral infections, all of which disrupt ER integrity and can act as inducers of ER (71, 74). The ER stress signaling network is controlled by three related transmembrane stress sensors of the UPR pathway located in the ER lumen. Acute and prolonged activations of UPR proteins can lead to the development of allergic airway inflammation (71, 76). Asthma-driven type 2 inflammation and expression of IFN-stimulated genes (ISGs) are both independently associated with elevated expression of ER stress genes (77).

Preliminary investigation has highlighted the link between the differentiation of neutrophils and mitochondrial ATP production through activation of an arm of the UPR pathway (78). UPR also mediates neutrophil cell apoptosis via the activation of the CHOP-PERK arm (79). Several studies have also revealed that ER stress and UPR activation play a critical role in the pathogenesis of severe or steroid-resistant bronchial asthma by regulating NF-κβ (80).

Approximately 85% of asthma exacerbations are caused by viral infections. During the Spring, Fall, and Winter seasons, viruses such as Rhinoviruses (RV), Influenza, and human respiratory syncytial virus (RSV), can increase acute symptoms of asthma. Although several viruses can provoke asthma symptoms, there is evidence that allergens can amplify the adverse effects of viruses on asthma symptom control. Most viruses appear to use the pro-survival anti-apoptotic arms of the UPR to enhance replication by increasing ER capacity (81-83).

Airway goblet cells express IRE1β, a key UPR sensor, which is responsible for inducing genes involved in mucus-secretion and cell differentiation, the mucins MUC5AC and MUC5B, and protein glycosylation (71, 84). During ovalbumin (OVA)-induced experimental asthma, IRE1β has a pivotal role in goblet cell metaplasia (GCM), a hallmark feature of asthma (71). During exposure to allergens, IRE1β modulates the level of ARG2 and MUC5AC; thus increasing the immune reactivity of IRE1β in the airway epithelium, which in turn induces expression of AGR2 (71, 84). Further, during allergic inflammation in $Agr2^{-/-}$ knockout mice, the production of mucins MUC5AC and MUC5B are reduced. And in $Ire1\beta^-$ and $Agr2^-$ knockout mice, the airway epithelium displays a lack of the mucin layer (71).

The other ER transmembrane sensors, PERK, counteracts IRE1β activity by downregulating mucus overproduction. The transcription factor Nrf2 plays an important role in

PERK-mediated cell survival. Nrf2 deficiency causes an increase in mucus cell hyperplasia and secretion of Th2 cytokines that results in allergen hypersensitivity (71, 76). The transcription factor 6 (ATF6), a component of ER stress, is induced by complex allergens such as HDM in airway epithelial cells (64, 65). Oasis is a member of the ATF6 family that participates in the differentiation of lung goblet cells, collagen production, and is involved in lung repair (84). In experimental asthma induced by OVA or HDM, higher activation of ATF6α and XBP1 leads to mucus metaplasia and pro-inflammatory responses. Also, overexpression of CHOP due to the activation of ATF6 during ER stress induces apoptosis in type II alveolar epithelial cells and subsequent lung fibrosis (64). ATF6 downregulates Nogo-B protein that plays an important role in shaping the tubular structure of the ER. And the expression of Nogo-B in the airway epithelium of asthmatic subjects is reduced significantly.

Along with the induction of ATF6α in response to HDM exposure, ER-resident protein 57 (ERp57) is upregulated in airway epithelial cells (64, 65). Hoffman et al. showed that ERp57 induces airway inflammation, AHR, and airway fibrosis in a mouse model of allergic asthma (64, 85). Subsequently, specific targeted deletion of ERp57 in lung epithelial cells attenuated the features of asthma in mice with allergic airways disease (86). Siddesha et al. showed that TUDCA (Tauroursodeoxycholic acid) acts as an ER-stress inhibitor chaperone. It extensively attenuates the expression of HDM (house dust mite)-induced ER-stress marker genes, including ATF-6, ERp57, XBP1s, CHOP, and GRP78, in lung lysates during preventive and therapeutic regimens (87).

Role of Orosomucoid like 3 (ORMDL3) in Asthma and the UPR

Calcium ions play an important role in many cellular functions. Further, maintenance of calcium homeostasis within the ER is vital for signal transduction pathways, protein translation/processing/trafficking, and cell division (71, 88). Any disruption in luminal calcium levels within the ER induces ER stress and activates the UPR (70, 89).

Orosomucoid-like3 (ORMDL3) is a critical transmembrane protein anchored in the ER (63). ORMDL3 has been associated with asthma and a series of autoimmune disorders and is involved in ER-mediated inflammatory responses. However, the crucial molecular mechanism underlying its expression is not clearly understood (90-92). This protein is a conserved family of ER-resident proteins that regulates Ca^{2+} homeostasis through a membrane protein known as the sarco-endoplasmic reticulum calcium-ATPase pump (SERCA). SERCA controls the amount of calcium ions that enter the ER (68, 71). ORMDL3, also known as ORMDL sphingolipid biosynthesis regulator 3, physiologically downregulates the biosynthesis of sphingolipids which are key elements in cell communication, trafficking, and inflammation (64, 70, 71). As mentioned previously, asthma is a complex disease with both genetic predisposition and environmental factors involved in its pathogenesis (67). Several genome-wide associated studies (GWASs) identified that the ORMDL3 gene as one of several important genes linked to the pathogenesis of asthma (66, 70, 71, 89). Based on genomic analyses, single nucleotide polymorphisms (SNPs) in the 17q21 region of the chromosome are associated with ORMDL3 (66, 70, 71). This gene is a potential risk factor for both childhood and adult asthma, and it is induced with exposure to allergens, including tobacco smoke, OVA, interleukin-3 (IL-3), and interleukin-4 (IL-4) in airway epithelial cells (71, 93-96).

The T allele of the ORMDL3 rs7216389 polymorphism carries an increased risk of developing asthma (97). This allele of the SNP at the 17q21 locus, rs7216389, is associated with significantly higher FeNO levels and peripheral eosinophil counts in adults (98). Higher levels of ORMDL3 expression in $CD8^+$ T-cell, B-cell and eosinophils compared to monocytes (in a cell-specific pattern of ORMDL3 expression) may explain the connection between this gene and asthma pathogenesis. ORMDL3 expression may act on eosinophil inflammatory functions and its trafficking, or it may contribute to asthma severity via increasing allergen-specific IgE in B-cells (91, 92).

ORMDL3 expression is also implicated in the adhesion of microbes on the epithelial surface via its involvement in the protein transcription of ICAM1, the major receptor for the human rhinovirus and several inhaled bacterial pathogens (99). Conversely, increased expression of ORMDL3 contributes to a boosted antiviral defense in times of amplified rhinoviral load (100). Knockdown of ORMDL3 in an asthmatic mouse model can reduce AHR, airway inflammation, and key markers of allergic inflammation such as concentrations of IL-4, IL-5 and IL-13 cytokines in BAL fluid. Alleviation of symptoms and airway remodeling appear to be the inactivation of the JNK1/2-MMP-9 pathway (101).

Of note, ORMDL3 expression correlates positively with the expression of SERCA2b (70), and the expression of ORMDL3 can activate ATF6 in the UPR pathway (70, 71, 93). Thus, the activation of ATF6 increases the expression of SERCA2b, which leads to ASM cell proliferation and airway remodeling in asthma. And insufficient expression of SERCA can also induce airway remodeling (68). Patients with asthma can overexpress ORMDL3 particularly in response to human rhinovirus (HRV) and in murine models during OVA challenge (63). Higher levels of ORMDL3 inhibits the expression of SERCA that results in perturbation of Ca^{2+}

homeostasis in the ER. ORMDL3 can then act as an endogenous inducer of the UPR (67). Consequently, dysregulation of ORMDL3 in airway epithelial cells leads to progression of ER stress, which increases the activation of the UPR pathway. These factors all together induce various inflammatory pathways that link together ORMDL3, UPR, and asthma in a complex web of responses (71, 80).

UPR Triggers in Asthma

Environmental triggers for allergic asthma can act as UPR activators in the airways. Common triggers include HDM, pollens, molds, animal dander, cockroach, fungal spores, chemical irritants, cigarette smoke, diesel particles, ozone gas, and viral or bacterial infections (71, 74). Emotional or psychological conditions such as anger, fear, stress and physical exercise can trigger inflammatory responses of asthma (74). All these factors can lead to dysregulation of innate immune function and ER homeostasis (71, 74, 80). A summary of the link between UPR and asthma pathogenesis is illustrated in Figure 2.

Emerging literature suggests that UPR is involved in both innate and adaptive immune responses (102). The adaptive arm of the UPR restores protein folding in the ER while UPR signaling is essential in many immune cell functions such as differentiation of B cells to plasma cells, immunoglobulin production (103), antigen presentation (104, 105), cell development, and survival of both conventional and plasmacytoid dendritic cells (106, 107), immune cell phenotype (108). Further to this, XBP1 splicing is important during the early stages of both B and T cell development while it is enhanced in antigen specific CD8^{+} T cells during viral and bacterial infection (109). In summary, ER stress triggered UPR activation is a requirement for

key components of the immune system and impairment of UPR may result in various immune disorders including asthma.

SUMMARY and FUTURE DIRECTIONS

Recent evidence indicates that ER stress can be triggered by several common pathogens affecting the lungs. In recent years the effects of ER stress and activated UPR have attracted broad research interest both as a cause and consequence of airway diseases such as asthma (110-112). Continuous exposure of pulmonary cells to diverse environmental stimulants can activate UPR pathways specifically in the lung. IRE1β expressed by Goblet cells has an essential role in the production of MUC5B and MUC5AC mucins. This expression is associated with increased mucin secretion in these disorders. On the other hand, ATF6 increases the expression of ER Ca^{2+} pump (SERCA2), interfering with the proliferation of the ASM in asthma. ATF6 is upregulated in airway epithelial cells with Th2 cytokines (113, 114). OASIS is the other transcription factor that is expressed in high levels in the lung, affecting the production of collagen, lung repair, and differentiation of secretory cells. Recent investigations indicate that IRE1 plays an important role in the regulation of the secretome (115) which can also subsequently affect organisms' microenvironment. Thus, the activation of IRE1 can be a key component of disease immune-regulation relevant to asthma pathogenesis. Therefore, it will be very important to address IRE1 activation in infiltrated immune cells, lung epithelial cells, and lung mesenchymal cells to gain a better understanding of disease mechanisms. This will allow for the future development of targeted therapeutics in asthma.

Disruptions of ER stress/UPR also manifest in several other pulmonary disorders. As reported, there is increased expression of IRE1β in respiratory epithelial cells not only in asthma

but also in cystic fibrosis and chronic obstructive pulmonary disease (COPD). In COPD, there is an increase in the expression of UPR transcription factors, such as p-eIF2α, CHOP, and several proteins involved in ERAD pathway. Although two arms of ATF6 and IRE1 are not affected, the expression of these UPR transcription factors positively correlates with the degree and severity of airflow obstruction in lung diseases (116-118). In patients with idiopathic pulmonary fibrosis (IPF), there is enhanced expression of GRP78, XBP1, IRE1, ATF6, CHOP in type II alveolar epithelial cells as well as induction of ATF4, CHOP, and BAX (119-121).

Cigarette smoke (CS) affects the lung in many diverse ways and contributes to the development of asthma, COPD, IPF, and bronchiectasis, to name a few. CS alters protein metabolism and the UPR cascade via oxidative stress, which damages a variety of lung proteins irreversibly. Acute exposure to cigarette smoke causes an increase in the expression of GRP78, Calnexin, Calreticulin, ATF4, PERK, p-e IF2α, and CHOP. However, persistent consumption of CS just increases the expression of GRP78, Calnexin, Calreticulin in human lung cells. In general, smoking not only increases the loading of proteins in ER, but also degrades the ability of ER capacity (122, 123). Overall, while several *in vivo* and *in vitro* studies have shown the pro-fibrotic effect of ER stress, our understanding of the exact role of UPR in the pathogenesis of lung diseases remains limited with many gaps in our knowledge.

Many investigators are working to find ways to target ER stress and components of the UPR to help treat lung diseases and their complications. For instance, the chemical chaperones 4-phenyl butyric acid (4-PBA) and taurohyodeoxycholic acid (TUDCA) have beneficial effects in a mouse model of fibrosis (117, 122, 124). Recent work from Nakada et al. explores how therapies based on conjugated bile acids can attenuate allergen-induced allergic airway disease in mice while also inhibiting UPR via targeting binding of ATF6α (125). Given the biological

complexity of signalling pathways in cells, identifying a specific diagnostic biomarker could help advance novel therapies in lung diseases affected by ER stress/UPR.

A greater understanding of these cell fate processes may lead to deeper insights into cellular dysfunction that manifests as disease pathophysiology. Since the discovery of UPR, great advances in cell biology have been made, but we are just beginning to apply this knowledge to human disease. The uniqueness of cell fate mechanisms is that these are cellular programs that commit the cell to an outcome and have directionality. Therefore, they provide a unique avenue to modulate such processes via pharmacological (and potentially other non-pharmacological) interventions. In recent investigations, for example, the role of the UPR in regulating the secretome of cells has been widely investigated in cancer (115). Such new knowledge could inform the field of lung diseases in important ways, and in the case of asthma, lead to novel ways of targeting inflammatory responses that hinge on the modulation of ER stress/UPR.

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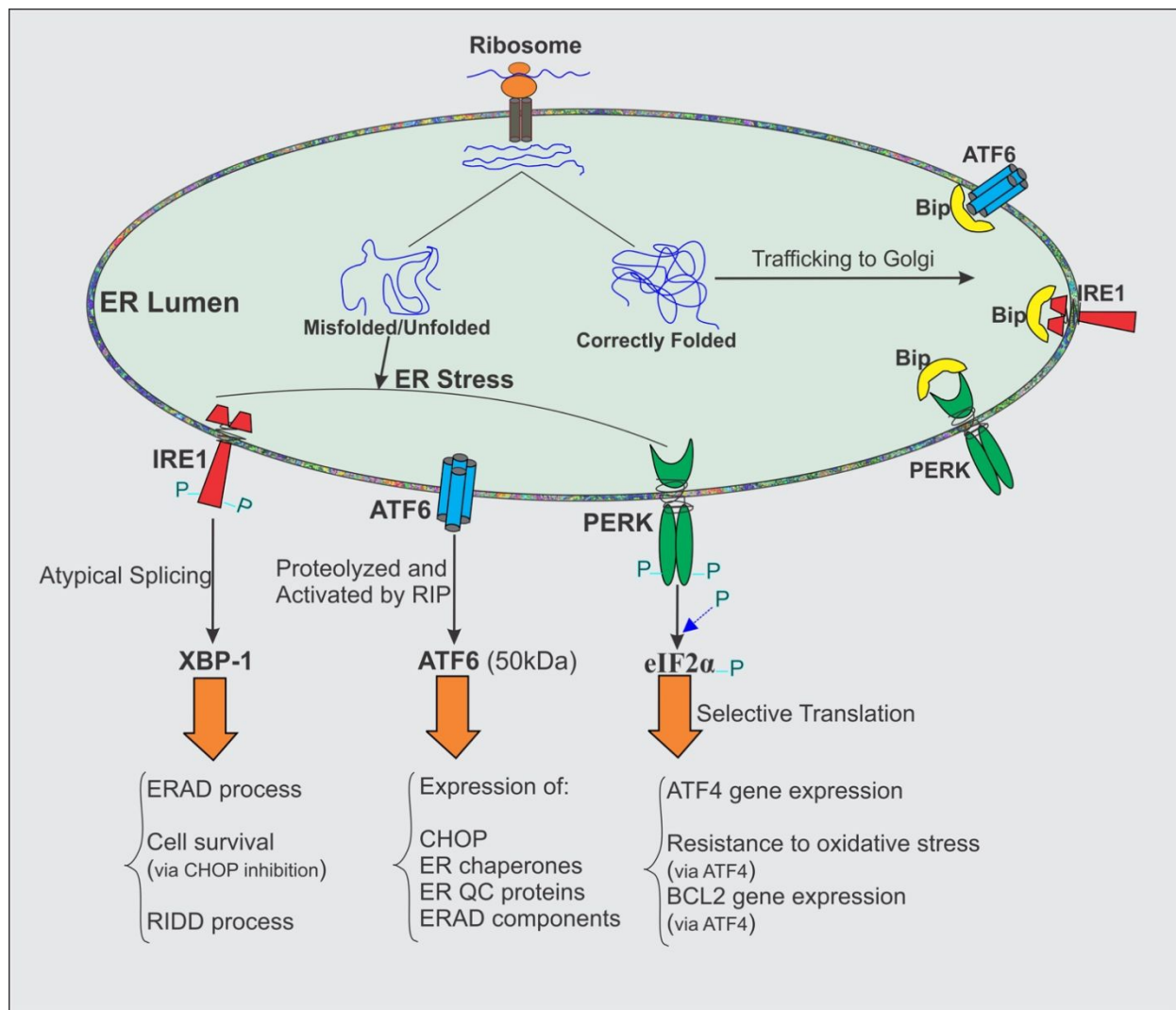
Figure 1. UPR Signaling Pathways. Proteins that are correctly folded in the endoplasmic reticulum (ER) are transferred to the Golgi for further modifications. Conversely, proteins that misfolded following synthesis promote ER stress and activate three independent pathways (three different UPR arms) driven by ATF6, PERK, and IRE1. This counteracts abnormal protein synthesis, folding, and modification. Each UPR arm activates specific downstream transcription factors that are involved in the regulation of cellular proliferation, apoptosis, and protein translation pathways.
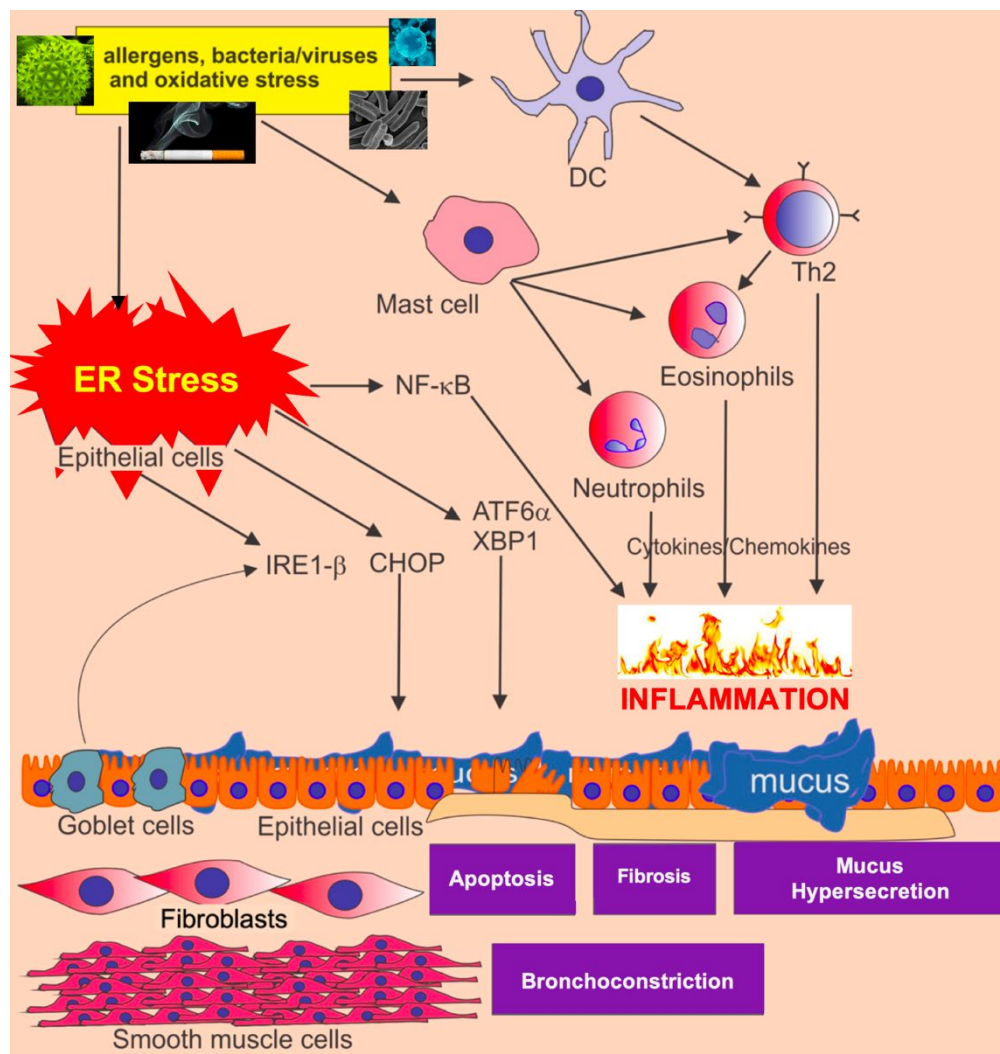
Figure 2. Asthma and UPR Crosstalk. Allergens and viral or bacterial infections induce eosinophilia and T helper type 2 (Th2 or type 2) inflammatory responses which could trigger asthma. Stimuli such as aeroallergens, oxidative stress, bacteria, pollutants, and viral infections can also disrupt ER integrity and act as inducers of ER. Pathogenic factors in asthma trigger ER stress and UPR pathways contributing to apoptosis and fibrosis in the lung. They also activate mast cells and Th2 cells to drive inflammation resulting in mucus hypersecretion, fibrosis, and bronchoconstriction following cytokine/chemokine release within the lung. *Abbreviations:* Dendritic cells (DCs), T helper (Th) cells, activating transcription factor-6 α (ATF6 α), CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP), Inositol-Requiring Enzyme-1 (IRE1), X-box-binding protein (XBP1), and Nuclear Factor Kappa B (NFκB).