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Subcellular Organelles in Immune Responses of Severe Asthma: The Roles of Mitochondria and Endoplasmic Reticulum

Yong Chul Lee and So Ri Kim

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Abstract

Subcellular organelles including mitochondria and endoplasmic reticulum are now considered as one major target for many therapeutic approaches. In fact, recent evidence has uncovered the roles of mitochondria as a direct inflammatory and immune controller and contributor to the diseases by metabolic dysfunction and/or their abnormal dynamics. In addition, one of the important subcellular organelles, endoplasmic reticulum, also plays as an immune responder in several diseases including bronchial asthma. Recently, we have reported that the endoplasmic reticulum stress and mitochondrial reactive oxygen species (ROS) contribute to the pathogenesis of steroid-resistant severe bronchial asthma through the modulation of immune responses such as production of regulatory cytokines and NLRP3 inflammasome activation. These findings indicate that the subcellular organelles and their complex can be a promising target for the development of novel therapeutic strategies including medicines to cure severe asthma. This chapter is aimed to present the state-of-art information regarding the role of subcellular organelles in severe asthma.

Keywords: subcellular organelles, mitochondria, endoplasmic reticulum, inflammasome, severe asthma

1. Introduction

Subcellular organelle is a specialized subunit within a cell that has a specific function. Individual organelles are usually separately enclosed within their own lipid bilayers [1]. Specifically in eukaryotic cells, the organelles include nucleus, mitochondrion, endoplasmic reticulum (ER), Golgi apparatus, peroxisome, and lysosome which are found in the cytoplasm, a viscous liquid

found within the cell membrane that houses the organelles and is the location of most of the action happening in a cell. Each organelle plays the specific functions such as DNA storage, energy production, production of lipid and proteins, export of the proteins from the cells, protein modification, and destruction of lipid and protein, respectively.

Among them, ER is the largest organelle in the cell and is a major site of protein synthesis and transport, protein folding, lipid and steroid synthesis, carbohydrate metabolism, and calcium storage [2–6]. One of the most prominent functions of the ER is protein synthesis. When ER is overloaded by increased demand in protein folding, cells initiate an adaptive response called unfolded protein response (UPR). In addition, the ER's secretory pathway of its products and the ER-associated degradation (ERAD) pathway try to keep the homeostasis with full activity [7, 8]. ER stress can be developed, if ER fails to overcome the overloads and UPR are not able to make the ER adapt to the stressful conditions, despite all ER's efforts and adaptive responses. Recent considerable studies have demonstrated that ER stress is associated with the pathogenesis of several diseases such as neurodegenerative disorders, metabolic disorders, cardiovascular diseases, malignancies, and respiratory disorders [9–12]. More specifically, in severe asthma or steroid-resistant asthma, the role of ER stress has been highlighted in terms of regulation and interaction of various signaling pathways linked to steroid resistance [13]. In addition, nowadays, changes of ER shapes and structure responded to ER stress are emerging as one of pathogenic mechanisms regarding several disorders [14].

Mitochondria are energy-producing organelles which are dynamic and possess mitochondrial own DNA distinct from nuclear genome [15]. Basically, they are in charge of the synthesis and catabolism of metabolites, generation and detoxification of reactive oxygen species (ROS), apoptosis, regulation of calcium, and generation of adenosine triphosphate (ATP) by oxidative phosphorylation [16]. Recently, a novel role for mitochondria has been revealed in various disorders such as infectious diseases, neurodegenerative diseases, cerebrovascular diseases, and metabolic diseases, especially in the association of innate immune and inflammatory responses [16–19]. In addition, our recent study has revealed that exceed generation of mitochondria ROS and alteration of mitochondrial DNA induced steroid-resistant neutrophilic asthmatic features through the activation of NLRP3 inflammasome in mice [20]. More interestingly, mitochondria are highly motile organelles. In fact, we know that mitochondria actively travel along the microtubule network in neurons and accumulate at sites of high-energy demands [21]. These mitochondrial dynamics and morphological changes are through constitutive cycles of fusion and fission [22]. Nowadays, impaired processes of mitochondrial dynamics have been accepted as a pathogenic contributor to various disorders, including lung diseases [23, 24].

The last decades have witnessed an explosion in the elucidation of the causative mechanisms implicated in bronchial asthma, especially severe or steroid-resistant asthma; however, the treatment of asthmatic patients is still challenging. One of the reasons can be that many newly developed therapeutic tools are single-targeted and linked to the shortage of broad clinical effect, although they might be effective in asthmatic patients with specific phenotypes. On the other hand, drugs with more widespread effects (e.g., kinase inhibitors) might be more effective pharmacologically, whereas the potential risk of side effects might increase [25].

Therefore, novel treatments should be considered to target the aspects of the multiple underlying allergic/immune/inflammatory processes and minimize the adverse effects on other systems. In terms of this point, targeting subcellular organelles, especially ER and mitochondria, has a strong competitive power for the development of future medications of asthma, especially steroid-resistant asthma, since restoration of their abnormal function to normal physiologic status of each subcellular organelle is going without serious adverse effects unlikely to the existing therapeutic approach of blocking or eliminating the pathogenic targets.

2. Severe asthma and its heterogeneity

For many years, the term severe asthma has been used interchangeably with other similar terms, and considerable effort has been concentrated to be uniform in the term and concept. Several academic societies and research groups have suggested the definition of severe asthma such as European Respiratory Society (ERS), American Thoracic Society (ATS), World Health Organization (WHO), and British Thoracic Society (BTS)/Scottish Intercollegiate Guideline Networks (SIGN) [26–30]. In various definitions of severe asthma with little differences, there is a common ground that severe asthma can be defined as a failure to achieve control with maximum doses of inhaled corticosteroid therapies [31]. Taken together, severe asthma contains the existing disease entities; steroid-insensitive asthma, steroid-resistant asthma, difficult asthma, and refractory asthma, and these subsets of asthmatics have been estimated up to 5–10% of all asthmatics.

Although there are still many different definitions for severe asthma available and difficulties in making an accurate definition for severe asthma, numerous data based on these definitions consistently demonstrate the heterogeneity of severe asthma in populations with asthma [32, 33]. In fact, in 2001, the National Heart, Lung, and Blood Institute initiated the Severe Asthma Research Program (SARP) to identify and characterize not only a large number of subjects with severe asthma but also to compare these subjects with those with mild to moderate asthma [34]. In the SARP adult clinical cluster analysis, five different groups of subjects with asthma were identified who differ in clinical and pathophysiologic parameters [33]. These five asthma phenotypes differ in lung function, age of asthma onset and duration, atopy, sex, symptom frequency, medication use, and health-care utilization. Clusters 1, 2, and 4 reflect the spectrum of allergic asthma from mild to severe airflow obstruction. The majority of these patients have early-onset disease, with history of atopy confirmed by skin prick testing and elevated total serum IgE. By contrast, Clusters 3 and 5 reflect the spectrum of adult-onset asthma characterized by older patients with less atopy, yet, high health-care utilization and poor quality of life [33]. The SARP clinical heterogeneity, even in severe asthma group, can provide a basis for the needs to investigate the different molecular and biological mechanisms and different therapeutic approaches for the patients with severe asthma. In addition, SARP cluster analysis revealed inflammatory heterogeneity through the evaluation of blood and sputum inflammatory cells. In addition, the data suggested that sputum eosinophils were increased in Cluster 4 subjects with severe allergic asthma, whereas both eosinophils and neutrophils were increased in subjects from Cluster 5 [33]. A sequential study has reported that

grouping of subjects based on their sputum inflammatory cell profile identified four groups of subjects with distinguishing clinical characteristics [35]. For instance, patients showing both eosinophil ($\geq 2\%$) and neutrophil ($\geq 40\%$) predominant pattern, called as mixed granulocytic pattern, had the most severe asthma with severe chronic airflow obstruction and increased symptoms with high health-care utilization. In addition, according to this paradigm, all five clinical clusters of SARP showed all four patterns of sputum inflammatory profiles without a dominant pattern in any one cluster [34]. The lack of association between the clinical clusters and sputum inflammatory cell patterns does not only make the heterogeneity of severe asthma more complex but also emphasize that future analyses must incorporate clinical, physiologic, and inflammatory measures into one analysis [36].

Very recently, an interesting study of cluster analysis data has been released using U-BIOPRED (Unbiased BIOmarkers in PREDiction of respiratory disease outcomes) severe asthma cohort [37]. In this study, three transcriptome-associated clusters (TACs) were defined: TAC1 characterized by immune receptors IL33R, CCR3, and TSLPR, TAC2 characterized by interferon-, tumor necrosis factor (TNF)- α , and inflammasome-associated genes, and TAC3 characterized by genes of metabolic pathways, ubiquitination, and mitochondrial function. Subjects with severe asthma were classified into these three clusters based on their sputum transcriptomics data. Each TAC group exhibits their own differential clinical features: one Th2-high eosinophilic phenotype TAC1 and two non-Th2 phenotypes TAC2 and TAC3, characterized by inflammasome-associated and metabolic/mitochondrial pathways, respectively. This analytic approach is unlikely to previous ones such as SARP which showed the lack of association between the clinical clusters and sputum inflammatory cell patterns. Considering that clustering using clinical features alone has not yielded information on the underlying biology as similar inflammatory cell profiles have been seen between these clinical clusters [34], this study is worthy to approach with the unconventional direction from inflammatory or biologic clustering to clinical phenotyping. This approach provides a fresh framework on which to phenotype asthma and a more precise targeting of specific treatments [38]. Specifically, the development of novel medications has been poorer, targeting non-Th2 or non-type-2 severe asthma than Th2 or Type-2 severe asthma. In terms of this issue, this novel-clustering analysis data are expected to be helpful for the development of the medicines targeting non-type 2 asthmatics. In fact, two non-Th2 phenotypes TAC2 and TAC3 are associated with inflammasome and mitochondrial pathway, respectively. In addition, while the majority of subjects in TAC2 group show neutrophilic predominant inflammatory pattern, the subjects in TAC3 group can be further divided into paucigranulocytic, eosinophilic, and neutrophilic pattern subgroups. Interestingly, the differential characteristic of eosinophilic TAC3 subjects from Th2-type TAC1 subjects is the elevated levels of inflammasome, suggesting that non-Th2 type asthma can also have eosinophilic-dominant inflammation partly through the activation of inflammasome.

Considering nowadays the concept of severe asthma and heterogeneity, the improvement of the detailed characterization of the patients is required to achieve appropriate therapeutic responses for severe asthma. It is expected that the correct determination of phenotype and molecular endotype leads to more effective precision medicine for severe asthma.

3. ER stress in severe asthma

As introduced, ER is a specialized organelle that plays as an important regulator of protein homeostasis in cells of an organism. The ER is rich in chaperones and enzymes that help to fold the protein properly. ER chaperones and enzymes are fragile to various stresses; thus several stressful or pathologic conditions (e.g., disease situation) may lead to the impaired ER protein-folding capacity leading to the accumulation of misfolded and unfolded proteins in the ER lumen. This out-of-controlled state of ER is usually called as ER stress [13, 39, 40].

Three ER transmembrane sensors are inositol-requiring enzyme 1 α (IRE1 α), double-stranded RNA-dependent protein kinase (PKR)-like ER kinase (PERK), and activating transcription factor 6 (ATF6). The functions of the ER membranous proteins include monitoring protein homeostasis of ER lumen and activation of canonical UPR pathways to deliver the information on the ER status to cytoplasm [40, 41]. According to the classic model of the activation of UPR, in basal conditions, these three transmembrane proteins are bound by a chaperone, BiP/glucose-regulated protein 78 (GRP78) [42, 43]. The development of ER stress causes the separation of BiP from these UPR sensors bound. The activation of IRE1 α and PERK is associated with the dimerization and auto-phosphorylation, while in case of ATF6, its translocation to the Golgi is required to get activated [7]. Activated forms of proteins mitigate ER stress through the reduction of protein synthesis, the enhancement of protein degradation, and the induction of production of ER chaperones. When the protective process fails to resolve ER stress, the cell is prepared for apoptosis which is also one of the biological protective mechanisms [44]. Recently, in addition to these canonical UPR, noncanonical aspects of UPR confer cells to interconnect protein homeostasis-related cellular apparatus to a wide array of cellular events including immunity and inflammation through various mechanisms, as substantially reviewed elsewhere [45, 46].

In addition, the complex roles of ER including protein synthesis and lipid synthesis, calcium regulation, and interactions with other organelles are reflected in an equally complex physical architecture. The ER is composed of a continuous membrane system that includes the nuclear envelope and the peripheral ER, defined by flat sheets and branching tubules [14]. While it is generally known how the basic shapes of ER sheets and tubules are determined, it is relatively unclear how changes in the shape or the ratio of sheets to tubules occur in response to specific cellular signals. In several conditions, increasing ER loads and ER stress such as mitosis, changes of ER structure, and shapes are noted. In fact, recent studies showed that splicing of XBP1 is activated during meiosis in both *Xenopus* and budding yeast [47, 48], suggesting that changes in ER structure during meiosis could be linked to the ER stress response. However, to date, it is remained unclear whether ER stress induces immediate restructuring of ER or not. In the same vein, it has not yet been determined whether the activation of ER stress-responsive-signaling pathways results in a modification of structural components of the ER [14].

Accumulating data have indicated that ER stress and UPR link to major inflammatory and stress-signaling networks including the nuclear factor kappa B (NF- κ B) pathway and oxidative stress. Recent studies have unveiled the role of ER stress in the pathogenesis of various

pulmonary disorders, including asthma, chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis, and acute lung injury [9, 49–52].

As for bronchial asthma including severe form, the role of ER stress has been reviewed elsewhere [13, 40, 52]. In particular, neutrophilic steroid-resistant severe asthma animal model, which is similar to human TAC2 group, exhibited the significant increases in ER stress markers, GRP78 and CCAAT/enhancer binding protein-homologous protein (CHOP), as well as UPR-related proteins in lung tissues and BAL cells [9]. The mice showed typical asthmatic manifestations including bronchial hyperresponsiveness and airway inflammation which were not attenuated by the treatment with oral dexamethasone. Intriguingly, an ER stress regulator, 4-phenylbutyric acid (4-PBA), effectively attenuated steroid refractory asthmatic features as well as increases in ER stress linked to NF- κ B activation which induces various severe inflammatory/immune responses in the lung. In addition, 4-PBA dramatically reduced the increased expression of IL-17, while it further enhanced the increase in IL-10 levels, leading to the attenuation of steroid-resistant asthmatic features. In another recent study using the same animal model [20], the activation of NLRP3 inflammasome was measured. The results revealed that NLRP3 inflammasome was significantly activated in lung tissues and BAL cells from neutrophilic-dominant severe asthma murine model and that the severe asthmatic symptoms were dramatically attenuated by the blockade of IL-1 β which is one of major effector cytokines by NLRP3 inflammasome activation. A more recent study using another neutrophilic-dominant steroid-resistant asthma animal model and human data also has revealed the significant role of NLRP3 inflammasome in the pathogenesis of neutrophilic-dominant severe asthma [53]. These findings suggest that the neutrophilic-dominant steroid-resistant or severe asthma animal models can represent the TAC2 group of human severe asthma characterized by IFN, TNF- α , and inflammasome-associated genes and that ER stress can be more associated with this clustered asthma. A recent study has also demonstrated that ER stress inducer, tunicamycin, aggravates ER stress in mouse bronchial epithelial cells and increased the expression of inflammation indicators such as IL-6, IL-8, and TNF- α in lung tissues of neutrophilic severe asthmatic mice [54]. The double-stranded RNA (dsRNA)-activated serine/threonine kinase R (PKR) is well characterized as an essential component of the innate antiviral response. In view of the relation with ER stress, PKR phosphorylates e-IF2 α , one of the branches for UPR, and at the same time, ER stress activates PKR which stimulates various inflammatory-signaling pathways [55, 56]. With this background, a recent interesting study showed that poly (I:C)-induced exacerbation of neutrophilic severe asthmatic mice was closely associated with PKR phosphorylation as well as increased ER stress in lung tissues including bronchial epithelial cells [56].

In addition to neutrophilic severe asthmatic phenotype, eosinophil-dominant severe asthma with fungal sensitization also showed the significant elevation of ER stress in mice [57]. In this study, *Aspergillus fumigatus* extract-inhaled mice showed typical asthmatic manifestations including eosinophilic airway inflammation and airway hyperresponsiveness which were not responded to treatment with oral steroid, while all asthmatic features and increased ER stress were very well controlled by 4-PBA, suggesting that ER stress is linked to the pathogenesis of eosinophilic-dominant severe asthma as well as neutrophilic-dominant one. Meanwhile, this animal model appeared to represent the TAC3 human severe asthma group. As described

earlier, TAC3 is characterized by genes of metabolic pathways, ubiquitination, and mitochondrial function, and the subjects of TAC3 exhibit various inflammatory cell types including paucigranulocytic, eosinophilic, and neutrophilic pattern subgroups. Thus, this fungal extract-inhaled eosinophilic severe asthma murine model can be considered as an eosinophilic pattern TAC3, non-Th2 eosinophilic asthma. Actually, in this study, mitochondrial ROS was significantly increased in the lung from *A. fumigatus* extract-inhaled mice [57].

These observations suggest that ER stress plays a critical role in the pathogenesis of various phenotypes of severe asthma including neutrophilic, eosinophilic, and viral infection-related types, supporting that the ER stress-targeting strategy seems to be able to overcome the steroid resistance in severe asthma.

4. Mitochondria in severe asthma

Asthma is characterized by ongoing inflammation and accompanied by increased oxidative stress and subsequent lung injury. ROS production, which leads to oxidative stress, is one of critical features in chronic airway disorders [58]. Two major sources of ROS induced by external stimuli are mitochondria and the Nox family of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in vivo. Besides, the mitochondrial respiratory chain is considered to be an important part of ROS production within most cells [59]. In addition, the considerable interplay between mitochondria and ER in several respiratory disorders has been demonstrated [59, 60]. In fact, fungal extract-inhaled mice exhibiting features of eosinophilic severe asthma or representing eosinophilic TAC3 showed significant increases in the production of mitochondrial ROS in BAL cells [57]. *A. fumigatus* extract-stimulated tracheal epithelial cells from the mice also markedly more generated mitochondrial ROS compared to the control cells [57]. In addition, treatment with NecroX-5, a potent mitochondrial ROS inhibitor, effectively attenuated increases in mitochondrial ROS in BAL cells, reduced increases in GRP78 and CHOP in lung tissues from *A. fumigatus* extract-inhaled mice, and ameliorated pathophysiologic features of fungal extract-induced eosinophilic severe asthma. In addition to eosinophilic severe asthma, recent experimental data using ovalbumin (OVA) and lipopolysaccharide (LPS)-sensitized and OVA-challenged mice (OVA-LPS mice) representing TAC2 revealed that the increased generation of mitochondrial ROS and the alteration of mitochondrial DNA induced steroid-resistant neutrophilic asthmatic features through the activation of NLRP3 inflammasome in lung and that the restoration of mitochondrial ROS levels using mitochondrial-specific ROS scavenger dramatically attenuated steroid-resistant airway hyperresponsiveness and inflammation in mice [20]. These findings suggest that mitochondrial metabolic dysfunctions such as mitochondrial ROS generation and mitochondrial DNA damage are linked to other subcellular organelles (e.g., ER) and immunologic complex (e.g., inflammasome) in the pathogenesis of steroid-resistant asthma and that mitochondrial ROS plays a key role in the induction and maintenance of neutrophilic and eosinophilic steroid-resistant severe asthma. As supporting data, when N-acetylcysteine (NAC), which is a representative conventional antioxidant, was administered to both types of steroid-resistant severe asthma murine models, for example, eosinophilic and neutrophilic types, NAC did not affect

steroid-resistant asthmatic features, mitochondrial ROS generation, and NLRP3 inflammasome activation (unpublished data), suggesting the importance of the role of mitochondrial ROS generation in the pathogenesis of steroid-resistant severe asthma as well as one of causes for the previous failures in the clinical trials for asthma patients to evaluate the effects of various conventional antioxidants.

Like this, mitochondria perform many roles beyond energy production, including the generation of ROS, redox molecules, and metabolites; regulation of cell signaling and cell death; and biosynthetic metabolism [61–63]. Thanks to these observations, mitochondria have recently become a promising target for the treatment of various inflammatory disorders, including bronchial asthma. However, most studies regarding mitochondria as a pathogenic contributor have dealt with mitochondrial metabolic dysfunction or mitochondrial genetic abnormality. As introduced, mitochondria are not static, highly dynamic in cells, and change the morphology.

Mitochondrial morphology is controlled by large guanosine triphosphatases in the dynamin family [64]. Among them, mitofusins 1 and 2 (MFN-1 and MFN-2) and optic atrophy protein 1 (OPA-1) are essential mediators of mitochondrial fusion [65]. By contrast, fission requires dynamin-related protein 1 (DRP-1) to be recruited from the cytosol to the mitochondrial surface [66]. Mitochondrial fission is known to be prevalent in diseased cells, with subsequent elimination of damaged mitochondria via mitophagy [67]. By contrast, mitochondrial fusion inhibits apoptotic cell death [22]. In fact, several reports have demonstrated that increased mitochondrial fission and decreased fusion are observed in cells from various lung diseases such as lung cancer [68, 69]. Interestingly, our preliminary data have revealed that mitochondrial dynamics were out of control in *A. fumigatus* extract-inhaled mice, and the restoration of abnormal mitochondrial dynamics could attenuate the steroid-resistant airway inflammation and airway hyperresponsiveness (unpublished data), providing the novel concept that a therapeutic strategy targeting mitochondrial dynamics can overcome steroid resistance in severe asthma. However, we are only beginning to evaluate and understand the related mechanisms and the role of mitochondrial dynamics in the pathogenesis of severe asthma. More future researches and studies are needed to support the role of mitochondria in the pathogenesis of severe asthma. In addition, the identification of the specific phenotype and/or endotype related to mitochondrial metabolic and morphologic dysfunction is eagerly required for the patient-oriented treatment or the precision medicine of severe asthma.

5. Potential clinical biomarkers and therapies related to mitochondria and ER

Biomarkers may facilitate the diagnosis and classification of severe asthma, predict efficacy of specific therapies, and assess medication response. Based on the data, to date, there are some potential biomarkers related to ER, mitochondria, and inflammasome in severe asthma. More specifically, the biomarker candidates are considered as the biomarkers for the prediction of efficacy of the subcellular organelle targeting therapies and for assessment therapeutic responses.

In fact, ER stress markers, GRP78 and CHOP, have been measured in BAL fluid from asthmatic patients [9]. Very interestingly, the levels were increased in BAL fluid from asthmatics compared to the levels from the healthy persons. The asthmatics were composed of patients who had been diagnosed and treated for asthma for more than 3 months with inhaled corticosteroid or combined inhaled corticosteroid and beta- β_2 -agonist. In addition, the patients exhibited uncontrolled asthmatic symptoms scored below 19 points by asthma control test (ACT) scoring system despite the standard treatment including inhaled corticosteroid. Although the protein expression levels of GRP78 and CHOP were not correlated with the lung function, the protein expression reflected the asthmatic-controlled status in humans supported by the data from animal experiments, in which steroid-responded asthmatic mice showed the decrease in the expression levels of GRP78 and CHOP in lung tissues by the treatment of steroid, while the steroid-resistant asthmatic mice were refractory to the treatment with steroid in terms of the protein levels. When an ER stress inhibitor, 4-PBA, was administered to the steroid-resistant asthmatic mice, the levels of GRP78 and CHOP were substantially reduced in lung tissues and BAL cells with the attenuation of asthma symptoms [9]. These findings suggest the potential of the use of GRP78 and CHOP as biomarkers, classifying the patients into steroid-responsive group and steroid-resistant group after the standard treatment including inhaled corticosteroid as well as predicting or monitoring the therapeutic responses of ER stress inhibitor as a medication for severe asthma.

Mitochondrial ROS can be another biomarker candidate. In asthma, there is an elevated airway expression of products of oxidative stress. Actually, exhaled breath condensate levels of oxidative stress-related biomarkers, such as hydrogen peroxide (H_2O_2), nitrite/nitrate, 8-isoprostane, and others vary with asthma exacerbations, disease severity, and medication use [70]. As mentioned earlier, mitochondrial ROS may be a more critical player in the pathogenesis of severe asthma compared to general or total cellular ROS generation. Nowadays, several tools including simple detection kits and staining indicators have been introduced for measuring the specific mitochondrial ROS levels which distinct from the total cellular ROS generation in vivo. Thus, in addition to cellular ROS, mitochondrial ROS in various biological samples such as exhaled breath condensate, sputum, and BAL fluid can be expected to be one of biomarkers of the next generation for the diagnosis of severe or steroid-resistant asthma. Moreover, the studies using recently developed mitochondrial ROS inhibitor, NecroX compounds, have reported the excellent efficacy of this chemical as a potent and specific mitochondria-targeted antioxidant in several disease models [71–75]. Even in human studies, a phase II clinical trial is currently being performed to evaluate the efficacy, safety, and pharmacokinetics of intravenous injection of NecroX-7 immediately before percutaneous coronary intervention in patients with myocardial infarction (ClinicalTrials.gov; NCT02770664). Therefore, it can be hypothesized to consider that NecroX compounds may be developed as a novel therapeutic agent to control or cure the steroid-resistant severe asthma in future.

Furthermore, mitochondrial ROS is closely associated with the assembly of inflammasome, specifically NLRP3 inflammasome, which is formed by various stimuli in the inflammatory state. NLRP3, one of the cytosolic pattern recognition receptors, plays as one of the components of the inflammasome and recognizes a variety of inflammatory stimuli, pathogen-associated molecular pattern molecules (PAMPs), and damage-associated molecular pattern molecules (DAMPs) in cells. Subsequently, the assembled and activated NLRP3 inflammasome controls the production of important pro-inflammatory cytokines such as IL-1 β and IL-18 [76]. Two

common events that are required for these activators of the NLRP3 inflammasome are potassium efflux and ROS generation [77]. Recent interesting studies have revealed that steroid-resistant neutrophilic asthmatic manifestations were significantly controlled by the NLRP3 inflammasome activation, and the severe asthmatic symptoms were dramatically attenuated by the blockade of IL-1 β or inflammasome inhibitor, MCC950 [20, 53]. Moreover, increased NLRP3 and IL-1 β sputum gene expression were strongly associated with increasing asthma severity in humans, suggesting that the NLRP3 inflammasome is important in human disease as well [53]. In addition, the protein expression levels of NLRP3 and caspase-1 were more increased in BALF from uncontrolled asthmatics compared to healthy subjects [20]. Taken together, these data suggest the potential of NLRP3, IL-1 β , or caspase-1 to use as diagnostic and therapeutic biomarkers in respiratory specimens and urge to perform the translational or clinical studies regarding this issue. In addition, until now, there are no interventional clinical data applying the agents targeting NLRP3 inflammasome such as MCC950 in steroid-refractory severe asthma; however, it can be a very promising target for the control of severe asthma.

Altogether, it is clear that there are huge needs for further researches and future translational and clinical studies to use the candidate markers and therapeutic agents related to subcellular organelles in clinical practice.

6. Conclusion and future directions

Severe asthma is characterized by uncontrolled symptoms and recurrent exacerbation with excessive chronic airway inflammation despite adequate and even maximum treatment with the current medications. Although multiple factors can cause poor responses and underlying pathogenic differences are being revealed explaining the various therapeutic responses including steroid insensitivity, effective therapeutic modalities for severe asthma still remain as a major unmet need [78]. To overcome these current obstacles, cluster analysis and research for the heterogeneity of severe asthma are actively ongoing. Recent unconventional approach to define the clusters of subjects with severe asthma using TACs seems to be more helpful for the development of precision medicine for severe asthma compared to conventional clinical feature-based clusters, although more future supporting researches are needed. In addition, the heterogeneity of severe asthma is going to be more complex as the cluster analytic tools are advanced.

Recently, accumulating findings suggest that the regulation of ER stress and the restoration of mitochondrial dysfunction are prospective molecular therapeutic approaches for various pulmonary disorders including bronchial asthma. More encouragingly, the inhibition of ER stress overcomes the failure of steroid in attenuating the severe asthmatic features of mice including non-Th2 neutrophilic type (similar to TAC2) as well as the eosinophilic type (similar to TAC3). Furthermore, as we described earlier, therapeutic approach to control ER stress is able to regulate multiple integrated signaling networks concomitantly known as the famous pathogenic mechanisms for steroid-resistant inflammatory responses. In addition, the link between ER stress and mitochondrial ROS generation is very interesting in severe asthma.

Interestingly, in non-Th2 neutrophilic severe asthma, NLRP3 inflammasome assembly is activated and consequently induces IL-1 β production and release. In addition, mitochondrial

ROS generation and the mitochondrial DNA damage are closely associated with NLRP3 inflammasome activation in this animal model of severe asthma. Meanwhile, in non-Th2 eosinophilic steroid-resistant asthma induced by fungal extract, mitochondrial dysfunction on their dynamics or morphology as well as ROS generation is observed, which resulted in steroid-resistant airway inflammation and hyperresponsiveness in mice. Therefore, the restoration and inhibition of mitochondrial dysfunction can be a novel promising target for the therapeutics of severe asthma. Considering the link among ER, mitochondria, and inflammasome, their interconnection can be suggested as a more powerful tool for the control of severe asthma (**Figure 1**).

Despite success in mice, to date, there is the shortage of information on molecular mechanisms, explaining these effects of the control for ER stress and mitochondria, and there are also no clinical trials that evaluate the therapeutic effects of the pharmacologic agents targeting subcellular organelles in humans. In addition, since the subcellular organelles play essential roles in the body, the adverse effects of the pharmacologic intervention targeting ER or mitochondria must be considered. However, as for the adverse effects, since this therapeutic approaching concept is aimed to restore the stressful or dysfunctional condition into the physiologic levels, not to block the function or to null, it seems to be superior to other new therapeutics pursuing the single specific target blockade.

In conclusion, the restoration of subcellular organelles in a disease state is a potentially exciting target for developing agents to achieve better management of severe asthma in which steroids and other current agents are less effective.

Figure 1. Schematic diagram of the possible interconnection among endoplasmic reticulum, mitochondria, and inflammasome in the pathogenesis of steroid-resistant asthma.

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Conflict of interest

The authors have declared that no conflict of financial interest exists.

Author details

Yong Chul Lee and So Ri Kim*

*Address all correspondence to: sori@jbnu.ac.kr

Division of Respiratory Medicine and Allergy, Department of Internal Medicine, Chonbuk National University Medical School, Jeonju, South Korea

References

- [1] Kerfeld CA, Sawaya MR, Tanaka S, Nguyen CV, Phillips M, Beeby M, et al. Protein structures forming the shell of primitive bacterial organelles. *Science*. 2005;**309**(5736):936-938
- [2] Reid DW, Nicchitta CV. Diversity and selectivity in mRNA translation on the endoplasmic reticulum. *Nature Reviews. Molecular Cell Biology*. 2015;**16**(4):221-231
- [3] Rapoport TA. Protein translocation across the eukaryotic endoplasmic reticulum and bacterial plasma membranes. *Nature*. 2007;**450**(7170):663-669
- [4] Braakman I, Hebert DN. Protein folding in the endoplasmic reticulum. *Cold Spring Harbor Perspectives in Biology*. 2013;**5**(5):a013201
- [5] Clapham DE. Calcium signaling. *Cell*. 2007;**131**(6):1047-1058
- [6] Westrate LM, Lee JE, Prinz WA, Voeltz GK. Form follows function: The importance of endoplasmic reticulum shape. *Annual Review of Biochemistry*. 2015;**84**:791-811
- [7] Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nature Reviews. Molecular Cell Biology*. 2007;**8**(7):519-529
- [8] Kim I, Xu W, Reed JC. Cell death and endoplasmic reticulum stress: Disease relevance and therapeutic opportunities. *Nature Reviews. Drug Discovery*. 2008;**7**(12):1013-1030

- [9] Kim SR, Kim DI, Kang MR, Lee KS, Park SY, Jeong JS, et al. Endoplasmic reticulum stress influences bronchial asthma pathogenesis by modulating nuclear factor kappaB activation. *The Journal of Allergy and Clinical Immunology*. 2013;**132**(6):1397-1408
- [10] Toth A, Nickson P, Mandl A, Bannister ML, Toth K, Erhardt P. Endoplasmic reticulum stress as a novel therapeutic target in heart diseases. *Cardiovascular & Hematological Disorders Drug Targets*. 2007;**7**(3):205-218
- [11] Kelsen SG, Duan X, Ji R, Perez O, Liu C, Merali S. Cigarette smoke induces an unfolded protein response in the human lung: A proteomic approach. *American Journal of Respiratory Cell and Molecular Biology*. 2008;**38**(5):541-550
- [12] Poppek D, Grune T. Proteasomal defense of oxidative protein modifications. *Antioxidants & Redox Signaling*. 2006;**8**(1-2):173-184
- [13] Kim SR, Lee YC. Endoplasmic reticulum stress and the related signaling networks in severe asthma. *Allergy, Asthma & Immunology Research*. 2015;**7**(2):106-117
- [14] Schwarz DS, Blower MD. The endoplasmic reticulum: Structure, function and response to cellular signaling. *Cellular and Molecular Life Sciences*. 2016;**73**(1):79-94
- [15] Emelyanov VV. Mitochondrial connection to the origin of the eukaryotic cell. *European Journal of Biochemistry*. 2003;**270**(8):1599-1618
- [16] Cloonan SM, Choi AM. Mitochondria: Commanders of innate immunity and disease? *Current Opinion in Immunology*. 2012;**24**(1):32-40
- [17] Akundi RS, Huang Z, Eason J, Pandya JD, Zhi L, Cass WA, et al. Increased mitochondrial calcium sensitivity and abnormal expression of innate immunity genes precede dopaminergic defects in Pink1-deficient mice. *PLoS One*. 2011;**6**(1):e16038
- [18] d'Avila JC, Santiago AP, Amancio RT, Galina A, Oliveira MF, Bozza FA. Sepsis induces brain mitochondrial dysfunction. *Critical Care Medicine*. 2008;**36**(6):1925-1932
- [19] Hopps E, Noto D, Caimi G, Averna MR. A novel component of the metabolic syndrome: The oxidative stress. *Nutrition, Metabolism, and Cardiovascular Diseases*. 2010;**20**(1):72-77
- [20] Kim SR, Kim DI, Kim SH, Lee H, Lee KS, Cho SH, et al. NLRP3 inflammasome activation by mitochondrial ROS in bronchial epithelial cells is required for allergic inflammation. *Cell Death & Disease*. 2014;**5**:e1498
- [21] Birsa N, Norkett R, Higgs N, Lopez-Domenech G, Kittler JT. Mitochondrial trafficking in neurons and the role of the Miro family of GTPase proteins. *Biochemical Society Transactions*. 2013;**41**(6):1525-1531
- [22] Westermann B. Mitochondrial fusion and fission in cell life and death. *Nature Reviews. Molecular Cell Biology*. 2010;**11**(12):872-884
- [23] Archer SL. Mitochondrial dynamics—Mitochondrial fission and fusion in human diseases. *The New England Journal of Medicine*. 2013;**369**(23):2236-2251

- [24] Aravamudan B, Thompson MA, Pabelick CM, Prakash YS. Mitochondria in lung diseases. *Expert Review of Respiratory Medicine*. 2013;**7**(6):631-646
- [25] Barnes PJ. New therapies for asthma: Is there any progress? *Trends in Pharmacological Sciences*. 2010;**31**(7):335-343
- [26] Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *The European Respiratory Journal*. 2014;**43**(2):343-373
- [27] Chung KF, Godard P, Adelroth E, Ayres J, Barnes N, Barnes P, et al. Difficult/therapy-resistant asthma: The need for an integrated approach to define clinical phenotypes, evaluate risk factors, understand pathophysiology and find novel therapies. ERS Task Force on Difficult/Therapy-Resistant Asthma. European Respiratory Society. *The European Respiratory Journal*. 1999;**13**(5):1198-1208
- [28] Proceedings of the ATS workshop on refractory asthma: Current understanding, recommendations, and unanswered questions. American Thoracic Society. *American Journal of Respiratory and Critical Care Medicine*. 2000;**162**(6):2341-2351
- [29] Bousquet J, Mantzouranis E, Cruz AA, Ait-Khaled N, Baena-Cagnani CE, Bleecker ER, et al. Uniform definition of asthma severity, control, and exacerbations: Document presented for the World Health Organization Consultation on Severe Asthma. *The Journal of Allergy and Clinical Immunology*. 2010;**126**(5):926-938
- [30] The 2016 BTS/SIGN British guideline on the management of asthma. <https://www.brit-thoracic.org.uk>
- [31] Barnes PJ. Severe asthma: Advances in current management and future therapy. *The Journal of Allergy and Clinical Immunology*. 2012;**129**(1):48-59
- [32] Wu W, Bleecker E, Moore W, Busse WW, Castro M, Chung KF, et al. Unsupervised phenotyping of Severe Asthma Research Program participants using expanded lung data. *The Journal of Allergy and Clinical Immunology*. 2014;**133**(5):1280-1288
- [33] Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *American Journal of Respiratory and Critical Care Medicine*. 2010;**181**(4):315-323
- [34] Moore WC, Fitzpatrick AM, Li X, Hastie AT, Li H, Meyers DA, et al. Clinical heterogeneity in the severe asthma research program. *Annals of the American Thoracic Society*. 2013;**10**(Suppl):S118-S124
- [35] Hastie AT, Moore WC, Meyers DA, Vestal PL, Li H, Peters SP, et al. Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. *Journal of Allergy and Clinical Immunology*. 2010;**125**(5):1028-1036 e13
- [36] Kupczyk M, Wenzel S. U.S. and European severe asthma cohorts: What can they teach us about severe asthma? *Journal of Internal Medicine*. 2012;**272**(2):121-132

- [37] Kuo CS, Pavlidis S, Loza M, Baribaud F, Rowe A, Pandis I, et al. T-helper cell type 2 (Th2) and non-Th2 molecular phenotypes of asthma using sputum transcriptomics in U-BIOPRED. *The European Respiratory Journal*. 2017;**49**(2). DOI: 10.1183/13993003.02135-2016
- [38] Chung KF. Defining phenotypes in asthma: A step towards personalized medicine. *Drugs*. 2014;**74**(7):719-728
- [39] Vannuvel K, Renard P, Raes M, Arnould T. Functional and morphological impact of ER stress on mitochondria. *Journal of Cellular Physiology*. 2013;**228**(9):1802-1818
- [40] Jeong JS, Kim SR, Cho SH, Lee YC. Endoplasmic reticulum stress and allergic diseases. *Current Allergy and Asthma Reports*. 2017;**17**(12):82
- [41] Hetz C. The unfolded protein response: Controlling cell fate decisions under ER stress and beyond. *Nature Reviews. Molecular Cell Biology*. 2012;**13**(2):89-102
- [42] Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nature Cell Biology*. 2000;**2**(6):326-332
- [43] Shen J, Chen X, Hendershot L, Prywes R. ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals. *Developmental Cell*. 2002;**3**(1):99-111
- [44] Hotamisligil GS. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell*. 2010;**140**(6):900-917
- [45] Grootjans J, Kaser A, Kaufman RJ, Blumberg RS. The unfolded protein response in immunity and inflammation. *Nature Reviews. Immunology*. 2016;**16**(8):469-484
- [46] Arensdorf AM, Diedrichs D, Rutkowski DT. Regulation of the transcriptome by ER stress: Non-canonical mechanisms and physiological consequences. *Frontiers in Genetics*. 2013;**4**:256
- [47] Cao Y, Knochel S, Oswald F, Donow C, Zhao H, Knochel W. XBP1 forms a regulatory loop with BMP-4 and suppresses mesodermal and neural differentiation in *Xenopus* embryos. *Mechanisms of Development*. 2006;**123**(1):84-96
- [48] Brar GA, Yassour M, Friedman N, Regev A, Ingolia NT, Weissman JS. High-resolution view of the yeast meiotic program revealed by ribosome profiling. *Science*. 2012;**335**(6068):552-557
- [49] Malhotra D, Thimmulappa R, Vij N, Navas-Acien A, Sussan T, Merali S, et al. Heightened endoplasmic reticulum stress in the lungs of patients with chronic obstructive pulmonary disease: The role of Nrf2-regulated proteasomal activity. *American Journal of Respiratory and Critical Care Medicine*. 2009;**180**(12):1196-1207
- [50] Korfei M, Ruppert C, Mahavadi P, Henneke I, Markart P, Koch M, et al. Epithelial endoplasmic reticulum stress and apoptosis in sporadic idiopathic pulmonary fibrosis. *American Journal of Respiratory and Critical Care Medicine*. 2008;**178**(8):838-846

- [51] Kim HJ, Jeong JS, Kim SR, Park SY, Chae HJ, Lee YC. Inhibition of endoplasmic reticulum stress alleviates lipopolysaccharide-induced lung inflammation through modulation of NF-kappaB/HIF-1alpha signaling pathway. *Scientific Reports*. 2013;**3**:1142-1151
- [52] Marciniak SJ. Endoplasmic reticulum stress in lung disease. *European Respiratory Review*. 2017;**26**(144)
- [53] Kim RY, Pinkerton JW, Essilfie AT, Robertson AAB, Baines KJ, Brown AC, et al. Role for NLRP3 inflammasome-mediated, IL-1beta-dependent responses in severe, steroid-resistant asthma. *American Journal of Respiratory and Critical Care Medicine*. 2017;**196**(3):283-297
- [54] Guo Q, Li H, Liu J, Xu L, Yang L, Sun Z, et al. Tunicamycin aggravates endoplasmic reticulum stress and airway inflammation via PERK-ATF4-CHOP signaling in a murine model of neutrophilic asthma. *The Journal of Asthma*. 2017;**54**(2):125-133
- [55] Cabanski M, Steinmuller M, Marsh LM, Surdziel E, Seeger W, Lohmeyer J. PKR regulates TLR2/TLR4-dependent signaling in murine alveolar macrophages. *American Journal of Respiratory Cell and Molecular Biology*. 2008;**38**(1):26-31
- [56] Kim SR, Lee YC, Kim DI, Park HJ. Effects of PKR inhibitor on poly (I:C)-induced exacerbation of severe asthma. *The European Respiratory Journal*. 2016;**48**:PA1099
- [57] Lee KS, Jeong JS, Kim SR, Cho SH, Kolliputi N, Ko YH, et al. Phosphoinositide 3-kinase- δ regulates fungus-induced allergic lung inflammation through endoplasmic reticulum stress. *Thorax*. 2016;**71**(1):52-63
- [58] Ciencewicky J, Trivedi S, Kleeberger SR. Oxidants and the pathogenesis of lung diseases. *The Journal of Allergy and Clinical Immunology*. 2008;**122**(3):456-468 quiz 69-70
- [59] Reddy PH. Mitochondrial dysfunction and oxidative stress in asthma: Implications for mitochondria-targeted antioxidant therapeutics. *Pharmaceuticals (Basel)*. 2011;**4**(3):429-456
- [60] Cheresh P, Kim SJ, Tulasiram S, Kamp DW. Oxidative stress and pulmonary fibrosis. *Biochimica et Biophysica Acta*. 2013;**1832**(7):1028-1040
- [61] Ernster L, Schatz G. Mitochondria: A historical review. *The Journal of Cell Biology*. 1981;**91**(3 Pt 2):227s-255s
- [62] Rizzuto R, Bernardi P, Pozzan T. Mitochondria as all-round players of the calcium game. *The Journal of Physiology*. 2000;**529**(Pt 1):37-47
- [63] Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. *Science*. 2004;**305**(5684):626-629
- [64] Okamoto K, Shaw JM. Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. *Annual Review of Genetics*. 2005;**39**:503-536
- [65] Song Z, Ghochani M, McCaffery JM, Frey TG, Chan DC. Mitofusins and OPA1 mediate sequential steps in mitochondrial membrane fusion. *Molecular Biology of the Cell*. 2009;**20**(15):3525-3532

- [66] Otera H, Wang C, Cleland MM, Setoguchi K, Yokota S, Youle RJ, et al. Mff is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells. *The Journal of Cell Biology*. 2010;**191**(6):1141-1158
- [67] Kubli DA, Gustafsson AB. Mitochondria and mitophagy: The yin and yang of cell death control. *Circulation Research*. 2012;**111**(9):1208-1221
- [68] Aravamudan B, Kiel A, Freeman M, Delmotte P, Thompson M, Vassallo R, et al. Cigarette smoke-induced mitochondrial fragmentation and dysfunction in human airway smooth muscle. *American Journal of Physiology. Lung Cellular and Molecular Physiology*. 2014;**306**(9):L840-L854
- [69] Rehman J, Zhang HJ, Toth PT, Zhang Y, Marsboom G, Hong Z, et al. Inhibition of mitochondrial fission prevents cell cycle progression in lung cancer. *The FASEB Journal*. 2012;**26**(5):2175-2186
- [70] Loukides S, Bouros D, Papatheodorou G, Panagou P, Siafakas NM. The relationships among hydrogen peroxide in expired breath condensate, airway inflammation, and asthma severity. *Chest*. 2002;**121**(2):338-346
- [71] Chung HK, Kim YK, Park JH, Ryu MJ, Chang JY, Hwang JH, et al. The indole derivative NecroX-7 improves nonalcoholic steatohepatitis in ob/ob mice through suppression of mitochondrial ROS/RNS and inflammation. *Liver International*. 2015;**35**(4):1341-1353
- [72] Park J, Park E, Ahn BH, Kim HJ, Park JH, Koo SY, et al. NecroX-7 prevents oxidative stress-induced cardiomyopathy by inhibition of NADPH oxidase activity in rats. *Toxicology and Applied Pharmacology*. 2012;**263**(1):1-6
- [73] Jin SA, Kim SK, Seo HJ, Jeong JY, Ahn KT, Kim JH, et al. Beneficial effects of necrosis modulator, indole derivative NecroX-7, on renal ischemia-reperfusion injury in rats. *Transplantation Proceedings*. 2016;**48**(1):199-204
- [74] Im KI, Kim N, Lim JY, Nam YS, Lee ES, Kim EJ, et al. The free radical scavenger NecroX-7 attenuates acute graft-versus-host disease via reciprocal regulation of Th1/regulatory T cells and inhibition of HMGB1 release. *Journal of Immunology*. 2015;**194**(11):5223-5232
- [75] Park JH, Seo KS, Tadi S, Ahn BH, Lee JU, Heo JY, et al. An indole derivative protects against acetaminophen-induced liver injury by directly binding to N-acetyl-p-benzoquinone imine in mice. *Antioxidants & Redox Signaling*. 2013;**18**(14):1713-1722
- [76] Davis BK, Wen H, Ting JP. The inflammasome NLRs in immunity, inflammation, and associated diseases. *Annual Review of Immunology*. 2011;**29**:707-735
- [77] Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature*. 2011;**469**(7329):221-225
- [78] Lee YC, Kim SR, Severe Asthma CSH. *Toward Personalized Patient Management*. Singapore: Springer; 2017