# Roles of Inflammasome in Cigarette Smoke-Related Diseases and Physiopathological Disorders: Mechanisms and Therapeutic Opportunities

Yiming Ma, Yingjiao Long[*†] and Yan Chen[*†]

*Department of Pulmonary and Critical Care Medicine, The Second Xiangya Hospital, Central South University, Changsha, China*

Cigarette smoke damages a wide range of immunological functions, including innate and adaptive immune responses. Emerging literature demonstrates that inflammasome constitutes an essential component in innate immune response. In this review, we focus on the cumulative mechanisms of inflammasome in cigarette smoke-related diseases and physiopathological disorders, and summarize potential therapeutic opportunities targeting inflammasome. This review suggests that inflammasomes (NLRP3, NLRP6, NLRP12 and AIM2) are involved in the pathogenesis of several cigarette smoke-related diseases (including COPD, ALI, atherosclerosis, kidney injury, bladder dysfunction, and oral leukoplakia) and physiopathological disorders (macrophage dysfunction, endothelial barrier dysfunction, podocyte injury, and ubiquitin-mediated proteasomal processing). MyD88/NF-κB, HMGB1, production of ROS, endoplasmic reticulum stress and mitochondrial dysfunction, and $Ca^{2+}$ influx are potentially involved in cigarette smoke induced-inflammasome activation. Strategies targeting ROS/NLRP3 inflammasome axis are most widely investigated and show potential therapeutic effects.

Keywords: inflammasome, cigarette smoke, NLRP3, ROS, innate immune

## INTRODUCTION

Cigarette smoke remains to be a threat to the health of the world's population, and a global survey has indicated that prevalence of daily tobacco smoking in the population older than 15 years is 31.1% for men and 6.2% for women (1). Previous studies have demonstrated that cigarette smoke is a major risk factor for occurrence and progress of diseases involving multiple systems throughout the body, such as respiratory system (2–4), cardiovascular system (4, 5), and nervous system (6, 7). Moreover, chronic cigarette smoke inhalation damages a wide range of immunological functions, including innate and adaptive immune responses (8).

Inflammasomes are multiprotein signaling platforms mediating inflammatory responses and coordinating antimicrobial host defenses (9–12). Assembly of an inflammasome complex requires cytosolic sensing of pathogen-associated molecular patterns or danger-associated molecular patterns by a nucleotide-binding domain and leucine-rich repeat receptor (NLR) or absent in

melanoma (AIM) 2-like receptor (13). Inflammasomes could activate proinflammatory caspase-1, and caspase-11 (in mice)/caspase-4 and caspase-5 (in humans), which further leads to maturation of interleukins 1β and 18 (IL-1β and IL-18) through proteolytic cleavage of pro-IL-1β and pro-IL-18 (14–16); besides, activated caspase-1, and caspase-11 (in mice)/caspase-4 and caspase-5 (in humans) also cleave and activate Gasdermin D (GSDMD), which induces a type of cell death called pyroptosis (15, 17, 18). The NLRP3 inflammasome has been under intensive study due to its extensive connection with a variety of human diseases and a two-signal model has been proposed for NLRP3 inflammasome activation; in this model, the first signal (priming) is provided by microbial or endogenous molecules that induce NLRP3 and pro-IL-1β expression through activation of NF-κB; the second signal (activation) is triggered by K+ efflux, $Ca^{2+}$ signaling, reactive oxygen species (ROS), mitochondrial dysfunction, and lysosomal rupture (19). In clinical practice, VX-765, an inhibitor that directly targets inflammasome downstream cytokines, is able to block the hypersensitive response to an inflammatory stimulus in monocytes from familial cold autoinflammatory syndrome patients, which provides a novel alternative in treating immune-associated diseases (20). In this review, we focus on the cumulative mechanisms of inflammasome in cigarette smoke-related diseases and physiopathological disorders, and summarize potential therapeutic opportunities targeting inflammasome.

# ROLES OF INFLAMMASOME IN CIGARETTE SMOKE-RELATED DISEASES

## Inflammasome in Cigarette Smoke-Related Lung Diseases

### Stable Chronic Obstructive Pulmonary Disease (COPD)

Roles of inflammasome in cigarette smoke-related diseases and physiopathological disorders are summarized in **Table 1**. The role of inflammasome in stable COPD has been widely investigated both *in vitro* and *in vivo* models, while the conclusion remains contradictory. In vitro, Mortaz et al. (38) found that cigarette smoke could increase the expression of caspase-1 and IL-1β in human alveolar epithelial cells, suggesting the potential role of inflammasome signaling in the pathogenesis of COPD. Thereafter, more studies further demonstrated cigarette smoke was able to activate NLRP3 inflammasome in human bronchial and alveolar epithelial cells, and the inflammasome activation was able to further increase the release of inflammatory cytokines (including IL-1β and IL-18) (21, 22, 26, 27). Particularly, Mahalanobish et al. (21) reported that the activation of NLRP3 inflammasome in alveolar epithelial might result from cigarette smoke induced endoplasmic reticulum (ER) stress and mitochondrial dysfunctions. Wang et al. (27) found that cigarette smoke-induced inflammasome activation was triggered by oxidative stress injury and $Ca^{2+}$ influx in human bronchial and alveolar epithelial cells. Besides the activation of NLRP3 inflammasome,

Singh et al. (31) observed that increase of NLRP10 and NLRP12 proteins in human alveolar type II epithelial cells challenged by cigarette smoke extract. Furthermore, Kaur et al. (32) proved that NLRP10 knockdown rescued cigarette smoke extract induced inflammatory responses in human alveolar type II epithelial cells, which might serve as an effective therapeutic target of COPD.

*In vivo*, COPD mouse model was established to explore the effect of cigarette smoke on inflammasome (21, 29, 32, 34, 37). In mouse lung tissue, increased expression of NLRP3 was observed after exposure to cigarette smoke (21, 29); moreover, it was also found that the release of downstream inflammation cytokines significantly increased in mouse bronchoalveolar lavage fluid (BALF) samples (21, 29, 34). Cao et al. (29) concluded that activation of NLRP3 inflammasome was related to cigarette smoke-induced ROS production. Nevertheless, an earlier study by Pauwels et al. (37) demonstrated that cigarette smoke-induced inflammation was independent on the activation of NLRP3/caspase-1/IL-1β axis. Moreover, NLRP10 activation might also be involved in the inflammation cytokine release induced by cigarette smoke (32).

Clinical samples from patients were also used to measure whether inflammasome played a role in the pathogenesis of stable COPD (33, 35). Inconsistent with results of studies *in vitro* and *in vivo*, Faner et al. (33) found that the NLRP3 inflammasome was primed, but not activated in the lung tissue of stable COPD patients; besides, both caspase-1 and ASC were mostly in inactive forms. Also, Di Stefano et al. (35) reported that the NLRP3 inflammasome is not activated in bronchial mucosa and BALF of stable COPD patients. However, gene expression analyses revealed that polymorphisms in NLRP1 rs12150220 were associated with COPD disease severity, which suggested the importance of NLRP1 inflammasome fine-tuning in maintaining lung tissue integrity and treating chronic airway inflammation (25). Significant heterogeneity of clinical presentation and disease progression exists in COPD patients, so phenotyping COPD patients is beneficial to identify patient subgroups with unique prognostic or therapeutic characteristics (52). The Spanish guidelines describe four clinical phenotypes for COPD, including chronic bronchitis phenotype, emphysema phenotype, asthma-COPD overlap syndrome phenotype, and non-exacerbator phenotype (53). Interestingly, chronic bronchitis phenotype of COPD is associated with worse respiratory symptoms and higher risk of exacerbations in contrast to other phenotypes (54). However, there have been no studies assessing activation levels of inflammasomes among different phenotypes of COPD patients. Importantly, it seems significant to evaluate whether a higher proportion of activated inflammasomes exists in chronic bronchitis phenotype of COPD patients compared with other phenotypes, as this may contribute a lot to the precision treatment of COPD.

## Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD)

Previous studies also proved that the activation of NLRP3 inflammasome was involved in the pathogenesis of AECOPD. Cigarette smoke could enhance the NLRP3 inflammasome

**TABLE 1** | Roles of inflammasome in cigarette smoke-related diseases and physiopathological disorders.

| Reference | Year | Disease/disorder | Sample/Subjects | Effect of cigarette smoke on inflammasome |
|---|---|---|---|---|
| Mahalanobish et al. (21) | 2020 | COPD | Mouse lung tissue and BALF, human alveolar epithelial cells | Induce endoplasmic reticulum stress and mitochondrial dysfunctions, and further activate NLRP3 inflammasome |
| Rumora et al. (22) | 2020 | COPD | Human bronchial epithelial cells, monocyte-derived macrophages, and THP-1 cells | Increase NLRP3 and IL-1β |
| Rumora et al. (23) | 2021 | AECOPD | Human bronchial epithelial cells, monocyte-derived macrophages, and THP-1 cells | Increase NLRP3 and IL-1β |
| Ji et al. (24) | 2020 | AECOPD | Rat lung tissue and BALF, human bronchial epithelial cells | Increase caspase-1, NLRP3, IL-1β and IL-18 |
| Ozretic et al. (25) | 2019 | COPD | Human peripheral blood mononuclear and lung fibroblast cells | NLRP1 rs12150220 coding polymorphisms are associated with COPD disease severity |
| Nachmias et al. (26) | 2019 | COPD/AECOPD | Human alveolar epithelial cells | Increase NLRP3 and IL-1β |
| Wang et al. (27) | 2019 | COPD | Human bronchial and alveolar epithelial cells | Induce oxidative stress injury, promote $Ca^{2+}$ influx, and increase caspase-1, NLRP3, IL-1β and IL-18 |
| Colarusso et al. (28) | 2019 | AECOPD | Human peripheral blood mononuclear cells | Increase AIM2/caspase-1/caspase-4 in IL-1α-induced TGF-β release |
| Cao et al. (29) | 2018 | COPD | Mouse lung tissue and BALF | Induce ROS production and increase NLRP3, cleaved-IL-1β and cleaved-caspase-1 |
| Wang et al. (30) | 2018 | AECOPD | Human peripheral blood mononuclear cells, bronchial tissues, serum and BALF | Increase NLRP3, caspase -1, ASC, IL-18 and IL-1β |
| Singh et al. (31) | 2018 | COPD | Human alveolar epithelial cells | Increase NLRP10, NLRP12, caspase-1, IL-1β, and IL-18 |
| Kaur et al. (32) | 2018 | COPD | Mouse lung tissue, human alveolar epithelial cells | Increase NLRP10, caspase-1, IL-1β, and IL-18 |
| Faner et al. (33) | 2016 | COPD/AECOPD | Human lung tissue of stable COPD, human sputum and plasma of AECOPD | Stable COPD: NLRP3 inflammasome is primed, but not activated; both caspase-1 and ASC were mostly inactive |
| Yang et al. (34) | 2016 | COPD | Mouse BALF | AECOPD: Caspase-1, oligomeric ASC, and associated cytokines (IL-1β, IL-18) were significantly increased |
| Di Stefano et al. (35) | 2014 | COPD | Human bronchial mucosa and BALF | Increase IL-1 and IL-18 |
| Rotta et al. (36) | 2013 | AECOPD | Mouse macrophage cells, human alveolar macrophages and human lung tissue | NLRP3 inflammasome is not activated in patients with stable COPD |
| Pauwels et al. (37) | 2011 | COPD | Mouse lung tissue | Increase NLRP3, caspase-1 and IL-1β |
| Mortaz et al. (38) | 2011 | COPD | Human bronchial epithelial cells | CS-induced inflammation occurred independently of IL-1β activation by the NLRP3/caspase-1 axis |
| Zhang et al. (39) | 2018 | ALI | Mouse lung tissue, mouse alveolar macrophages | Increase caspase-1 and IL-1β<br>Increase NLRP3, caspase-1 and IL-1β |
| Mehta et al. (40) | 2020 | Atherosclerosis | Human THP-1 monocytes, macrophages, and foam cells | Activate MyD88/NF-κB pathway and increase NLRP3, caspase-1, IL-1β, and IL-18. |
| Wu et al. (41) | 2018 | Atherosclerosis | Mouse aortic tissue, human aortic endothelium cells | Induce ROS production and increase NLRP3, ASC, caspase-1, pro-caspase-1, IL-1β, and IL-18 |
| Yao et al. (42) | 2019 | Atherosclerosis | Rat vascular smooth muscle cells, rat aortic tissue | Induce ROS production and increase NLRP3 |
| Zheng et al. (43) | 2020 | Kidney injury | Mouse kidney tissue, human kidney cells | Induce NLRP6 inflammasome activation via alpha7 nicotinic acetylcholine receptor |
| Wu et al. (44) | 2020 | Bladder dysfunction | Human bladder tissue, human bladder urothelial cells | Induce oxidative stress injury and the activation of NLRP3 inflammasome |
| Buscetta et al. (45) | 2020 | Macrophage dysfunction | Human monocyte-derived macrophages and THP-1 cells | Inhibit NLRP3, caspase-1, IL-1β, and IL-18 acting mainly at the transcriptional level, and increase the caspase-1 activity via an NLRP3-independent and TLR4-TRIF-caspase-8-dependent pathway |
| Singh et al. (46) | 2019 | Podocyte injury | Mouse podocyte cells | Induce ROS production and increase the colocalization of NLRP3 with ASC, caspase-1 activity, and IL-1β production |
| Zhang et al. (47) | 2019 | Endothelial barrier dysfunction | Mouse microvascular endothelial cells and mouse coronary arterial endothelium | Increase HMGB1 and enhance cathepsin B-dependent NLRP3 inflammasome activation |
| Chen et al. (48) | 2019 | Endothelial barrier dysfunction | Human umbilical vein endothelial cells | Increase caspase-1, NLRP3, and IL-1β |
| Wang et al. (49) | 2019 | Endothelial dysfunction | Rat carotid artery tissue, human umbilical vein endothelial cells | Activate ROS/NLRP3 axis |
| Ye et al. (50) | 2019 | Oral leukoplakia | Rat oral mucosal epithelium | Reduce expression of the NLRP3 and diminish the secretion of IL-1β and IL-18 maturing by the NLRP3 inflammasome |
| Han et al. (51) | 2017 | Ubiquitin-mediated proteasomal processing | Human monocyte THP-1 cells and mouse lung tissue | Decrease NLRP3 protein abundance via increased ubiquitin-mediated proteasomal processing |

*COPD, chronic obstructive pulmonary disease; BALF, bronchoalveolar lavage fluid; NLRP, nucleotide binding oligomerization domain and leucine, rich repeat containing receptor; IL, interleukin; AECOPD, acute exacerbation of chronic obstructive pulmonary disease; AIM, absent in melanoma; TGF, transforming growth factor; ASC, apoptosis associated speck like protein containing a caspase recruitment domain; TLR, Toll like receptor; TRIF, Toll/IL, 1receptor domain containing adaptor inducing interferon, beta; ROS, reactive oxygen species; HMGB1, high mobility group box 1.*

activation, and further promote the downstream inflammatory cytokines' release (including IL-1β and IL-18) in human bronchial epithelial cells (23, 24), and human alveolar epithelial cells (26). Of note, inducing factors of acute exacerbations varied in different studies, such as lipopolysaccharide (23, 26), lipoteichoic acid (23), extracellular heat shock protein 70 (23), and influenza A virus (24) etc. In addition, Rotta et al. (36) verified that there was an upregulation of the NLRP3 in murine macrophage cells and human alveolar macrophages from *Nontypeable Haemophilus Influenzae* infection (NTHi)-induced AECOPD disease model, which imitated the pathogenesis of bacterial exacerbations in COPD. In vivo, animal experiments showed that NLRP3 inflammasome activation existed in AECOPD rat lung tissue and BALF (24).

A growing number of studies demonstrated that there were significant increases of NLRP3 and IL-1β in human peripheral blood mononuclear cells from AECOPD patients (23, 30). Furthermore, potential NLRP3 inflammasome activation accompanied with inflammation cytokine release were also observed in human bronchial tissues (30, 36), serum (30), BALF (30), sputum (33), and plasma (33). Interestingly, Colarusso et al. (28) found that AIM2 inflammasome was also activated in human peripheral blood mononuclear cells derived from AECOPD patients, and could further lead to the release of IL-1α and TGF-β.

### Cigarette smoke-induced acute lung injury (ALI)

As for smoke inhalation-induced acute lung injury, Zhang et al. (39) reported that cigarette smoke was able to augment the formation of NLRP3 inflammasome, the activation of caspase-1 and IL-1β induced in mouse alveolar macrophages.

## Inflammasome in Cigarette Smoke-Related Cardiovascular Diseases

Recently, there have been studies investigating the role of inflammasome in cigarette smoke-induced atherosclerosis, and these studies mainly focused on NLRP3 inflammasome (40–42, 55). Mehta et al. (55) used human THP-1 monocytes, macrophages and foam cells to represent crucial stages of initiation, progression and development in cigarette smoke-induced atherogenesis, and it was found that cigarette smoke exposure could activate NLRP3 inflammasome in these cells with a stage-specific manner; furthermore, they proved that MyD88/NF-kappa B pathway was an upstream regulator of NLRP3 inflammasome (40). In rat vascular smooth muscle cells and rat aortic tissue, activation of reactive oxygen species (ROS)-NLRP3 inflammasome-C reactive protein (CRP) axis significantly enhanced after nicotine treatment (42). Moreover, nicotine induced ROS-NLRP3-mediated endothelial cell pyroptosis in human aortic endothelial cells (HAECs), which was evidenced by cleavage of caspase-1, production of downstream interleukin IL-1β and IL-18 (41). Importantly, one recent study found that transcriptional and translational expression of NLRP3 inflammasome markers (including caspase-1, pro-IL-1β, IL-1β, pro-IL-18 and IL-18) in mononuclear cells were significantly increased (2 to 7-fold) in

smokers with coronary artery disease (CAD) in contrast to non-smokers with CAD (56).

## Inflammasome in Cigarette Smoke-Related Urinary Diseases

Two recent researches concentrated on the change of inflammasome in cigarette smoke-related urinary diseases, including kidney injury and bladder dysfunction (43, 44). Zheng et al. (43) proposed that nicotine induced NLRP6 inflammasome activation *via* alpha7 nicotinic acetylcholine receptor in human kidney cells and mouse kidney tissue. Besides, Wu et al. (44) found that cigarette smoke induced the pyroptosis of urothelial cells through ROS/NLRP3/caspase-1 signaling pathway in bladder dysfunction models.

## INFLAMMASOME IN CIGARETTE SMOKE-RELATED PHYSIOPATHOLOGICAL DISORDERS

Inflammasome might also play an important role in some other physiopathological disorders induced by cigarette smoke, such as macrophage dysfunction (45), endothelial barrier dysfunction (47–49), podocyte injury (46), ubiquitin-mediated proteasomal processing (51), and oral leukoplakia (50) etc.

With regard to macrophage dysfunction, the study by Buscetta et al. (45) manifested that cigarette smoke restrained the expression of NLRP3, caspase-1, IL-1β, and IL-18 acting mainly at the transcriptional level in human monocyte-derived macrophages and THP-1 cells, and increased the caspase-1 activity *via* an NLRP3-independent and Toll-like receptor 4 (TLR4)-Toll/IL-1receptor domain containing adaptor inducing IFN-β (TRIF)-caspase-8-dependent pathway. On the contrary, Rumora et al. (22, 23) found that cigarette smoke was an activated factor of NLRP3 inflammasome in human monocyte-derived macrophages and THP-1 cells.

Endothelial barrier injury has been increasingly considered as an important pathophysiological process in COPD. Chen et al. (48) verified that cigarette smoke could increase the expression of caspase-1, NLRP3, and IL-1β in human umbilical vein endothelial cells. In mouse microvascular endothelial cells and mouse coronary arterial endothelium, nicotine was proved to increase high mobility group box 1(HMGB1) expression, cause NLRP3 inflammasome complex formation and enhance the inflammasome activity as demonstrated by increased cleavage of pro-caspase-1, and IL-1β production (47). In addition, Wang et al. (49) pointed out that cigarette smoke could activate ROS/NLRP3 axis in rat carotid artery tissue and human umbilical vein endothelial cells.

In addition, Singh et al. (46) found that nicotine instigated mouse podocyte cell injury *via* inducing ROS production and activating NLRP3 inflammasome. Han et al. (51) proved that cigarette could decrease NLRP3 protein abundance *via* increased ubiquitin-mediated proteasomal processing. Another interesting finding was that long-term cigarette smoking suppressed NLRP3

inflammasome activation in oral mucosal epithelium and attenuated host defense against Candida albicans in a rat model (50).

Inconsistent conclusions of cigarette smoke on NLRP3 expression among different studies may result from the following reasons. Firstly, Baroja-Mazo et al. (57) reported that the NLRP3 inflammasome particle was released from macrophages after inflammasome activation; thus, the decreased cellular level of NLRP3 protein may be due to the secretion of NLRP3 protein as extracellular oligomeric complexes. Secondly, the preparation method and treated concentration of cigarette smoke extract could not be homogenized among different experiments.

# THERAPEUTIC STRATEGIES TO TARGET INFLAMMASOME IN CIGARETTE SMOKE-RELATED DISEASES AND PHYSIOPATHOLOGICAL DISORDERS

**Table 2** illustrates therapeutic strategies to target inflammasome in cigarette smoke-induced diseases and physiopathological disorders. Scavenging ROS has become the most widely investigated strategy to inhibit NLRP3 inflammasome activation induced by cigarette smoke. N-Acetyl-L-cysteine (NAC) is an amino-acid derivative of cysteine and a precursor of the antioxidant enzyme glutathione (58). The application of NAC as an antioxidant to diminish ROS generation has been proved in previous studies (59, 60). In clinical practice, NAC has been used as a mucolytic to help clear mucus in patients with respiratory diseases (61). Several studies verified that NAC could decrease ROS generation and further inhibit NLRP3 inflammasome activation, and NAC-mediated disease/disorder involved atherosclerosis (41), bladder damage (44), and podocyte injury (46). Besides NAC, other therapies antagonizing ROS were also explored to modulate inflammasome activation in cigarette smoke-induced diseases. Melatonin (N-acetyl-5-methoxytryptamine) is a neuroendocrine hormone synthesized by tryptophan and serotonin metabolism, and secreted from the pineal gland (62, 63). Melatonin was found to play an important role in relieving oxidative stress injury (64, 65). One study by Wang et al. (49) illustrated that melatonin inhibited ROS production, NLRP3 inflammasome activation and pyroptosis in cigarette smoke-treated endothelial cells. Another effect of melatonin on NLRP3 inflammasome activation was to suppress endoplasmic reticulum stress and mitochondrial dysfunction in COPD (21). Inhibitors of transient receptor potential protein (TRP) ion channels (TRPA1 and TRPV1) might also attenuate NLRP3 inflammasome activation by reducing oxidative stress and blocking $Ca^{2+}$ influx (27). Moreover, rosmarinic acid and lipoxin receptor agonist (BML-111) were also capable of mitigating ROS production and restraining NLRP3 inflammasome activation in cigarette smoke-induced diseases (29, 42).

There were also some other strategies to target NLRP3 inflammasome in cigarette smoke-related diseases. Concerning endothelial barrier dysfunction induced by cigarette smoke, Zhang et al. (47) found that blockade of HMGB1 could inhibit NLRP3 inflammasome activation; similarly, mitoquinone was also able to diminish NLRP3 inflammasome activation in cigarette smoke-induced endothelial barrier dysfunction (48). With respect to cigarette smoke induced-ALI, suppressor of cytokine signaling-1 (SOCS-1) might dampen the formation of

TABLE 2 | Therapeutic strategies to target inflammasome in cigarette smoke-induced diseases and physiopathological disorders.

| Reference | Year | Disease/disorder | Therapy | Mechanism |
|---|---|---|---|---|
| Wu et al. (41) | 2018 | Atherosclerosis | NAC | Inhibit ROS |
| Wu et al. (44) | 2020 | Bladder damage | NAC | Inhibit ROS |
| Singh et al. (46) | 2019 | Podocyte injury | NAC | Scavenge ROS |
| Wang et al. (49) | 2019 | Endothelial dysfunction | Melatonin | Inhibit ROS/NLRP3 axis |
| Mahalanobish et al. (21) | 2020 | COPD | Melatonin | Suppress endoplasmic reticulum stress, mitochondrial dysfunction and further inhibit NLRP3 inflammasome activation |
| Wang et al. (27) | 2019 | COPD | Transient receptor potential protein ion channel inhibitors | Reduce oxidative stress, block $Ca^{2+}$ influx, and inhibit NLRP3 inflammasome activation |
| Yao et al. (42) | 2019 | Arteriosclerosis | Rosmarinic acid | Inhibit the ROS-NLRP3 inflammasome-CRP axis |
| Cao et al. (29) | 2018 | COPD | Lipoxin receptor agonist | Inhibit ROS production and prevent NLRP3 inflammasome activation |
| Ji et al. (24) | 2020 | AECOPD | Shufeng Jiedu Capsule (SFJDC), oseltamivir | Inhibit NLRP3 inflammasome activation |
| Zhang et al. (47) | 2019 | Endothelial barrier dysfunction | Blockade of HMGB1 | Inhibit NLRP3 inflammasome activation |
| Chen et al. (48) | 2019 | Endothelial barrier dysfunction | Mitoquinone | Inhibit NLRP3 inflammasome activation |
| Zhang et al. (39) | 2018 | ALI | Suppressor of cytokine signaling-1 (SOCS-1) | Dampen the formation of NLRP3 inflammasome and the activation of caspase-1 and IL-1β |
| Singh et al. (31) | 2018 | COPD | Poly-unsaturated fatty acids (PUFA) | Inhibit membrane recruitment of NLRP10 and NLRP12 |

*NAC, N, Acetyl, L, cysteine; ROS, reactive oxygen species; NLRP, nucleotide binding oligomerization domain and leucine, rich repeat containing receptor; COPD, chronic obstructive pulmonary disease; CRP, C reactive protein; AECOPD, acute exacerbation of chronic obstructive pulmonary disease; HMGB1, high mobility group box 1; ALI, acute lung injury; IL, interleukin.*

NLRP3 inflammasome and the activation of caspase-1 and IL-1β (39). Futhermore, Ji et al. (24) pointed out that Shufeng Jiedu Capsule (SFJDC) and oseltamivir significantly decreased NLRP3 inflammasome activation in influenza virus A-induced AECOPD disease models.

Targeting NLRP10 and NLRP12 inflammasome, poly-unsaturated fatty acids (PUFA) was capable of rescuing A549 cells from cigarette smoke extract (CSE)-mediated membrane recruitment of NLRP10 and NLRP12, and also from inflammatory responses (31). Finally, **Supplemental Figure 1** summarized effects of cigarette smoke on NLRP3 inflammasome, and potential therapeutic strategies.

## SUMMARY

Collectively, emerging evidence demonstrates that inflammasomes (NLRP3, NLRP6, NLRP12 and AIM2) are involved in the pathogenesis of several cigarette smoke-related diseases (including COPD, ALI, atherosclerosis, kidney injury, bladder dysfunction, and oral leukoplakia) and physiopathological disorders (macrophage dysfunction, endothelial barrier dysfunction, podocyte injury, and ubiquitin-mediated proteasomal processing). MyD88/NF-κB, HMGB1, production of ROS, endoplasmic reticulum stress and mitochondrial dysfunction, and Ca$^{2+}$ influx are potentially involved in cigarette smoke induced-inflammasome activation. Strategies targeting ROS/NLRP3 inflammasome axis are most widely investigated and show potential therapeutic effects. Although this review reveals the potential relationship between cigarette smoke and inflammasome, more studies may be still needed to further confirm more detailed mechanisms so as to provide effective alternatives for treating cigarette smoke-related diseases.

## AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

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**Supplementary Figure 1 |** Effect of cigarette smoke on NLRP3 inflammasome, and potential therapeutic strategies. HMGB, high mobility group box 1; NAC, N-Acetyl-L-cysteine; ROS, reactive oxygen species; TRP, transient receptor potential protein; ER, endoplasmic reticulum; NLRP, Nucleotide binding oligomerization domain and leucine-rich repeat containing receptor.

## REFERENCES

1. Ng M, Freeman MK, Fleming TD, Robinson M, Dwyer-Lindgren L, Thomson B, et al. Smoking Prevalence and Cigarette Consumption in 187 Countries, 1980-2012. *Jama-J Am Med Assoc* (2014) 311(2):183–92. doi: 10.1001/jama.2013.284692

2. Ambrose JA, Barua RS. The Pathophysiology of Cigarette C-V Smoking and Cardiovascular Disease - An Update. *J Am Coll Cardiol* (2004) 43(10):1731–7. doi: 10.1016/j.jacc.2003.12.047

3. Yoshida T, Tuder RM. Pathobiology of Cigarette Smoke-Induced Chronic Obstructive Pulmonary Disease. *Physiol Rev* (2007) 87(3):1047–82. doi: 10.1152/physrev.00048.2006

4. Pope CA III, Burnett RT, Turner MC, Cohen A, Krewski D, Jerrett M, et al. Lung Cancer and Cardiovascular Disease Mortality Associated With Ambient Air Pollution and Cigarette Smoke: Shape of the Exposure-Response Relationships. *Environ Health Perspect* (2011) 119(11):1616–21. doi: 10.1289/ehp.1103639

5. Huxley RR, Woodward M. Cigarette Smoking as a Risk Factor for Coronary Heart Disease in Women Compared With Men: A Systematic Review and Meta-Analysis of Prospective Cohort Studies. *Lancet* (2011) 378(9799):1297–305. doi: 10.1016/s0140-6736(11)60781-2

6. Hernan MA, Takkouche B, Caamano-Isorna F, Gestal-Otero JJ. A Meta-Analysis of Coffee Drinking, Cigarette Smoking, and the Risk of Parkinson's Disease. *Ann Neurol* (2002) 52(3):276–84. doi: 10.1002/ana.10277

7. Heneka MT. Inflammasome Activation and Innate Immunity in Alzheimer's Disease. *Brain Pathol* (2017) 27(2):220–2. doi: 10.1111/bpa.12483

8. Sopori M. Effects of Cigarette Smoke on the Immune System. *Nat Rev Immunol* (2002) 2(5):372–7. doi: 10.1038/nri803

9. Broz P, Dixit VM. Inflammasomes: Mechanism of Assembly, Regulation and Signalling. *Nat Rev Immunol* (2016) 16(7):407–20. doi: 10.1038/nri.2016.58

10. Allen IC, Scull MA, Moore CB, Holl EK, McElvania-TeKippe E, Taxman DJ, et al. The NLRP3 Inflammasome Mediates In Vivo Innate Immunity to Influenza A Virus Through Recognition of Viral RNA. *Immunity* (2009) 30 (4):556–65. doi: 10.1016/j.immuni.2009.02.005

11. Man SM, Kanneganti T-D. Converging Roles of Caspases in Inflammasome Activation, Cell Death and Innate Immunity. *Nat Rev Immunol* (2016) 16 (1):7–21. doi: 10.1038/nri.2015.7

12. Kanneganti T-D. The Inflammasome: Firing Up Innate Immunity. *Immunol Rev* (2015) 265(1):1–5. doi: 10.1111/imr.12297

13. Man SM, Kanneganti TD. Regulation of Inflammasome Activation. *Immunol Rev* (2015) 265(1):6–21. doi: 10.1111/imr.12296

14. Malik A, Kanneganti TD. Inflammasome Activation and Assembly at a Glance. *J Cell Sci* (2017) 130(23):3955–63. doi: 10.1242/jcs.207365

15. Kayagaki N, Stowe IB, Lee BL, O'Rourke K, Anderson K, Warming S, et al. Caspase-11 Cleaves Gasdermin D for non-Canonical Inflammasome Signalling. *Nature* (2015) 526(7575):666–71. doi: 10.1038/nature15541

16. Lamkanfi M, Dixit VM. Mechanisms and Functions of Inflammasomes. *Cell* (2014) 157(5):1013–22. doi: 10.1016/j.cell.2014.04.007

17. Pang Y, Zhang PC, Lu RR, Li HL, Li JC, Fu HX, et al. Andrade-Oliveira Salvianolic Acid B Modulates Caspase-1-Mediated Pyroptosis in Renal Ischemia-Reperfusion Injury *via* Nrf2 Pathway. *Front Pharmacol* (2020) 11:541426. doi: 10.3389/fphar.2020.541426

18. Liu X, Zhang Z, Ruan J, Pan Y, Magupalli VG, Wu H, et al. Inflammasome-Activated Gasdermin D Causes Pyroptosis by Forming Membrane Pores. *Nature* (2016) 535(7610):153–8. doi: 10.1038/nature18629

19. He Y, Hara H, Nunez G. Mechanism and Regulation of NLRP3 Inflammasome Activation. *Trends Biochem Sci* (2016) 41(12):1012–21. doi: 10.1016/j.tibs.2016.09.002

20. Stack JH, Beaumont K, Larsen PD, Straley KS, Henkel GW, Randle JC, et al. IL-Converting Enzyme/Caspase-1 Inhibitor VX-765 Blocks the Hypersensitive Response to an Inflammatory Stimulus in Monocytes From Familial Cold Autoinflammatory Syndrome Patients. *J Immunol* (2005) 175 (4):2630–4. doi: 10.4049/jimmunol.175.4.2630

21. Mahalanobish S, Dutta S, Saha S, Sil PC. Melatonin Induced Suppression of ER Stress and Mitochondrial Dysfunction Inhibited NLRP3 Inflammasome Activation in COPD Mice. *Food Chem Toxicol* (2020) 144:111588. doi: 10.1016/j.fct.2020.111588

22. Rumora L, Somborac-Bačura A, Hlapčić I, Hulina-Tomašković A, Rajković MG. Cigarette Smoke and Extracellular Hsp70 Induce Secretion of ATP and Differential Activation of NLRP3 Inflammasome in Monocytic and Bronchial Epithelial Cells. *Cytokine* (2020) 135:155220. doi: 10.1016/j.cyto.2020.155220

23. Rumora L, Hlapčić I, Hulina-Tomašković A, Somborac-Bačura A, Bosnar M, Rajković MG. Pathogen-Associated Molecular Patterns and Extracellular Hsp70 Interplay in NLRP3 Inflammasome Activation in Monocytic and Bronchial Epithelial Cellular Models of COPD Exacerbations. *APMIS: Acta Pathol Microbiol Immunol Scandinavica* (2021) 129(2):80–90. doi: 10.1111/apm.13089

24. Ji S, Bai Q, Wu X, Zhang DW, Wang S, Shen JL, et al. Unique Synergistic Antiviral Effects of Shufeng Jiedu Capsule and Oseltamivir in Influenza A Viral-Induced Acute Exacerbation of Chronic Obstructive Pulmonary Disease. *Biomed Pharmacother Biomed Pharmacother* (2020) 121:109652. doi: 10.1016/j.biopha.2019.109652

25. Ozretić P, da Silva Filho MI, Catalano C, Sokolović I, Vukić-Dugac A, Šutić M, et al. Association of NLRP1 Coding Polymorphism With Lung Function and Serum IL-1β Concentration in Patients Diagnosed With Chronic Obstructive Pulmonary Disease (COPD). *Genes* (2019) 10(10):783. doi: 10.3390/genes10100783

26. Nachmias N, Langier S, Brzezinski RY, Siterman M, Stark M, Etkin S, et al. NLRP3 Inflammasome Activity Is Upregulated in an in-Vitro Model of COPD Exacerbation. *PloS One* (2019) 14(5):e0214622. doi: 10.1371/journal.pone.0214622

27. Wang MY, Zhang YB, Xu MM, Zhang H, Chen YQ, Chung KF, et al. Roles of TRPA1 and TRPV1 in Cigarette Smoke-Induced Airway Epithelial Cell Injury Model. *Free Radic Biol Med* (2019) 134:229–38. doi: 10.1016/j.freeradbiomed.2019.01.004

28. Colarusso C, Terlizzi M, Molino A, Imitazione P, Somma P, Rega R, et al. AIM2 Inflammasome Activation Leads to IL-1α and TGF-β Release From Exacerbated Chronic Obstructive Pulmonary Disease-Derived Peripheral Blood Mononuclear Cells. *Front Pharmacol* (2019) 10:257. doi: 10.3389/fphar.2019.00257

29. Cao Y, Zhou X, Yin Z, Yu X, Yang Q, Guo Q, et al. The Anti-Inflammatory Effect of BML-111 on COPD may be Mediated by Regulating NLRP3 Inflammasome Activation and ROS Production. *Prostaglandins Other Lipid Mediators* (2018) 138:23–30. doi: 10.1016/j.prostaglandins.2018.08.001

30. Wang H, Lv C, Wang S, Ying H, Weng Y, Yu W. NLRP3 Inflammasome Involves in the Acute Exacerbation of Patients With Chronic Obstructive Pulmonary Disease. *Inflammation* (2018) 41(4):1321–33. doi: 10.1007/s10753-018-0780-0

31. Singh DP, Kaur G, Bagam P, Pinkston R, Batra S. Membrane Microdomains Regulate NLRP10- and NLRP12-Dependent Signalling in A549 Cells Challenged With Cigarette Smoke Extract. *Arch Toxicol* (2018) 92(5):1767–83. doi: 10.1007/s00204-018-2185-0

32. Kaur G, Bagam P, Pinkston R, Singh DP, Batra S. Cigarette Smoke-Induced Inflammation: NLRP10-Mediated Mechanisms. *Toxicology* (2018) 398-399:52–67. doi: 10.1016/j.tox.2018.02.010

33. Faner R, Sobradillo P, Noguera A, Gomez C, Cruz T, López-Giraldo A, et al. The Inflammasome Pathway in Stable COPD and Acute Exacerbations. *ERJ Open Res* (2016) 2(3):00002-2016. doi: 10.1183/23120541.00002-2016

34. Yang W, Ni H, Wang H, Gu H. NLRP3 Inflammasome is Essential for the Development of Chronic Obstructive Pulmonary Disease. *Int J Clin Exp Pathol* (2015) 8(10):13209–16.

35. Di Stefano A, Caramori G, Barczyk A, Vicari C, Brun P, Zanini A, et al. Innate Immunity But Not NLRP3 Inflammasome Activation Correlates With Severity of Stable COPD. *Thorax* (2014) 69(6):516–24. doi: 10.1136/thoraxjnl-2012-203062

36. Rotta Detto Loria J, Rohmann K, Droemann D, Kujath P, Rupp J, Goldmann T, et al. Nontypeable Haemophilus Influenzae Infection Upregulates the NLRP3 Inflammasome and Leads to Caspase-1-Dependent Secretion of Interleukin-1β - A Possible Pathway of Exacerbations in COPD. *PloS One* (2013) 8(6):e66818. doi: 10.1371/journal.pone.0066818

37. Pauwels NS, Bracke KR, Dupont LL, Van Pottelberge GR, Provoost S, Vanden Berghe T, et al. Role of IL-1α and the Nlrp3/caspase-1/IL-1β Axis in Cigarette Smoke-Induced Pulmonary Inflammation and COPD. *Eur Respir J* (2011) 38(5):1019–28. doi: 10.1183/09031936.00158110

38. Mortaz E, Henricks PA, Kraneveld AD, Givi ME, Garssen J, Folkerts G. Cigarette Smoke Induces the Release of CXCL-8 From Human Bronchial Epithelial Cells *via* TLRs and Induction of the Inflammasome. *Biochim Biophys Acta* (2011) 1812(9):1104–10. doi: 10.1016/j.bbadis.2011.06.002

39. Zhang L, Xu C, Chen X, Shi Q, Su W, Zhao H. SOCS-1 Suppresses Inflammation Through Inhibition of NALP3 Inflammasome Formation in Smoke Inhalation-Induced Acute Lung Injury. *Inflammation* (2018) 41(4):1557–67. doi: 10.1007/s10753-018-0802-y

40. Mehta S, Dhawan V. Molecular Insights of Cigarette Smoke Condensate-Activated NLRP3 Inflammasome in THP-1 Cells in a Stage-Specific Atherogenesis. *Int Immunopharmacol* (2020) 88:107013. doi: 10.1016/j.intimp.2020.107013

41. Wu XX, Zhang HY, Qi W, Zhang Y, Li JM, Li ZG, et al. Nicotine Promotes Atherosclerosis *via* ROS-NLRP3-Mediated Endothelial Cell Pyroptosis. *Cell Death Dis* (2018) 9:12. doi: 10.1038/s41419-017-0257-3

42. Yao Y, Mao J, Xu S, Zhao L, Long L, Chen L, et al. Rosmarinic Acid Inhibits Nicotine-Induced C-Reactive Protein Generation by Inhibiting NLRP3 Inflammasome Activation in Smooth Muscle Cells. *J Cell Physiol* (2019) 234(2):1758–67. doi: 10.1002/jcp.27046

43. Zheng CM, Lee YH, Chiu IJ, Chiu YJ, Sung LC, Hsu YH, et al. Nicotine Causes Nephrotoxicity Through the Induction of NLRP6 Inflammasome and Alpha7 Nicotinic Acetylcholine Receptor. *Toxics* (2020) 8(4):92. doi: 10.3390/toxics8040092

44. Wu Z, Liu Q, Zhu K, Liu Y, Chen L, Guo H, et al. Cigarette Smoke Induces the Pyroptosis of Urothelial Cells Through ROS/NLRP3/caspase-1 Signaling Pathway. *Neurourol Urodynamics* (2020) 39(2):613–24. doi: 10.1002/nau.24271

45. Buscetta M, Di Vincenzo S, Miele M, Badami E, Pace E, Cipollina C. Cigarette Smoke Inhibits the NLRP3 Inflammasome and Leads to Caspase-1 Activation *via* the TLR4-TRIF-Caspase-8 Axis in Human Macrophages. *FASEB J: Off Publ Fed Am Soc Exp Biol* (2020) 34(1):1819–32. doi: 10.1096/fj.201901239R

46. Singh GB, Kshirasagar N, Patibandla S, Puchchakayala G, Koka S, Boini KM. Nicotine Instigates Podocyte Injury *via* NLRP3 Inflammasomes Activation. *Aging* (2019) 11(24):12810–21. doi: 10.18632/aging.102611

47. Zhang Y, Chen Y, Zhang Y, Li PL, Li X. Contribution of Cathepsin B-Dependent Nlrp3 Inflammasome Activation to Nicotine-Induced Endothelial Barrier Dysfunction. *Eur J Pharmacol* (2019) 865:172795. doi: 10.1016/j.ejphar.2019.172795

48. Chen S, Wang Y, Zhang H, Chen R, Lv F, Li Z, et al. The Antioxidant MitoQ Protects Against CSE-Induced Endothelial Barrier Injury and Inflammation by Inhibiting ROS and Autophagy in Human Umbilical Vein Endothelial Cells. *Int J Biol Sci* (2019) 15(7):1440–51. doi: 10.7150/ijbs.30193

49. Wang X, Bian Y, Zhang R, Liu X, Ni L, Ma B, et al. Melatonin Alleviates Cigarette Smoke-Induced Endothelial Cell Pyroptosis Through Inhibiting ROS/NLRP3 Axis. *Biochem Biophys Res Commun* (2019) 519(2):402–8. doi: 10.1016/j.bbrc.2019.09.005

50. Ye P, Wang X, Ge S, Chen W, Wang W, Han X. Long-Term Cigarette Smoking Suppresses NLRP3 Inflammasome Activation in Oral Mucosal Epithelium and Attenuates Host Defense Against Candida Albicans in a Rat Model. *Biomed Pharmacother Biomed Pharmacother* (2019) 113:108597. doi: 10.1016/j.biopha.2019.01.058

51. Han S, Jerome JA, Gregory AD, Mallampalli RK. Cigarette Smoke Destabilizes NLRP3 Protein by Promoting its Ubiquitination. *Respir Res* (2017) 18(1):2. doi: 10.1186/s12931-016-0485-6

52. Barnes PJ. Inflammatory Mechanisms in Patients With Chronic Obstructive Pulmonary Disease. *J Allergy Clin Immunol* (2016) 138(1):16–27. doi: 10.1016/j.jaci.2016.05.011

53. Rubio MC, Casamor R, Miravitlles M, Grp FS. Identification and Distribution of COPD Phenotypes in Clinical Practice According to Spanish COPD Guidelines: The FENEPOC Study. *Int J Chronic Obstructive Pulmonary Dis* (2017) 12:2373–83. doi: 10.2147/copd.s137872

54. Kim V, Han MK, Vance GB, Make BJ, Newell JD, Hokanson JE, et al. The Chronic Bronchitic Phenotype of COPD: An Analysis of the COPDGene Study. *Chest* (2011) 140(3):626–33. doi: 10.1378/chest.10-2948

55. Mehta S, Dhawan V. Exposure of Cigarette Smoke Condensate Activates NLRP3 Inflammasome in THP-1 Cells in a Stage-Specific Manner: An

Underlying Role of Innate Immunity in Atherosclerosis. *Cell Signalling* (2020) 72:109645. doi: 10.1016/j.cellsig.2020.109645

56. Mehta S, Vijayvergiya R, Dhawan V. Activation of NLRP3 Inflammasome Assembly is Associated With Smoking Status of Patients With Coronary Artery Disease. *Int Immunopharmacol* (2020) 87:106820. doi: 10.1016/j.intimp.2020.106820

57. Baroja-Mazo A, Martín-Sánchez F, Gomez AI, Martínez CM, Amores-Iniesta J, Compan V, et al. The NLRP3 Inflammasome is Released as a Particulate Danger Signal That Amplifies the Inflammatory Response. *Nat Immunol* (2014) 15(8):738–48. doi: 10.1038/ni.2919

58. Bretti C, Cardiano P, Irto A, Lando G, Milea D, Sammartano S. Interaction of N-Acetyl-L-Cysteine With Na(+), Ca(2+), Mg(2+) and Zn(2+). Thermodynamic Aspects, Chemical Speciation and Sequestering Ability in Natural Fluids. *J Mol Liquids* (2020) 319:114164. doi: 10.1016/j.molliq.2020.114164

59. Gupta A, Vijayaraghavan R, Gautam A. Combination Therapy of N-Acetyl-L-Cysteine and S-2(2-Aminoethylamino) Ethylphenyl Sulfide for Sulfur Mustard Induced Oxidative Stress in Mice. *Toxicol Rep* (2021) 8:599–606. doi: 10.1016/j.toxrep.2021.03.011

60. Lee CY, Su CH, Tsai PK, Yang ML, Ho YC, Lee SS, et al. Cadmium Nitrate-Induced Neuronal Apoptosis is Protected by N-Acetyl-L-Cysteine *via* Reducing Reactive Oxygen Species Generation and Mitochondria Dysfunction. *Biomed Pharmacother Biomed Pharmacother* (2018) 108:448–56. doi: 10.1016/j.biopha.2018.09.054

61. Holdiness MR. Clinical Pharmacokinetics of N-Acetylcysteine. *Clin Pharmacokinetics* (1991) 20(2):123–34. doi: 10.2165/00003088-199120020-00004

62. Shao A, Gao S, Wu H, Xu W, Pan Y, Fang Y, et al. Melatonin Ameliorates Hemorrhagic Transformation *via* Suppression of ROS-Induced NLRP3 Activation After Cerebral Ischemia in Hyperglycemic Rats. *Oxid Med Cell Longevity* (2021) 2021:6659282. doi: 10.1155/2021/6659282

63. Zhou R, Ma Y, Tao Z, Qiu S, Gong Z, Tao L, et al. Melatonin Inhibits Glucose-Induced Apoptosis in Osteoblastic Cell Line Through PERK-Eif2α-ATF4 Pathway. *Front Pharmacol* (2020) 11:602307. doi: 10.3389/fphar.2020.602307

64. Zhu HL, Shi XT, Xu XF, Zhou GX, Xiong YW, Yi SJ, et al. Melatonin Protects Against Environmental Stress-Induced Fetal Growth Restriction *via* Suppressing ROS-Mediated GCN2/ATF4/BNIP3-Dependent Mitophagy in Placental Trophoblasts. *Redox Biol* (2021) 40:101854. doi: 10.1016/j.redox.2021.101854

65. Shi L, Liang F, Zheng J, Zhou K, Chen S, Yu J, et al. Melatonin Regulates Apoptosis and Autophagy Via ROS-MST1 Pathway in Subarachnoid Hemorrhage. *Front Mol Neurosci* (2018) 11:93. doi: 10.3389/fnmol.2018.00093