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Pivotal Role of Mitogen-Activated Protein Kinase-Activated Protein Kinase 2 in Inflammatory Pulmonary Diseases

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Abstract

Mitogen-activated protein kinase (MAPK)-activated protein kinase (MK2) is exclusively regulated by p38 MAPK *in vivo*. Upon activation of p38 MAPK, MK2 binds with p38 MAPK, leading to phosphorylation of TTP, Hsp27, Akt and Cdc25 that are involved in regulation of various essential cellular functions. In this review, we discuss current knowledge about molecular mechanisms of MK2 in regulation of TNF- α production, NADPH oxidase activation, neutrophil migration, and DNA-damage-induced cell cycle arrest which are involved in the molecular pathogenesis of acute lung injury, pulmonary fibrosis, and non-small-cell lung cancer. Collectively current and emerging new information indicate that developing MK2 inhibitors and blocking MK2-mediated signal pathways is a potential therapeutic strategy for treatment of inflammatory and fibrotic lung diseases and lung cancer.

Keywords

Acute lung injury; inflammation; lung cancer; MK2; MAPK; pulmonary fibrosis

1. INTRODUCTION

The p38 MAPK mediated signal pathway has an important role in cell proliferation, differentiation, migration, and metabolism; therefore p38 MAPK has been considered as a prime drug target for treatment of severe sepsis, rheumatoid arthritis, cancer, diabetes, and pulmonary inflammatory diseases [1]. However, direct inhibition of p38 MAPK or p38 MAPK deficiency leads to unintended severe side effects, such as liver toxicity and severe heart diseases [2], which have limited any potential human applications related to directly

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

targeting p38 MAPK. Thus, any possible interdiction in the p38 MAPK signal pathway will of necessity require targeting downstream signals which have greater selectivity for limiting tissue inflammation and damage without disrupting normal physiological function. In this review article, we will focus on one of the key downstream kinases for p38 MAPK, mitogen-activated protein kinase (MAPK)-activated protein kinase (MK2). A focus on MK2 is warranted because MK2 is directly and exclusively activated by p38 MAPK *in vivo* [3], which makes it potentially an ideal drug target for harnessing the beneficial impact of blocking p38 MAPK and minimizing disruption of normal physiology. Furthermore, recent studies have shown that MK2 is involved in many disease processes, such as skin inflammation [4], tumor development [5], asthma [6], and pulmonary fibrosis [7]. Unlike p38 MAPK deficient mice which are embryonically lethal [8], MK2 deficient mice display normal embryonic development and fertility, which further supports the notion that targeting MK2 obviates the severe side effects that result from directly targeting p38 MAPK [9]. As a result, MK2 becomes a potential drug target and several pharmaceutical companies have been developing several therapeutic inhibitors specific targeting MK2 [10] although the impact of these therapeutic inhibitors is still under proprietary investigation.

Within the innate immune response, MK2 serves as an important kinase in regulation of inflammatory cell activation [3]. Exogenous microbial components termed pathogen-associated molecular patterns (PAMPs) or endogenous inflammatory factors released from necrotic cells bind to the germline-encoded pattern recognition receptors (PRRs) including Toll-like receptors (TLRs), NOD-like receptors (NLRs), and C-type lectin receptors (CLRs), which triggers the activation of MAPK cascades via the adaptor proteins myeloid differentiation primary-response protein 88 (MyD88) and TIR domain-containing adaptor protein inducing IFN β (TRIF) [11]. In the canonical signal transduction, p38 MAPK is selectively phosphorylated by MAPKKs (MKK3 and MKK6) which are in turn activated by MAPKKKs including TGF β -activated kinase 1 (TAK1), apoptosis signal-regulating kinase 1 (ASK1), mixed-lineage kinase 2 (MLK2), or MLK3. The p38 MAPK-mediated signals initiate the activation of several transcriptional factors including CREB, ATF2, and Myc, as well as other kinases including MK2, but also MK3, MNK1/2, and MSK1/2 [12]. Among these distal kinases, the role of MK2 has been painstakingly determined to be essential for the regulation of innate immune responses including modulating production of inflammatory cytokines and chemokines, reactive oxygen species (ROS), and nitric oxide. Given the evidence that various MK2 dependent inflammatory mediators are essential in regulating a variety of pulmonary diseases, it is highly likely that inhibition of MK2 would be beneficial in as a treatment for inflammatory pulmonary diseases. This review will highlight recent findings regarding the molecular mechanisms of MK2 in regulating inflammatory processes including cytokine and chemokine production, ROS production, neutrophil migration, and DNA-damage-induced cell cycle arrest, as well as the preclinical data showing the contribution of MK2 to several pulmonary diseases including sepsis-induced acute lung injury, pulmonary fibrosis, and lung cancer.

2. ROLE OF MK2 IN INFLAMMATORY CYTOKINE PRODUCTION (FIG. 1)

Inflammatory cytokines and chemokines are important in orchestrating immune cell activation and leukocyte recruitment in pulmonary diseases. Accumulating evidence

indicates that MK2 is involved in regulation of TNF- α and other cytokine biosynthesis by enhancing mRNA stability [13]. The AU-rich elements (AREs) located in the 3'-untranslated region (UTR) of TNF- α mRNA modulate its translation essentially blunting transcriptional regulation. In contrast, depletion of AREs enhances stabilization of TNF- α mRNA and increases TNF- α production in mice through amplification of transcriptional regulation [14]. Tristetraprolin (TTP), a zinc finger protein, and human antigen R (HuR) competitively bind to AREs to modulate the stability of TNF- α mRNA [14, 15]. In response to lipopolysaccharides (LPS) stimulation, TTP deficiency results in increased half-life of TNF- α mRNA in macrophages, indicating the inhibitory role of TTP on TNF- α mRNA post-transcription, which is strictly regulated by p38 MAPK/MK2 signal transduction [16]. In contrast, HuR enhances TNF- α transcription by binding to TNF- α mRNA AREs [15]. *in vivo* experiments indicate that LPS-induced TNF- α production is nearly abrogated in MK2 deficient spleen cells and macrophages [17]. Following TLR-mediated signal transduction, MK2 down-regulates TTP affinity to AREs of TNF- α mRNA via directly phosphorylating Ser52 and Ser178 of TTP [18], which facilitates HuR binding to AREs of TNF- α mRNA to promote TNF- α transcription [19] (Fig. 2). Since TTP binding to ARE within the 3'-UTR is also a general regulatory mechanism for IL-6 [20], IL-10 [21], GM-CSF [22], and CXCL1 [23], MK2 is also involved in regulation of these cytokines. Furthermore, butyrate response factor 1 (BRF1) is another potent stimulator that participates in AREs-mediated mRNA decay that is connected to MK2 kinase activity. MK2 inhibits BRF1 function via phosphorylation of Ser54, Ser92 and Ser203 of BRF1 [24]. Thus, MK2 can regulate inflammatory cytokine production in a synergistic manner via phosphorylating of TTP and BRF1.

There are several alternative mechanisms of MK2 inducing TNF- α production other than direct phosphorylation of TTP. MK2 deficiency results in p38 MAPK degradation, because MK2 can maintain p38 MAPK protein stability through direct interaction [25]. Although MK3, another downstream kinase of p38 MAPK, cannot directly regulate TNF- α , MK3 facilitates MK2 enhancement of TNF- α translation, since MK2 and MK3 double knockout macrophages produce less TNF- α than MK2 knockout macrophages in response to LPS treatment [26]. It is of possibility that MK3 increases the binding stability between p38 MAPK and MK2 [27]. Furthermore, MK2 can bridge crosstalk between p38 MAPK and Akt signals via forming a complex with p38 MAPK, Akt, and Hsp27; while this direct interaction is dissociated in MK2 deficient MEF cells [28]. Future studies are needed that focus on whether MK2 serves as an adaptor protein but not a kinase in this process. MK2/3 double deficient macrophages display attenuated phosphorylation of Ser473 and Thr308 of Akt in response to TLR stimulation, in which MK2/3 phosphorylate Akt through activating PDK1 and mTORC2, which are two upstream kinases for Akt and facilitate Akt membrane translocation [25]. Recent publications show that MK2 is also involved in endoplasmic reticulum (ER) stress responses. MK2 can directly phosphorylate Ser184 of Ube2j1, an ER-associated ubiquitin-conjugating E2 enzyme, which in turn contributes to LPS-mediated TNF- α expression. Although the molecular mechanism hasn't been demonstrated, Ube2j1 may provide a specific docking platform to enhance the translation of TNF- α mRNA on ER [29]. All together, these data indicate that LPS-mediated MK2 activation leads to several alternative signals for positively actuating cytokine production.

TLR-mediated signals are elegantly coordinated to avoid excessive inflammatory damage. In addition to the positive role of MK2, it serves as a negative feedback regulatory molecule in macrophage activation. In response to LPS stimulation, MK2 deficient macrophages display decreased expression of TTP [26, 30], indicating MK2 is required for inhibiting TTP expression. On one hand, MK2 induces TTP expression via a positive feedback mechanism, in which MK2 induces the phosphorylation of TTP that in turn binds to ARE region of itself mRNA and stabilizes TTP expression [31]. On the other hand, MK2 may regulate TTP expression via stabilization of p38 MAPK in macrophages, given TTP is an immediate early gene in response to different stimuli, in which p38 MAPK-mediated signal transduction contributes to TTP transcription [32]. Ectopic over expression of MK2 can rescue the transcription of TTP mRNA in MK2/3-deficient cells, further indicating that MK2 induces *de novo* synthesis of TTP at the transcriptional level [30].

MAPK phosphatases (MKPs) are protein phosphatases that negatively regulate immune responses via dephosphorylating activated MAPKs [33]. Depletion of MK2 attenuates the expression of MKP1 that can dephosphorylate p38 MAPK and JNK in macrophages [34]. Although p38 MAPK/MK2 does not regulate mRNA level of MKP1, MK2 is required for the post-transcriptional modulation of MKP1 expression [34]. All in all, MK2 is involved in both positive and negative feedback signals for cytokine production, which seems controversial but is orchestrated based on spatial and temporal regulation in macrophage activation (Fig. 1).

3. ROLE OF MK2 IN CELL MIGRATION AND NADPH OXIDASE ACTIVATION

Neutrophils, monocytes and macrophages constitute the frontier of the cellular component of the innate immune defenses against invading pathogens and foreign particles, and eliminating necrotic or apoptotic dying cells. Inflammatory cell migration and NADPH oxidase activation, two basic functions of phagocytes, contribute to eliminate foreign bacteria [35], but can also cause the tissue damage [36]. Thus, coordinative regulation of cell migration and NADPH oxidase activation can efficiently eliminate invaded bacteria as well as avoid the organ injury. Leukocyte-specific protein 1 (LSP1), a F-actin binding molecule, is involved in actin remodeling including actin filament assembly and disassembly and the interaction between actin filaments and myosin thick filaments [37]. *In vitro* kinase assay shows that MK2 directly phosphorylates the C-terminal domain of LSP1, indicating that MK2 regulates cytoskeletal structure and function [38]. Further study identifies that Ser243 of LSP1 in murine neutrophils or Ser252 in human neutrophils is the major phosphorylation site for MK2 [39]. Chemotaxis assay indicates that MK2 deficient neutrophils lack directionality, but display higher migration speed in response to Formyl-Methionyl-Leucyl-Phenylalanine (fMLF) stimulation [40]. On the contrary, p38 α MAPK deficient neutrophils have an attenuated capability of migration speed, in which p38 α MAPK directly phosphorylates the formyl peptide receptor 1 (FPR1) and drives neutrophil migration [41]. Given that MK2 is exclusively activated by p38 α MAPK, the possible interpretation is that p38 α MAPK acts as an “engine” and provides “power” for neutrophil chemotaxis, whereas MK2 serves as a “steering wheel” that directs neutrophils to reach target sites. Remarkably, the interaction between p38 MAPK and MK2 forms a stable complex [42], and the expression of p38 MAPK is reduced in MK2 deficient mice [3]. Future studies are necessary

to determine whether the stable complex is necessary for neutrophil function and interdiction of the interaction between p38 MAPK and MK2 can inhibit neutrophil migration.

In both *in vivo* and *in vitro* experiments, the p38 MAPK/MK2 signal pathway can dominantly phosphorylate HSP27, an ATP-independent chaperone that is implicated in cell migration and cell survival [43]. In resting state, several HSP27s bind together and form large chaperone multimers. When cells receive the active signals from TLR agonists, IL-1 β or TNF- α , MK2 is activated and phosphorylates and depolymerizes the complex of HSP27 that facilitates signal transduction, which promotes actin polymerization and increases cell migration [44]. As a dominant kinase for HSP27 phosphorylation, MK2 modulates cell migration in various cell types in response to different stimuli. Upon treatment of fMLF, TNF- α , PDGF, or VEGF, MK2 deficient macrophages display decreased formation of filopodia [45]. In addition, MK2 enhances fibronectin-mediated migration in embryonic fibroblasts (MEFs) and smooth muscle cells [45, 46]. MK2 promotes actin filament remodeling in endothelial cells and enhances endothelial cell migration via phosphorylating HSP27 and releasing its inhibition of actin polymerization [47]. Interleukin-1 β (IL-1 β)-induced proangiogenic processes in endothelial cells is dependent on p38 MAPK/MK2 signal pathway that regulates tube formation and endothelial cell migration [48]. Alternatively, MK2 can mediate the phosphorylation of LIM-kinase 1 (LIMK) at Ser-323, which in turn inhibits the function of cofilin, an actin-depolymerizing factor and modulates VEGF-mediated actin reorganization and migration of endothelial cells [49]. In addition to p38 MAPK directly activating MK2, MK2 SUMOylation is another alternative regulatory mechanism for cell migration. TNF- α induces SUMOylation of MK2 at lysine (K)-339 to modulate actin filament dynamics in endothelial cells [50]. These data indicate that MK2 plays an essential role in cell migration of neutrophil, macrophage, fibrosis and endothelial cell which are important for pulmonary diseases.

NADPH oxidase activation is involved in host defense against invading microorganisms [51]. In inflammatory disease, neutrophils release large quantities of superoxide anions, which can be directly converted to a variety of destructive ROS [35]. Phagocyte NADPH oxidase is a multi-protein phox complex consisting of membrane-associated proteins p22^{phox} and gp91^{phox}, and four cytosolic components: p47^{phox}, p67^{phox}, p40^{phox} and the small GTPase Rac [52]. Upon activation, p47^{phox}, p67^{phox}, and p40^{phox} are phosphorylated at multiple sites. When these phosphorylated phox proteins translocate to the membrane and interact with p22^{phox} and gp91^{phox}, electrons are transferred from NADPH and delivered to molecular oxygen to generate superoxide anions [53]. In particular, p47^{phox} phosphorylation on multiple serine/threonine residues, which recruits cytosolic components to the membrane, is required for NADPH oxidase activation [54] (Fig. 3A). Several kinases, including protein kinase C (PKC), Akt, and MAPKs, are able to induce NADPH oxidase activation via directly phosphorylating p47^{phox} [54] (Fig. 3A). Among these pathways, MAPK signal pathways have been implicated in both priming and activation processes. TNF- α , granulocyte-macrophage colony-stimulating factor (GM-CSF), or complement component 5a (C5a) can activate p47^{phox} phosphorylation through p38 MAPK by directly phosphorylating Ser345/Ser348 (human) and Thr356 (mouse) of p47^{phox} [36, 55]. In human

neutrophils, phorbol myristate acetate (PMA) and fMLF induce MK2 phosphorylation at Ser334 and further increase NADPH oxidase activation; while MK2 inhibitory peptide limits superoxide production, confirming the regulation role of MK2 in ROS generation [56]. Angiotensin II induces vascular injury by releasing inflammatory chemokines and ROS; down-regulation of MK2 by siRNA can attenuate Angiotensin II-induced NADPH oxidase activity and inhibits p47^{phox} membrane translocation in vascular smooth muscle cells (VSMCs) [57]. These data indicate that MK2 is involved in the modulation of NADPH oxidase activation. However, the regulatory mechanisms have yet been defined. Given that HSP27 serves as a scaffold protein to facilitate the interaction between MK2 and Akt and enhances the MK2-mediated Akt Ser473 phosphorylation, MK2 is considered as an atypical PDK2 for Akt activation and continuously provides survival signals for human neutrophils [58]. Because Akt2 can specifically induce p47^{phox} phosphorylation and membrane translocation [59], MK2 may regulate NADPH oxidase activation via activation of the Akt2-mediated signal. Since p38 MAPK also participates in p47^{phox} phosphorylation [36, 55], MK2 might also be involved in NADPH oxidase activation through feedback regulation of p38 MAPK activation. Therefore, the direct and indirect regulatory signal pathways of MK2 for NADPH oxidase activation should be addressed by investigators in the field in the future.

NADPH oxidase activation is important for host defense against a wide range of infection. The p47^{phox} knockout mice that are deficient in production of ROS display reduced elimination of bacteria after intravenous (i.v.) injection of *Escherichia coli* (ATCC-25922) (Fig. 3B). Patients with chronic granulomatous disease (CGD), in which phagocytes could not produce enough ROS, are more sensitive to pathogen infection [60]. Therefore, enhancing NADPH oxidase activation is able to benefit patients with bacterial clearance. However, excessive production of ROS is also harmful for tissue, which can exacerbate the inflammatory processes and cause tissue damage [51]. Thus, the “double-edged sword” of ROS production needs to be tightly regulated in order to maintain homeostasis and provide an effective host defense as well. During these processes, MAPK phosphatase 5 (MKP5) is a “braking” molecule for neutrophil functions via dephosphorylating p38 MAPK, which restrains NADPH oxidase activation and thereby prevents excessive ROS-mediated tissue injury [36]. Although p38 MAPK has been proven to modulate NADPH oxidase activation, the role of MK2 in neutrophil NADPH oxidase activation is still unknown and its regulatory mechanisms need to be determined in the future by using genetically deficient mice.

4. ROLE OF MK2 IN DNA DAMAGE-INDUCED CELL CYCLE ARREST

Maintaining genome integrity requires elegant and highly accurate regulation with interlocking mechanisms in cells [61]. Disorder of chromosomal stability causes nucleotide substitutions, insertions and deletions, which ultimately results in the formation of tumor [62]. Ultraviolet (UV) light, DNA ionizing radiation (IR), chemotherapeutic drugs, smoking, and ROS induce DNA lesion including double strand breaks (DSBs) and single strand breaks (SSBs) that separately initiate two major PI3-kinase-like kinases ataxia-telangiectasia mutated (ATM) and ataxia-telangiectasia and Rad3-related (ATR) [63]. ATM/Chk2 and ATR/Chk1 constitute parallel signal pathways to control the G1/S, the intra-S and the G2/M cell cycle checkpoints which interdicts DNA replication and initiates series of enzymes for repairing DNA lesion [63]. Cdc25 phosphatase family (Cdc25A, Cdc25B and Cdc25C)

plays an essential role to drive cells into S phase and M phase by activating CyclinB-Cdk1, CyclinA-Cdk2 and CyclinE-Cdk2 [64]. Both Chk2 and Chk1 serve as the canonical DNA damage kinases to phosphorylate Cdc25 and block cell cycle progression [65]. ATM and ATR induce the phosphorylation of p53, the most important tumor suppression molecule, which turns on a wide range of genes that leads to DNA repair, cell arrest or apoptosis [66]. ATM can also directly phosphorylates histone H2AX (γ H2AX), a mark for DnA damage response (DDR), that occurs on DNA repair and tethers broken DNA ends together [67].

Emerging data indicate that p38 MAPK-mediated signal pathway plays a non-redundant role in DNA damage-induced cell cycle arrest [68]. Topoisomerase II (topo II) inhibitors activate p38 MAPK that controls ATM independent G2/S checkpoint and delays from entering mitosis [68c]. Chemotherapeutic methylating agent temozolomide induces p38 MAPK activation which in turn inactivates Cdc25C and its downstream target Cdc2, suggesting p38 MAPK is involved in the regulation of chemotherapeutic methylating agent-induced G2 checkpoint bypass [68b]. The regulatory mechanism has been implicated with the activation of p53. DSBs-induced p38 MAPK signaling pathway mediates p53-dependent G2/M checkpoint that allows DNA repair and inhibits cellular transformation [69]. In addition, in response to UV radiation, p38 MAPK directly binds and phosphorylates Cdc25B at Ser309 that facilitates the binding of 14-3-3 proteins and initiates G2/M checkpoint [70]. These data indicate, besides Chk1 and Chk2, p38 MAPK-induced signaling pathway contributes to the cell cycle checkpoint.

UV light-mediated DNA damage leads to p38 MAPK/MK2 activation that acts as non-canonical checkpoint. Reduction of MK2 inhibits UV-induced G2/M, intra S, and G1 checkpoints and enhances DNA damage-induced cell death. MK2 directly phosphorylates Cdc25B and facilitates the binding between phosphorylation of Cdc25B and 14-3-3 proteins that trigger the stop of cell cycle and the start of G2/M checkpoint. Thus MK2 serves as a new DNA damage checkpoint kinase and is independent on canonical ATR/Chk1 and ATM/Chk2-mediated checkpoints [71]. Genotoxic drugs such as cisplatin, doxorubicin and camptothecin can activate p38 MAPK/MK2 signal pathway, which plays a vital role in DNA damage-mediated cell cycle arrest. DNA damage-induced cell cycle checkpoint is deficient in MK2-depleted p53 knockout MEFs but is normal in MK2-depleted p53 wild-type MEFs. The ATR/Chk1-mediated signal is involved in the activation of p38 MAPK/MK2 that finally targets Cdc25A/B and regulates intra S and G2/M checkpoint. Therefore, MK2 can substitute p53 to initiate DNA damage-induced checkpoint and facilitate DNA repair, which indicates targeting MK2 is a potential strategy for eliminating p53-defective tumor cells [72]. Upon stimulation of genotoxic drugs, ATR/Chk1 is important for establishing the G2/M checkpoint, whereas MK2 plays a vital role in prolong checkpoint maintenance via blocking Gadd45 α mRNA degradation and sequestering Cdc25B/C in the presence of DNA damage [73]. DnA damage-induced apoptosis and survival are orchestrated by the crosstalk between MK2 and p53. MK2 directly phosphorylates the transcriptional regulator apoptosis-antagonizing transcription factor (AATF) at Thr366 that binds to BAX and BAK promoter regions to protect from p53-mediated apoptosis [74]. In pancreatic ductal adenocarcinoma (PDAC), MK2 directly phosphorylates p53-binding protein ATDC at Ser550 and protects PDAC against ionizing radiation treatment, which is dependent on ATM-mediated signal pathway [75]. Down-regulation of MK2 attenuates the phosphorylation of H2AX and

protects cells from DNA-damage-mediated cell death in response to UV irradiation, gemcitabine or Chk1 inhibitor treatment, in which MK2 is required for DNA damage signaling and inhibits DNA replication [76]. Although there are contradictions in different groups, no doubt p38 MAPK/MK2 is a new signal pathway for controlling the checkpoint response.

5. ROLE OF MK2 IN PULMONARY DISEASES

5.1. Role of MK2 in Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS)

ALI is a clinical syndrome of permeability pulmonary edema and neutrophilic inflammation that causes arterial hypoxemia, impaired carbon dioxide excretion and dyspnea, which finally leads to refractory hypoxemia [77]. Pulmonary endothelial cells, epithelial cells and alveolar macrophages interact to maintain alveolar-capillary barrier and physiological homeostasis. Disorders of these cells that impair homeostasis can induce ALI and may cause patient death. In pathological conditions, invaded pathogen-induced sepsis and pneumonia, major trauma and drug reactions lead to injury of alveolar-capillary barrier and lung microvascular filtration that causes increased lung permeability and inflammatory cell infiltration [78]. Gaesterl's laboratory group generated MK2 deficient mice that are protected against LPS/D-galactosamine-induced acute liver injury. Production of TNF- α and nitric oxide (NO) was also significantly attenuated in these MK2 KO mice, and *ex vivo* experiment showed that production of TNF- α , IL-1 β , IL-6, IL-10, and INF- γ are decreased in LPS-challenged spleen cells [17a]. Further studies indicate that MK2 modulates the classic activation of macrophages, which are critical innate immune cells in TLR-mediated acute lung injury, in which MK2 is required for the production of both pro-inflammatory cytokines and anti-inflammatory cytokines [14, 16-17, 79]. MK2 not only regulates innate immune responses but also modulates endothelial apoptosis as evidenced by MK2 deficient mice resist to LPS-induced pulmonary vascular permeability and LPS-induced apoptosis in the whole lung tissue. Although MK2 deficient and wild-type mice have no difference in caspase 3 activation upon treatment with LPS, translocation of cleaved caspase 3 to nucleus to induce apoptosis is attenuated in MK2 deficient mice compared to wild-type mice. Similarly in human lung microvascular endothelial cells, down-regulation of MK2 prevents LPS-induced nuclear translocation of cleaved caspase 3 and resists to apoptosis. Therefore, MK2 has an important role in the regulation of apoptosis and pulmonary vascular permeability in sepsis-induced acute lung injury [80]. In response to LPS stimulation, Gr-1^{high} monocyte subsets generate more TNF, IL-6, iNOS, and COX-2 than Gr-1^{low} monocytes that are associated with enhanced p38 MAPK/MK2 activation [81]. Interestingly, MK2 plays an paradoxical response to different stimulus induced sepsis; although MK2 deficient mice show a decreased mortality in response to *i.v.* injection of LPS, MK2 deficiency is more sensitive to TNF or cecal ligation and puncture (CLP)-induced systemic inflammation which is associated with enhanced production of IL-1 β , IL-6 and nitric oxides and decreased number of F-actin structures [82]. These contradictory results indicate that MK2 is involved in regulation of specific signal pathways and has different functions in various cells. It is well known that LPS triggers TLR-4 signal pathways inducing severe cytokine storm and causing septic shock through NF- κ B and MAPK signal pathway [83].

Therefore, as one of the p38 MAPK downstream kinases, MK2 may preferentially regulate macrophage activation and lead to tissue injury by mediating LPS-induced cytokine production. However, in response to TNF treatment, MK2 may preferentially modulate endothelial barrier function and resist to TNF-induced tissue injury. Therefore, the regulatory mechanisms of MK2 in sepsis-induced acute lung injury need to be determined in the future studies.

Mechanical ventilation is a fundamental strategy for supporting patients with hypoxemic and hypercapnic respiratory failure, but also can result in ventilator associated lung injury (VALI). Mechanical stress induces the activation of p38 MAPK-MK2-Hsp25 signal pathway that causes actin polymerization and endothelial barrier dysfunction. Inhibition of p38MAPK or MK2 attenuates high tidal volume mechanical ventilation-induced acute lung injury and prevents pulmonary capillary permeability [84]. Similarly, in pulmonary microvascular endothelial cells, the p38 MAPK-MK2-Hsp27 signal pathway is activated in response to hypoxia that modulates formation of actin cytoskeleton [85]. Therefore, MK2 is involved in reorganization of the cytoskeleton formation in pulmonary endothelial cells and causes ventilator or hypoxia induced lung injury.

In response to stress, the signal axis of MKK3/p38 MAPK/MK2 is strictly regulated, in which MKK3 specifically phosphorylates p38 MAPK, but not JNK or ERK, while p38 MAPK preferentially phosphorylates MK2 [86]. Indisputably, the MKK3/p38 MAPK/MK2 signal axis has a critical role in regulation of sepsis-induced acute lung injury. Depletion of p38 α MAPK in macrophages cause significantly decreased production of TNF- α , IL-12 and IL-18, but it has no effect on production of IL-6, in response to LPS treatment. p38 α MAPK regulates the activation of C/EBP- β and CREB, but doesn't modulate NF- κ B, JNK and ERK signal pathway in LPS-induced macrophage activation. Macrophage-specific p38 α MAPK deficient mice resist to LPS or CLP-induced mouse sepsis, which indicates that p38 α MAPK plays a critical role in septic inflammation [87]. As predicted, MKK3 deficiency protects against LPS-induced acute lung injury and MKK3 deficient mice display attenuated inflammatory responses, mitochondrial ROS production and endothelial injury. In endothelial cells, MKK3 is required for ICAM-1 and VCAM-1 expression that mediate inflammatory cell infiltration. Therefore, MKK3 deficient endothelial cells exhibit less inflammatory cell adhesion and accumulation [88]. These results confirm that MKK3/p38 MAPK/MK2 signal pathway plays a critical role in acute lung injury.

In general, MAP kinase phosphatases (MKPs) are important negative regulators for MAPK signal pathways [89]. MKP1 deficient mice display enhanced inflammatory responses including more production of TNF- α , IL-6, IL-10, and iNOS as well as increased mortality in response to LPS and heat killed *Staphylococcus aureus* [90]. MKP1 deficiency prolongs the activation of p38 MAPK and JNK as well as enhances production of TNF- α and IL-6 in macrophages [90b]. In addition, MKP1 deficient mice exhibit accelerated high ventilation-induced lung injury that is associated with increased inflammatory cell infiltration and damaged endothelial barriers [91]. Interestingly, as a highly homologous phosphatase of MKP1, MKP2 deficient mice protect against LPS or CLP-induced sepsis that is associated with decreased production of TNF- α , IL-1 β and IL-6 [92]. LPS-induce acute lung injury is attenuated in MKP2 deficient mice with decreased neutrophil infiltration and cytokine

production [93]. Meanwhile, MKP2 deficient macrophages exhibit increased expression of MKP1 [93], which indicates that MKP2 regulates inflammatory responses via inhibiting MKP1 expression. MKP5 is another phosphatase and is involved in both adaptive and innate immune responses [94]. MKP5 deficient mice display increased ALI and adoptive transfer of MKP5 deficient macrophages can also lead to enhanced pulmonary inflammation. In response to LPS, MKP5 deficiency causes up-regulation of p38 MAPK, JNK and ERK in macrophages [95]. Given that MKP5 is constitutively expressed in macrophages [95], whereas MKP1 is inducible in response to LPS [90b], MKP5 and MKP1 may cooperatively inhibit p38 MAPK/MK2 signal pathway and prevent sepsis-induced ALI.

5.2. Role of MK2 in Idiopathic Pulmonary Fibrosis (IPF)

IPF is a chronic progressive disease in which the extracellular matrix (ECM) is generated and deposited in pulmonary interstitial fluid leading to breathing difficulty. Environmental exposures (e.g. asbestos, inorganic dusts, drug toxicity, or radiation) and genetic predisposition contribution (e.g. mutations of surfactant protein or telomerase components) have been considered as major reasons for IPF [96]. Pathogenesis of IPF is an abnormal pathological progress of wound healing. After type I alveolar epithelial cells (AECs) are injured, fibroblasts migrate to damage site and transform into α -smooth muscle actin (SMA)-expressing myofibroblasts that generate ECM proteins to develop fibrosis [97]. Proliferation and differentiation of fibroblasts, epithelial-mesenchymal transition (EMT) and transition of progenitors to fibroblasts are main mechanisms for generating fibroblasts and myofibroblasts [98]. In comparison to normal human lung tissue, lung tissue from IPF patients exhibits increased activation of MK2, in which the phosphorylation of MK2 Thr334 is up-regulated in epithelial cells and fibroblasts. This clinic relevant evidence implicates that MK2 plays a critical role in the pathogenesis of IPF. MK2 inhibitor, a 22 amino-acid cell-permeable peptide, significantly ameliorates the bleomycin-induced pulmonary fibrosis via attenuating TGF- β -induced α -SMA expression and myofibroblast differentiation. More importantly, pulmonary fibrosis is therapeutically attenuated by intraperitoneal injection with MK2 inhibitor 7 day or 14 day after bleomycin administration; suggesting MK2 is an excellent drug target for IPF patients [99]. By using MK2 deficient mouse embryonic fibroblasts, TGF- β -induced differentiation from fibroblasts to myofibroblasts is significantly attenuated in MK2 deficient fibroblasts, which is associated with decreased expression of α -SMA, indicating that MK2 inhibits myofibroblast transformation and promotes the expression of α -SMA expression in fibroblasts [100]. MK2 also play a critical role in the migration of mouse embryonic fibroblasts and smooth muscle cells that are required for the pathological formation of pulmonary fibrosis [45]. Interestingly, *in vivo* experiments, paradoxical phenotypes have been reported in MK2 deficient mice. In response to bleomycin treatment, MK2 deficient mice display more severe pulmonary fibrosis that is associated with more collagen accumulation and more fibrotic region formation in the lungs, compared to wild-type mice. *Ex vivo* experiments indicate that MK2 deficiency enhances fibroblast proliferation and increases the production of collagen, but inhibits fibroblast migration [7]. The possible interpretation is that the MK2 inhibitor only blocks MK2 activation but not affects the expression of MK2 in cells, while MK2 is completely lost in knockout mice, indicating MK2 has another regulatory mechanism that may be independent on MK2 kinase activation.

5.3. Role of MK2 in Lung Cancer

Lung Cancer is the leading cause of cancer death in the United States and worldwide [101]. Mutations in tumor repressor gene p53 are the most common reason for lung cancers including non-small-cell lung cancer (about 85% of all lung cancers) and small-cell lung cancer (about 15% of all lung cancers) [102]. Studies show that MK2 not only control p53 stability but also its protein expression level via HDM2. As a RING domain protein of E3 ligase, HDM2 regulates p53 stability via ubiquitinating p53 to mediate its degradation, which can directly phosphorylated by MK2 on serine 157 and 166 [103]. In addition, MK2 is required for the accumulation of phosphorylated histone H2AX in response to UV irradiation. Depletion of MK2 prevents cells from UV-induced apoptosis [76]. Furthermore, miR-34c can be induced by p38 MAPK signal, which inhibits the expression of c-Myc and DNA-damage-induced genome instability [104]. Although these mechanisms indicate that MK2 is a critical gene involved in cancer by controlling genome stability, the role of MK2 in lung cancer in humans has not yet demonstrated.

Given activation of p38 MAPK is consistently increased in non-small lung cell lung cancers [105] and MK2 is ubiquitous expressed in various cells and is strictly activated by p38 MAPK [3], it is predictable that MK2 is involved in regulation of lung cancers. By using an autochthonous model of non-small-cell lung cancer in which inducible expression of oncogenic Kras^{G12D} leads to the development of adenomas that is independent on p53 mutation [106], Yaffe's group identified that MK2 and p53 cooperatively regulate the development of non-small-cell lung cancer [5]. Although MK2 proficiency or MK2 deficiency don't affect the tumor initiation in Kras^{G12D}/p53^{-/-} mice, it does modulate tumor progression. MK2^{-/-}/p53^{-/-} tumor cells exhibit increased tumor progression, compared to MK2^{-/-}/p53^{+/+} tumor cells. Interestingly, in p53-deficient human NSCLC cell line H1299, MK2 deficient cells display more sensitivity to cisplatin treatment; whereas, in p53-functional cell line A549, down-regulation of MK2 has no effect on it. Furthermore, in mouse NSCLC model, MK2 and p53 co-deficiency extremely enhance the chemosensitivity to cisplatin treatment, but MK2 deficiency couldn't enhance the chemosensitivity in p53-functional tumor cells [5]. Given that p53 mutation exists in most of human lung cancer cells, it is no doubt that MK2 is an intriguing drug target for cancer therapy. Therefore, blockage of MK2 might be able to enhance chemotherapy or radiotherapy for p53 mutation NSCLC.

CONCLUSION

There is an emerging data set that MK2 has various functions in regulation of cytokine production, NADPH oxidase activation, cell migration, and DNA damage-induced cell cycle arrest in different cell types. MK2, an essential downstream kinase of p38 MAPK-mediated signal pathways, plays a critical role in TNF- α , IL-6, and IL-10 production via phosphorylating TTP and reducing its affinity for TNF- α mRNA AREs. In human neutrophils, MK2 enhances NADPH oxidase activation. Based on the data that p38 MAPK and Akt are involved in regulation of NADPH oxidase activation [55, 59] and MK2 can bind to p38 MAPK and activate Akt by binding with Hsp27 [44], it is possible that MK2 regulates NADPH oxidase activation through feedback modulation of p38 MAPK and Akt, which might also contribute to neutrophil migration. Therefore, the regulatory mechanisms

of the interaction and activation among p38 MAPK, Akt, MK2, and Hsp27 still need to be determined in the future. DNA-damage-induced cell cycle arrest allows cells to repair damaged DNA and prevents mutated gene to entry cell cycle. In addition to classic ATM/Chk2 and ATR/Chk1 signal pathways, p38 MAPK/ MK2 servers as an alternative signal pathway for Cdc25 phosphorylation and leads to cell arrest. Although MK2 and p53 cooperatively control cell cycle arrest, the cross talk between p53 and MK2 is required for determination.

Because MAPK-mediated signal pathway is involved in regulation of a lot of diseases, targeting this signal pathway is attractive for pharmaceutical companies; more specifically, targeting p38 MAPK is an initial idea for drug development. Due to p38 MAPK is important for normal physiological functions, specific p38 MAPK inhibitors lead to severe liver and CNS problems, which make these inhibitors fail to pass clinical trials [107]. Therefore, downstream molecules of p38 MAPK seem to be promising drug targets. Due to MK2 deficient mice exhibits attenuated phenotypes in various disease models, including collagen-induced rheumatoid arthritis [108], cutaneous inflammation [109], atherosclerosis [110], asthma [6], and sepsis-induced tissue injury [17a], MK2 is considered as an attractive pharmacological target for treatment of above diseases. PF-3644022 (Pfizer) is an ATP-competitive MK2 inhibitor that inhibits TNF- α production; however, it is terminated in clinical development because of poor serum availability and hepatotoxicity in animal models [10c]. Other than that, it is feasible to develop a non-ATP-competitive inhibitor to target p38 MAPK/ MK2 signal pathways. Collectively, in term of the pivotal role of MK2 in inflammatory pulmonary diseases, it is undoubted that MK2 is a potential drug target for reducing the burden of ALI, IPF and lung cancer.

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Biography

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Fig. (1).

MK2-mediated signaling in cytokine production. Toll-like receptors (TLRs) trigger the MK2-mediated signaling by in turn cascade activation of MAPKKK (TAK1/ASK1/MLK2/MLK3), MAPK (MKK3/MKK6), and p38 MAPK. As one of downstream kinases of p38 MAPK, MK2 directly phosphorylates tristetraprolin (TTP) and reduces its affinity for the cytokine AU-rich elements (AREs), which facilitates the binding of HuR to cytokine AREs and enhances cytokine mRNA stability including TNF- α , IL-6, IL-10, and GM-CSF. Alternatively, MK2 induces Akt activation through direct phosphorylation of Akt or indirect activation of Akt upstream molecules mTORC and PDK1. In addition, MK2 also mediates a negative feedback regulatory signal of p38 MAPK by inducing MKP1 expression.

Fig. (2). Regulation of cytokine mRNA stability by MK2-mediated signal. Tristetraprolin (TTP) binds to the ARE in 3' untranslated region of cytokine mRNA and facilitates mRNA degradation. After receiving LPS-mediated signal, MK2 directly phosphorylates and removes TTP from ARE region that allowing HuR to bind ARE and promote cytokine translation.

Fig. (3).

NADPH oxidase activation and its protective role in elimination of bacterial infection. **(A)** Phagocyte NADPH oxidase consists of membrane-associated proteins p22^{phox} and gp91^{phox} (Nox2), and cytosolic components: p47^{phox}, p67^{phox} and the small GTPase Rac. When phagocytes are stimulated with agonists such as fMLF and C5a, p47^{phox} and p67^{phox} are phosphorylated by PKCs, Akt, MAPKs, and MK2, and translocated to the membrane to form an active oxidase with p22^{phox}, gp91^{phox} and Rac-GTP. One electron is transferred from NADPH to molecular oxygen to produce a superoxide anion. **(B)** WT and p47^{phox} deficient mice were intravenously injected with 50 million *E. coli* (ATCC-25922) per mouse. After 4 hours, peripheral blood was collected and the number of bacteria (colony-forming unit, CFU) was calculated. Blood from p47^{phox} deficient mice displayed more CFU of *E. coli* than that from wild-type mice. **, $P < 0.01$.