NEK7: a potential therapy target for NLRP3-related diseases

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SUMMARY NLRP3 inflammasome plays an essential role in innate immunity, yet the activation mechanism of NLRP3 inflammasome is not clear. In human or animal models, inappropriate NLRP3 inflammasome activation is implicated in many NLRP3-related diseases, such as tumors, inflammatory diseases and autoimmune diseases. Until now, a great number of inhibitors have been used to disturb the related signaling pathways, such as IL-1β blockade, IL-18 blockade and caspase-1 inhibitors. Unfortunately, most of these inhibitors just disturb the signaling pathways after the activation of NLRP3 inflammasome. Inhibitors that directly regulate NLRP3 to abolish the inflammation response may be more effective. NEK7 is a multifunctional kinase affecting centrosome duplication, mitochondrial regulation, intracellular protein transport, DNA repair and mitotic spindle assembly. Researchers have made significant observations on the regulation of gene transcription or protein expression of the NLRP3 inflammasome signaling pathway by NEK7. Those signaling pathways include ROS signaling, potassium efflux, lysosomal destabilization, and NF-κB signaling. Furthermore, NEK7 has been proved to be involved in many NLRP3-related diseases in humans or in animal models. Inhibitors focused on NEK7 may regulate NLRP3 to abolish the inflammation response and NEK7 may be a potential therapeutic target for NLRP3-related diseases.

Keywords inhibitors, NF-κB signaling, NLRP3-related diseases, NEK7, NLRP3 inflammasome

1. Introduction

Innate immunity provides the first line of defense to recognize pathogens and endogenous stress via the pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) (1). The danger signals can be identified by pattern recognition receptors (PRRs). Inflammasomes are activated to form a "danger" by innate immune cell defense through the maturation and secretion of pro-inflammatory cytokines, like IL-1β. Among all the known inflammasomes, the NLRP3 inflammasome plays a central role in innate immunity (2). NLRP3 inflammasome is a multiple protein complex composed of NLRP3, apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and caspase-1 (3,4). The activation of caspase-1 results in the maturation and secretion of IL-1β and IL-18 (5,6) and the cleavage of GasderminD triggers pyroptosis (7-9). Thereafter, an inflammatory response is formed against the antigens of environmental or host origin.

The components of NLRP3 inflammasome often lead to susceptibilities to inflammatory diseases and cancer in humans (10). Until now, a great number of inhibitors have been used to disturb the related signaling pathways, such as IL-1β blockade, IL-18 blockade and caspase-1 inhibitors. Unfortunately, most of these inhibitors just disturb the signaling pathways after the activation of NLRP3 inflammasome. Inhibitors that directly regulate NLRP3 to abolish the inflammation response may be more effective. NIMA-related kinase 7 (NEK7) directly targets at NLRP3 inflammasome. Researchers have made significant observations on the regulation of gene transcription or protein expression of the NLRP3 inflammasome signaling pathway by NEK7. Those signaling pathways include ROS signaling, potassium efflux, lysosomal destabilization, and NF-κB signaling. Furthermore, NEK7 has been proved to be involved in many NLRP3-related diseases in humans or in animal models. Inhibitors focused on NEK7 may regulate NLRP3 to abolish the inflammation response and NEK7 may be a potential therapeutic target for NLRP3-related diseases.

2. The structure of NEK7 and its mechanism of action in basic biological functions (Table 1)

2.1. NEK7 structure and regulation
The NEK family was isolated by Ron Morris and his colleagues in cell division cycle mutants of Aspergillus nidulans (16). The NIMA-related kinase constitutes approximately 2% of all human kinases. NEK7 located in the centrosome is one of the smallest members of 11 NEK kinases, and is a highly conserved serine/threonine kinase among 302 kinases (17). NEK7 is expressed in a variety of organs, such as heart, brain, liver, lung and spleen (18). Human NEK7 consists of a non-conserved and disordered N-terminal regulatory domain as well as a conserved C-terminal catalytic domain (19). NEK7 promotes recruitment of pericentriolar material (PCMs) to centrosomes in G1 and S phase. The number of PCMs directly affects centrosome duplication efficiency (20). NEK7 is a multifunctional kinase affecting centrosome duplication, mitochondrial regulation, intracellular protein transport, DNA repair and mitotic spindle assembly (21-24). NEK7-deficient mice results in lethality in late embryogenesis or in early postpartum stages to severe growth retardation, which shows that NEK7 plays a critical role in growth and survival (27).

2.2. NEK7 in mitosis (Figure 1)

NEK9, NEK6, and NEK7 are required for the module of downstream kinases implicated in mitotic signal transduction (28). Structurally, human NEK7 and NEK6 share 86% identity in their C-terminal domains and only 20% identity in their disordered N-terminal extensions (29,30). The mitotic regulatory factors cycle dependent kinase 1 (CDK1) and Polo-like kinase 1 (Plk1) activate NEK9 to bind directly to NEK6 and NEK7, thus controlling cytokinesis (23,31). In early mitosis, NEK9, NEK7, NEK6, and the kinesin Eg5 form a signaling module downstream of Cdk1 and Plk1 required for centrosome separation and bipolar spindle formation (32-34). Both NEK7 and NEK6 kinase can phosphorylate α- and β-tubulin in vitro, as well as regulate the half-life of microtubules in interphase (35). NEK6 phosphorylates KIF11 to target centrosomes in order to promote centrosome separation (36). Nek6 controls microtubule binding activity of the central spindle in the telophase of Mklp2 (23). NEK9 activates NEK7 and NEK6 during mitosis to coordinate microtubule dynamics so as to promote spindle formation, centrosome nucleation and cytokinesis (35). Notably, both NEK6 and NEK9 do not interact with NLRP3 and are also dispensable for NLRP3 inflammasome activation.

3. The role of NEK7 in NLRP3-related diseases (Table 2)

3.1. NLRP3-related diseases and the regulation mechanism of NLRP3 inflammasome activation

Among the currently known inflammasomes, the nucleotide-binding oligomerization domain (NOD) and leucine-rich repeat pyrin (LRR) 3 domain (NLRP3) inflammasome are most widely studied (37). NLRP3 contains a pyrin domain at the N-terminus (PYD), a LRR at the C-terminus and the intermediate nucleotide triphosphatase domain (NACHT) mediated oligomerization (37,38). NLRP3 scaffold, apoptosis-
associated speck-like protein (ASC) and pro-caspase-1 are assembled into the NLRP3 inflammasome, activated caspase-1 induces secretion, the maturation of IL-1β and IL-18 as well as the induction of pyroptosis (39, 40).

The activation of NLRP3 inflammasome requires two steps. The priming signal enhances the production of NLRP3 and pro-IL-1β through the nuclear factor NF-κB pathway (41). The second signal is inflammasome activation, where caspase-1 induces the maturation of IL-1β and IL-18 for secretion, as well as pyroptosis (42). The exact mechanism of activating NLRP3 inflammasome remains unclear. Inactive NLRP3 inflammasome may be induced by Potassium (K⁺) efflux (43), increased intracellular Ca²⁺, decreased cellular cyclic AMP (cAMP) (44), oxidized mitochondrial DNA release (mtDN) (45), lysosomal destabilization, mitochondrial dysfunction as well as oxygen species (ROS) production (46, 47).

Recently, NEK7 has been proved to be involved in the activation of NLRP3 inflammasome in several pathways, such as ROS signaling, potassium efflux, lysosomal destabilization, and NF-κB signaling (18). Researchers have made significant observations on the regulation of gene transcription or protein expression of NLRP3 inflammasome signaling pathway by NEK7 in NLRP3-related diseases, including tumors, inflammatory diseases, and autoimmune diseases, such as squamous cell carcinoma of head and neck (11), hepatocellular carcinoma (13), and systemic lupus erythematosus (14), gout, atherosclerosis, type 2 diabetes, metabolic syndrome, age related macular degeneration, Alzheimer's disease, multiple sclerosis, and inflammatory bowel disease (15, 18).

### 3.2. NEK7 and tumors

#### 3.2.1. NEK7 and tumors

Chronic inflammation plays a significant role in the occurrence and development of cancer (48, 49). The tumor microenvironment contains many different inflammatory cells and mediators. Tumor pro-inflammation is considered to be one of the favorable features for cancer development. Both inflammation and innate immunity are essential causes of cancer (50). Numerous studies have shown that NLRP3 inflammasome is involved in the progression of liver failure and liver disease (51-55). The down-regulation of NLRP3 inflammasome in liver cancer inhibits the proliferation and metastasis of hepatoma cells (13, 54). The activation of NLRP3 inflammasome leads to severe hepatic inflammation and fibrosis, and induces hepatocellular carcinoma apoptosis and pyroptosis (55-57).

Delayed cell cycle progression in knockout of NEK7 in head and neck squamous cell carcinoma suggests that NEK7 may play an important role in development of squamous cell carcinoma of head and neck through mitosis (58). NLRP3 is upregulated in head and neck squamous cell carcinoma as well as delays tumorigenesis in head and neck squamous cell carcinoma mice by reducing IL-1β secretion (11, 59). MCC950 blocks interaction between NEK7 and NLRP3. MCC950 inhibits activation of NLRP3 inflammasome and IL-1β secretion in mice with head and neck squamous cell carcinoma, as well as enhances anti-tumor immunity (60). The combination of MCC950+oATP does not induce cell death in normal cells, yet it can specifically kill cancer cells of head and neck squamous cell carcinoma without harming normal cells (59). Kooi et al. (25) indicated that NEK7 expression is generally up-regulated in retinoblastoma cell lines when compared to normal retinal pigment epithelial cells. Downregulation of NEK7 inhibits cell proliferation by inducing cell cycle arrest in retinoblastoma cells (61).

### 3.2. NEK7 and inflammatory diseases

#### 3.2.2. NEK7 and inflammatory diseases

Diabetic retinopathy is a highly specific neurovascular complication associated with inflammation which can cause severe visual impairment or even blindness (62, 63). NLRP3, caspase-1 and IL-18 levels are significantly elevated in patients with diabetic retinopathy (63, 64). NLRP3 inflammasome plays a vital role in the late stage of diabetic retinopathy in Akimba Mice (65). Limiting IL-1β affects retinal microglial activation and proliferation, which may affect neurological changes in diabetic patients (66). Downregulation of NEK7 by

### Table 2. The role of NEK7 in NLRP3-related diseases

| Research in animal model or in humans | Tumor | Inflammatory diseases | Autoimmune diseases | Ref |
|---------------------------------------|-------|----------------------|---------------------|-----|
| MCC950 blocks the interaction between NEK7 and NLRP3. MCC950 inhibits the activation of NLRP3 inflammasome and IL-1β secretion in mice with head and neck squamous cell carcinoma, as well as enhances anti-tumor immunity. | The downregulation of NEK7 by siRNA inhibits the activation of NLRP3 inflammasome in diabetic retinopathy; MCC950 attenuates high glucose-induced retinal endothelial cell dysfunction by disrupting the binding of NEK7 to NLRP3. | The expression of NEK7-NLRP3 complex may play a protective role in the pathogenesis of systemic lupus erythematosus and is negatively correlated with disease activity; NEK7 interacts with NLRP3 to modulate the pyroptosis in inflammatory bowel disease via NF-κB signaling | 12, 14, 60, 93-95 |
siRNA inhibits the activation of NLRP3 inflammasome in diabetic retinopathy, and MCC950 attenuates high glucose-induced retinal endothelial cell dysfunction by disrupting the binding of NEK7 to NLRP3 (12). The pulmonary ischemia-reperfusion injury is characterized by acute aseptic inflammation, alveolar damage and vascular permeability (67). Studies have shown that MCC950 significantly reduces the lung ischemia-reperfusion injury by blocking the interaction between some NEK7-NLRP3 (65). The mice deficient in NLRP3 inflammasome can regulate the inflammation of lung ischemia-reperfusion injury by reducing IL-1β production (68). Ozone protects lung from lung ischemia-reperfusion by reducing NR3 and alleviating lung damage caused by oxidative stress and inflammation (69).

3.2.3. NEK7 and autoimmune diseases

Autoimmune diseases are characterized by excessive immune responses that cause damage and dysfunction in specific organs or tissues (70). Although the treatment of the disease continues to strengthen, the prognosis of most patients is not satisfactory. Therefore, new targets for clinical treatment are urgently sought. Current study finds that the expression of NEK7-NLRP3 complex may play a protective role in the pathogenesis of systemic lupus erythematosus and is negatively correlated with disease activity (14). With the progression of lupus nephritis, NLRP3 gene expression is significantly increased in MRL/lpr mice and inhibiting the activation of NLRP3 inflammasome in MRL/lpr mice can reduce lupus nephritis (71,72). Mice lacking caspase-1 are resistant to systemic lupus erythematosus-associated vascular injury (73). Improvement of survival and proteinuria in IL-18-deficient MRL/lpr mice or MRL/lpr is seen in mice treated with anti-IL-18 (74). The serum IL-18 levels are positively correlated with the lupus nephritis disease activity (75).

Although the specific cause of inflammatory bowel disease remains unclear, studies have shown that NLRP3 inflammasome plays a key role in the development of chronic intestinal inflammation, and the secretion of IL-1β and IL-18 is positively correlated with the colitis disease activity (76,77). Existing studies find the NEK7 mRNA, protein expression and cell-cause-related factors, including Caspase-1 (p45, p20), NLRP3 and GSDMD in inflammatory bowel disease. Currently, our study demonstrates the relationship between NEK7 and inflammatory bowel disease. Our research found that NEK7 interacts with NLRP3 to modulate the pyroptosis in inflammatory bowel disease via NF-κB signaling.

4. Mechanism and regulation of NEK7-NLRP3 inflammasome activation

4.1. The mechanism of NEK7 regulating the activation of NLRP3 inflammasome (Figure 2)

Although there is abundant evidence that the activation of NLRP3 inflammasome regulates inflammatory diseases, its upstream regulatory mechanism remains unclear. Recent studies have shown that NEK7 can directly regulate the activation of NLRP3 inflammasome (78-80). The N-terminal region of NEK7 interacts with the C-terminal leucine-rich repeats (LRRs) and the nucleotide-binding domain (NOD) of NLRP3 (78,80). NEK7 plays a key role in the reduction of microtubule dynamic stability during interphase and phosphorylation of α- and β-tubulin in vitro. Acetylated α-tubulin mediates the dynein-dependent transport of mitochondria as well as the subsequent apposition of ASC on mitochondria to NLRP3 on the endoplasmic reticulum. The integrity of the cellular microtubule network is crucial for the activation of NLRP3 inflammasome (81,82). The increase of α-tubulin acetylation in NEK7-knockout cells indicates that NEK7 is involved in microtubule acetylation during the activation of NLRP3 inflammasome. All these results indicate a close correlation between NEK7 and NLRP3 inflammasome.

Although NEK7 is related to NLRP3 inflammasome, the exact mechanism between NEK7 and NLRP3 still remains unknown. K+ efflux is essential for inducing the activation of NLRP3 inflammasome through multiple stimuli. The assembly of NLRP3 inflammasome can be activated by a variety of exogenous stimuli, including nigericin, gramicidin, extracellular ATP, pore-forming protein toxins, and so forth. These NLRP3-activated stimuli directly disrupt the permeability of plasma membrane to K+ and reduce [K+] in the cytoplasmic membrane (83). Recent results indicate that Ne7k is an essential mediator of NLRP3 activation downstream of K+ efflux (78). Ne7k deficiency mice result in reduced IL-1β secretion, attenuated recruitment of immune cells and decreased disease severity when compared to wild-type mice (78,80). Ne7k binds to the NLRP3 LRRs in a kinase-independent manner downstream from the induction of mitochondrial ROS (49,80). ROS is a mediator that activates the binding of NEK7 and NLRP3 (84). Chloride intracellular channels (CLIC) act downstream of the K+ efflux-mitochondrial ROS axis to promote the activation of NLRP3 inflammasome, and the CLICs-dependent chloride efflux is indispensable for the activation of NLRP3 inflammasome (85).

4.2. NEK7 as a switch for mitosis and NLRP3 inflammasome activation

Under homeostasis conditions, NEK7 appears to be in a low activity status. Nevertheless, when homeostasis is disturbed, NEK7 will show abnormal expression (18). The abnormal expression of NEK7 induces the production of abnormal cells, including apoptotic cells and multinucleate cells which are closely related to mitosis and inflammation (58,80). NEK7 is involved in the formation of microtubule structures...
as well as the regulation of microtubule dynamic stability during interphase (86, 87). The microtubule-mediated assembly and the activation of the NLRP3 inflammasome (81, 82), and the NEK7-NLRP3 interaction are related to microtubule polymerization. Besides, NEK7 is required for the activation of both mitosis and NLRP3 inflammasome (49, 80). The levels of acetylated α-tubulin are similar in NEK7-deficient and NEK7-sufficient macrophages, which suggests that NEK7 does not activate NLRP3 inflammasome by regulating the microtubule dynamics signals during mitosis (49, 80). Nek6 and Nek9 are not associated with the activation of NLRP3 inflammasome. Thus, the activation of NLRP3 inflammasome is not associated with NEK7 kinase activity (78). The amount of NEK7 binding to NLRP3 in interphase is more than mitosis. Hence, the amount of NEK7 can only support mitosis or the activation of NLRP3 inflammasome (80). NEK7 acts as a cell switch during mitosis to avoid ineffective or potentially harmful inflammatory reactions (80). The expression of NEK7 in liver cancer is significantly higher than that in normal liver tissue, and the 5-year survival rate of patients with high expression of NEK7 is significantly lower (88). It is suggested that NEK7 may regulate the proliferation and metastasis of hepatoma cells by regulating the activation of NLRP3 inflammasome.

5. Discussion and future perspectives

As various diseases are linked to NLRP3-induced pyroptosis and IL-1β/18 secretion. IL-1β and IL-18 inhibitors have been widely used to disturb the related signaling pathways (89-91). Reported inhibitors include IL-1β blockade (neutralizing IL-1β antibody canakinumab, recombinant IL-1 receptor antagonist anakinra, the soluble decoy IL-1 receptor rilonacept), IL-18 blockade (GSK1070806), targeting inflammasome constituents (Parthenolide, Bay 11-7082, β-Hydroxybutyrate, VX-740 and VX-765), indirect NLRP3 inhibitors (Glyburide, 16673-34-0, JC124, FC11A-2), ASC inhibitors (cysteinyl leukotriene receptor antagonist), and P2X7 antagonists (AZD9056). (92-93) However, these inhibitors just disturb the signaling pathways of NLRP3 Inflammasome activation rather than directly regulate NLRP3 to abolish the inflammation response. Furthermore, inhibitors aimed at IL-1β or IL-18 can result in unintentional immunosuppressive effects. Therefore, inhibitors targeting the NLRP3 inflammasome only may be
a better option for treatment of NLRP3-associated diseases.

Until now, there are some reported direct inhibitors of NLRP3 Protein, including MCC950, 3,4-Methylenedioxy-β-nitrostyrene, Tranilast, and Oridonin. Among these inhibitors, oridonin binds to cysteine 279 of NLRP3 to abolish NLRP3-NEK7 interaction, and then blocks the activation of NLRP3 inflammasome (94).

Based on the knowledge of available structure of NLRP3, future studies should focus on the development of structure-guided direct inhibitors and inhibitors, which may regulate NLRP3 to abolish the inflammation response. Recent research has made great progress in the mechanism of NEK7 in the activation of mitosis and NLRP3 inflammation. NLRP3-induced pyroptosis has been reported, and our own research also found that NEK7 interacts with NLRP3 to modulate the pyroptosis in inflammatory bowel disease via NF-kB signaling (95). Inhibitors focused on NEK7 may regulate NLRP3 to abolish the inflammation response with improved specificity and potency (18).

The source of NEK7 activation and regulation remains unclear. Understanding the molecular mechanisms of NEK7 assembly and activation may lead to novel therapeutic targets to formulate therapies in the treatment of NLRP3-related diseases.

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