

# NLRP3 inflammasome in health and disease (Review)

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**Abstract.** Activation of inflammasomes is the activation of inflammation-related caspase mediated by the assembly signal of multi-protein complex and the maturity of inflammatory factors, such as IL-1 $\beta$  and IL-18. Among them, the Nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome is the most thoroughly studied type of inflammatory corpuscle at present, which is involved in the occurrence and development of numerous human diseases. Therefore, targeting the NLRP3 inflammasome has become the focus of drug development for related diseases. In this paper, the research progress of the NLRP3 inflammasome in recent years is summarized, including the activation and regulation of NLRP3 and its association with diseases. A deep understanding of the regulatory mechanism of NLRP3 will be helpful to the discovery of new drug targets and the development of therapeutic drugs.

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## 1. Introduction

Nod-like receptor (NLR) family pyrin domain containing 3 (NLRP3) inflammasome is mainly expressed in the cytoplasm of natural immune cells such as macrophages, and it is the most deeply studied inflammatory corpuscle complex at present. When NLRP3 is specifically stimulated, it recruits the connexin apoptosis-associated spot-like protein (ASC) and the effector protein pro-caspase-1 to start the assembly and activation of the inflammatory complex. The activated inflammatory body of NLRP3 mediates the self-shearing of pro-caspase-1 and produces caspase-1 with enzyme activity. On the one hand, caspase-1 can cleave pro-IL-1 $\beta$  and pro-IL-18, and promote the maturation and secretion of IL-1 $\beta$  and IL-18; on the other hand, caspase-1 can cleave gasdermin D (GSDMD) and release its N-terminus. The N-terminus of GSDMD forms a hole in the cell membrane and induces inflammatory cell death, that is, pyroptosis (1). The NLRP3 inflammasome is activated not only by a variety of ligands from pathogens and environmental sources, such as microbial cell wall components, nucleic acids, Alum, silica, aluminium hydroxide, nanoparticles, crystalline silicon dioxide, carbon nanotubes and chitosan, but also by endogenous danger signals, such as lipopolysaccharides (LPS), adenosine triphosphate (ATP), uric acid crystal, serum amyloid, prion protein, biglycan, hyaluronan, islet amyloid polypeptide, hydroxyapatite, haeme, oxidized mitochondrial DNA and membrane attack complex (2-4). The activation of the NLRP3 inflammasome plays an important role in pathogen clearance and adaptive immune response induction. However, the abnormal activation of the NLRP3 inflammasome leads to excessive inflammatory response, which in turn promotes the occurrence and development of numerous inflammatory diseases. Therefore, it is involved in the occurrence and development of many major human diseases, including Alzheimer's disease (AD), autoimmune diseases and arteriosclerosis (5,6). Therefore, the NLRP3 inflammasome is an important target to treat these major human diseases. It is of great significance to clarify the activation and regulation mechanism of NLRP3 inflammatory corpuscles and develop therapeutic drugs for the NLRP3 inflammasome. In this paper, the activation mechanism of the NLRP3 inflammasome and a series of related diseases are reviewed in order to provide a theoretical basis and new ideas for the prevention and treatment of inflammation-related diseases.

## 2. Structural characteristics of the NLRP3 inflammasome

The NLRP3 inflammasome is a high-molecular-weight intracellular multi-protein complex, which consists of NLRP3, apoptosis-associated speck-like protein containing card (ASC) and pro-caspase-1. NLRP3 is a member of the NLRs protein family, including three domains: An N-terminal pyrin domain (PYD), a central NBD-containing ATPase domain called NACHT and a C-terminal Leucine-rich repeat (LRR). ASC is a scaffold protein connecting NLRP3 and pro-caspase-1, and pro-caspase-1 is an inactive precursor of caspase-1. Caspase-1 produced by its activation is the effector protein of the NLRP3 inflammatory body. NLRP3 recruits ASC under the action of various agonists to form the NLRP3 inflammasome by combining ASC with pro-caspase-1 (7-9).

## 3. Activation mechanism of NLRP3 inflammasome

The NLRP3 inflammasome is closely related to numerous major human diseases, so it is important to clarify its activation mechanism. Since the concept of the NLRP3 inflammasome was first put forward in 2002, Kelley *et al* (10) discovered the classical, non-classical and alternative pathways of NLRP3 inflammasome activation. In this chapter, the activation mechanisms of these three pathways will be briefly introduced.

### *Classical activation pathway of the NLRP3 inflammasome.*

The classical activation pathway of the NLRP3 inflammasome includes priming and activation. The priming process means that cells are exposed to various stimuli, such as Toll-like receptors (TLRs) and NLD-like receptors, and then the transcription factor NF- $\kappa$ B is activated, thus upregulating the expression of pro-IL-1 $\beta$  and NLRP3 (10,11). The activation process means that cells treated by the priming process are activated by pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), and proteins such as NLRP3, ASC and pro-caspase-1 are assembled into inflammatory corpuscles, thus inducing cell apoptosis and IL-1 $\beta$  and IL-18 to activate the NLRP3 inflammasome. It is generally thought that there are three kinds of molecular and cellular signaling events induced by NLRP3 agonists (11,12) (Fig. 1).

**Ion flow.** Intracellular K<sup>+</sup> outflow is the first signaling pathway to activate the NLRP3 inflammasome (13). Previous studies have confirmed that low-K solution promotes the activation of the NLRP3 inflammasome, while high-K solution can inhibit the activation of the NLRP3 inflammasome by blocking K<sup>+</sup> outflow, and numerous NLRP3 activators, such as bacteriocin, ATP, granular molecules and crystalline substances, directly cause intracellular to extracellular flow of K<sup>+</sup>, thus activating the NLRP3 inflammasome (13). Further mechanistic research confirmed that the purinergic receptor (P2X7) on the cell membrane and the K family member 6/TWIK2 of the K<sup>+</sup> double-pore channel are jointly responsible for regulating the intracellular K<sup>+</sup> outflow, which leads to the activation of the NLRP3 inflammasome (14). However, previous research shows that certain small molecules activate the NLRP3 inflammasome without relying on potassium ion outflow, such as imiquimod, CL097 and peptidoglycan-induced activation of the NLRP3 inflammasome, and accordingly, K<sup>+</sup>

outflow is a sufficient condition for activation of the NLRP3 inflammasome, but it is not a necessary condition (15,16). It is worth noting that the outflow of K<sup>+</sup> is closely related to the potassium-acid balance in the body, and acidosis will lead to the release of K<sup>+</sup> from the inside to the outside of the cell, thus increasing the concentration of K<sup>+</sup> in the blood. Rajamäki *et al* (17) confirmed that extracellular acidosis activates NLRP3 inflammatory corpuscles in macrophages, which leads to enhanced caspase-1 processing and IL-1 $\beta$  secretion. Acute exposure to an acidic environment did not induce the activation of NLRP3 inflammatory bodies, which indicates that an acidic pH is not a direct activation factor (18).

The role of Ca<sup>2+</sup> flow in the activation of inflammatory corpuscles in NLRP3 is still controversial. As a second messenger, Ca<sup>2+</sup> has an important role in cell signal transduction and the disorder of Ca<sup>2+</sup> flow has disastrous consequences for cells. Ca<sup>2+</sup> receptor activator C promotes the expression of inositol 1,4,5-triphosphate and induces the release of Ca<sup>2+</sup> from the endoplasmic reticulum, thus activating the NLRP3 inflammasome (19). Early studies have confirmed that Ca<sup>2+</sup> chelating agent BAPTA-AM inhibited the activation of the NLRP3 inflammasome and the secretion of IL-1 $\beta$  (20-22). Furthermore, Katsnelson *et al* (23) also found that numerous NLRP3 agonists cause changes of intracellular Ca<sup>2+</sup>. However, certain studies have also found that the Ca<sup>2+</sup> chelating agent BAPTA-AM is independent of Ca<sup>2+</sup> flow, indicating that Ca<sup>2+</sup> flow may not be necessary for the activation of NLRP3 inflammasome, but it may play a regulatory role in this process.

One study indicated that a decrease in the Cl<sup>-</sup> concentration enhanced the activation of the NLRP3 inflammasome and the secretion of IL-1 $\beta$  (24). On the contrary, an increase in the concentration of extracellular Cl<sup>-</sup> inhibited the secretion of IL-1 $\beta$ . Certain studies further confirmed that Cl<sup>-</sup> channel inhibitors inhibit the activation of NLRP3 inflammasome (24,25). Furthermore, chloride intracellular channels (CLIC), the Cl<sup>-</sup> channel, is involved in the activation of the NLRP3 inflammasome. As the next event of mitochondrial reactive oxygen species (ROS), the outflow of Cl<sup>-</sup> mediated by CLICs induces the activation of NLRP3 inflammasome by promoting the interaction between NIMA-related kinase 7 (NEK7) and NLRP3 (24). However, it remains elusive how the outflow of Cl<sup>-</sup> enhances the interaction between NEK7 and NLRP3. In addition, another study found that WNK lysine-deficient protein kinase 1 regulates Cl<sup>-</sup>/cation synergistic transporter and reduces the outflow of Cl<sup>-</sup> to inhibit the activation of NLRP3 inflammasome, but Cl<sup>-</sup>/cation co-transporter not only regulates the outflow of Cl<sup>-</sup>, but also affects the outflow of K<sup>+</sup> (26). Furthermore, the intracellular Cl<sup>-</sup> efflux induced by low-Cl<sup>-</sup> solution does not activate the NLRP3 inflammasome, and accordingly, Cl<sup>-</sup> efflux may trigger the activation of NLRP3 inflammasome by cooperating with other signaling pathways (27).

Na<sup>+</sup> was previously reported to be involved in the activation of the NLRP3 inflammasome. The results showed that decreasing the concentration of extracellular Na<sup>+</sup> could inhibit the activation of the NLRP3 inflammasome induced by Nigericin and bacitracin, but Na<sup>+</sup> could not affect the activation of NLRP3 inflammasome induced by ATP and other factors, indicating that Na<sup>+</sup> influx alone is not sufficient to activate the

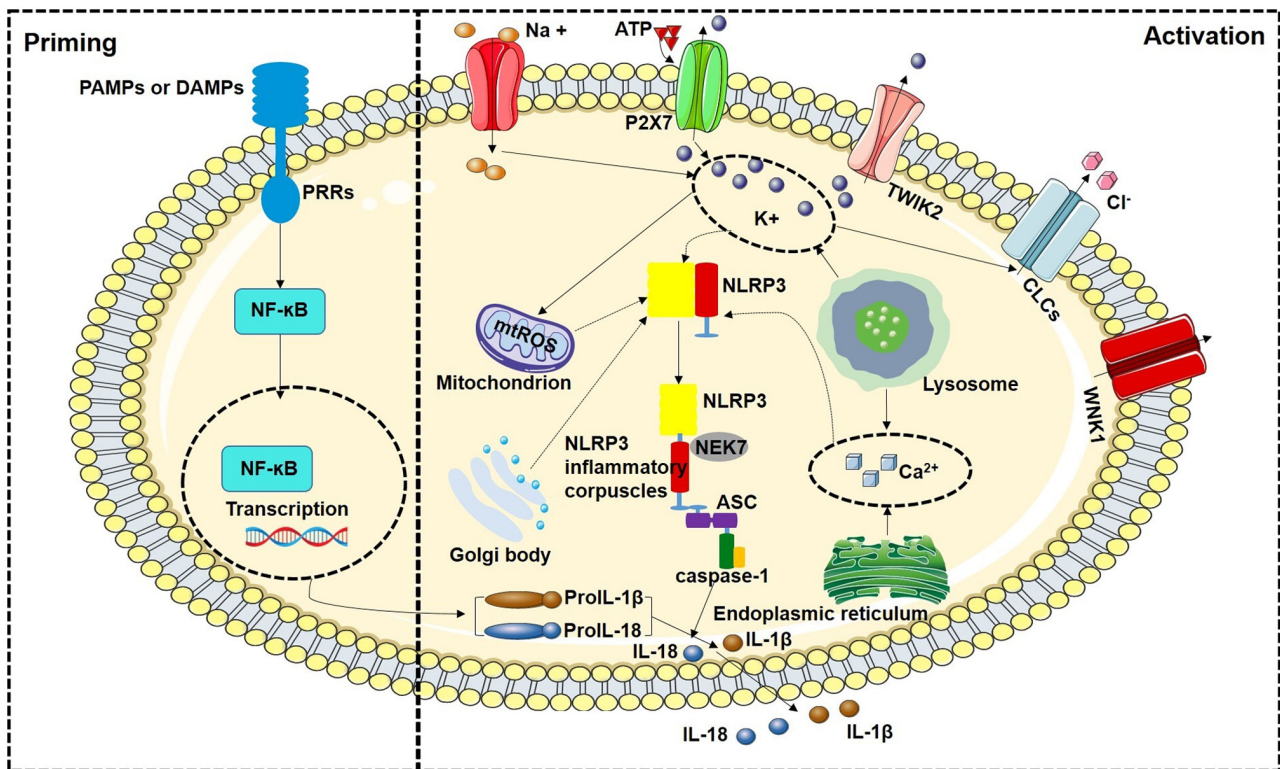


Figure 1. Schematic diagram of classical pathway regulation mechanism of NLRP3 inflammasome activation. The activation of NLRP3 inflammasome includes two processes: Priming and activation. Priming process: NF-κB signal transduction induced by PAMPs or DAMPs upregulates the transcription levels of NLRP3, Pro-IL-1β and Pro-IL-18. Activation process: It includes the composition of NLRP3 inflammasome and the activation of Caspase-1. After activation of Caspase-1, Caspase-1 transforms Pro-IL-1β and Pro-IL-18 stored in cells into active IL-1β and IL-18 and releases them to the outside of cells. PAMPs, pathogen-associated molecular patterns; DAMPs, damage-associated molecular patterns; NLRP3, Nod-like receptor family pyrin domain containing 3; mtROS, mitochondrial reactive oxygen species; NF-κB, nuclear factor κB; ASC, connexin apoptosis-associated spot-like protein; PRRs, pattern recognition receptors; TWIK2, the two-pore domain Weak Inwardly rectifying K<sup>+</sup> channel 2; CLC, chloride intracellular channels; WNK1, recombinant WNK lysine deficient protein kinase 1; NEK7, NIMA-related kinase 7.

NLRP3 inflammasome, which may be due to the synergistic effect of Na<sup>+</sup> influx and intracellular K<sup>+</sup> outflow, and the activation of the NLRP3 inflammasome by Na<sup>+</sup> influx depends on K<sup>+</sup> (13). Therefore, Na<sup>+</sup> influx may indirectly activate the NLRP3 inflammasome by triggering intracellular K<sup>+</sup> outflow.

**Generation of ROS.** The production of ROS is an important signal for the activation of the NLRP3 inflammasome. Most NLRP3 agonists induce cells to produce ROS. Under normal physiological conditions, there is a low level of ROS in the body, which participate in the process of cell proliferation and apoptosis. However, when the body is in a pathological state, mitochondrial damage leads to an increase in ROS production, thus activating the NLRP3 inflammasome. In 2011, Zhou *et al* (28) discovered for the first time that the accumulation of damaged mitochondria would increase the production of ROS, which in turn would activate the NLRP3 inflammasome. Subsequently, a large number of studies confirmed that the increase of ROS induced by various factors would lead to the activation of NLRP3 inflammasome (29). In the follow-up study, Shimada *et al* (30) found that certain NLRP3 agonists promote the release of mitochondrial DNA into the cytoplasm in a way that depends on mitochondrial ROS, and oxidized mitochondrial DNA was indicated to be the key substance to activate NLRP3 inflammasome. However, certain scholars have found that linezolid and bacitracin promote the activation of the NLRP3 inflammasome in a way independent

of mitochondrial ROS, and even certain serum β-amyloid proteins and viruses can activate NLRP3 inflammasome independent of ROS (13,29,31).

**Lysosome rupture.** In 2008, it was found for the first time that amyloid β protein induced lysosomes to dissolve, which in turn activated the NLRP3 inflammasome (32). Furthermore, Lima *et al* (33) found that a series of granular substances, such as urate crystals and asbestos, could damage lysosomes after being swallowed by cells, which led to the leakage of lysosomes into the cytoplasm, and then activated the NLRP3 inflammasome. When particulate matter (such as urate crystals) is swallowed into lysosomes, ion flow and intracellular osmotic pressure will change, bile will lead to K<sup>+</sup> outflow and activation of NLRP3 inflammasome, and tissue protease inhibitors also inhibit particle-induced activation of the NLRP3 inflammasome (34). Cathepsin B was originally considered the key lysosomal enzyme, as its inhibitor inhibits the activation of the NLRP3 inflammasome, and particulate matter also promotes the release of cathepsin when activating the NLRP3 inflammasome (32,35). However, in a cell experiment with cathepsin B deficiency, Dostert *et al* (36) found the activation level of NLRP3 inflammasome to be equivalent to that of wild-type cells, which is also contrary to previous research results. Orłowski *et al* (37) have also found that, besides inhibiting cathepsin B, the expression of cathepsin X, L or S genes has no effect on the activation of the NLRP3

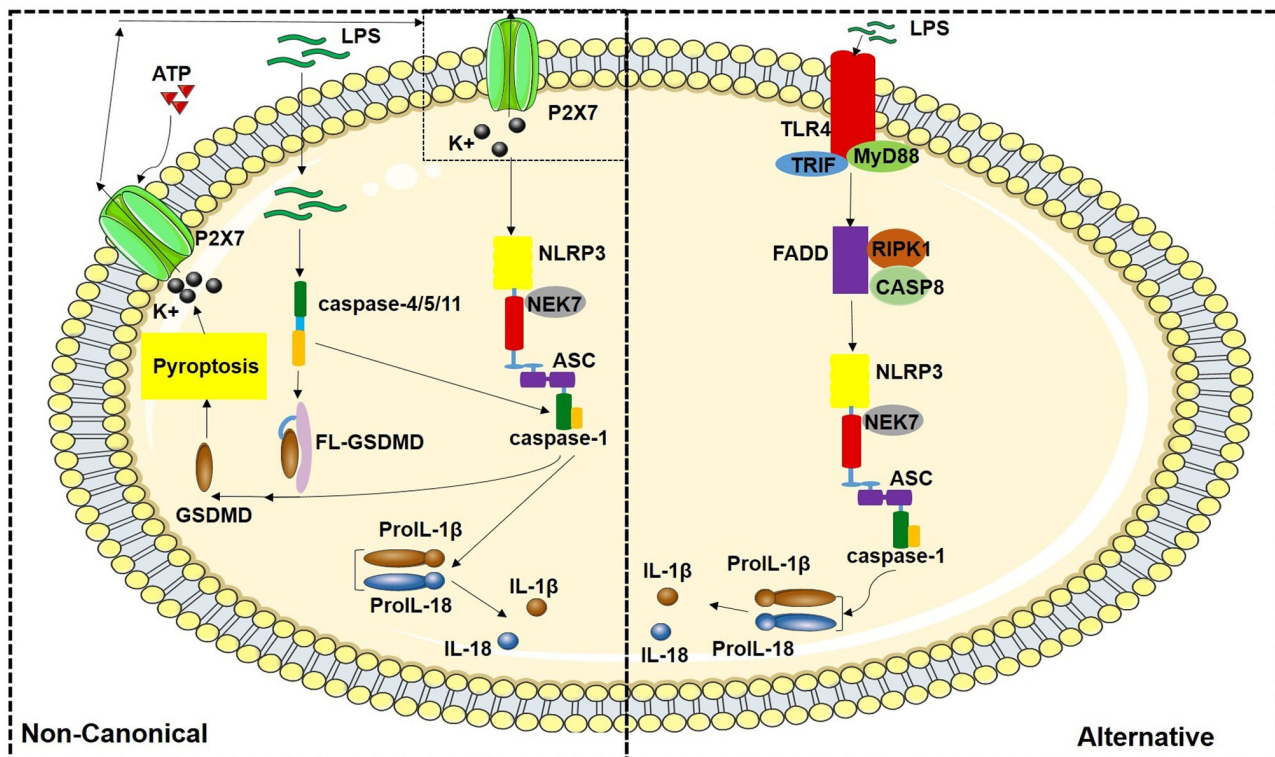


Figure 2. Schematic diagram of the non-classical pathway of NLRP3 inflammasome activation and regulation mechanism of the alternative pathway. LPS in cytoplasm can activate non-classical NLRP3 inflammatory corpuscles mediated by human caspase-4/5 or mouse caspase-11. LPS stimulation alone can activate NLRP3 inflammasome of the alternative pathway through TLR4 signaling. NLRP3, Nod-like receptor family pyrin domain containing 3; LPS, lipopolysaccharide; TLR, Toll-like receptor; GSDMD, gasdermin D; ASC, connexin apoptosis-associated spot-like protein; CASP, caspase; FL-GSDMD, full-length GSDMD; NEK7, NIMA-related kinase 7; TRIF, TIR-domain-containing adaptor-inducing interferon- $\beta$ ; MyD88, myeloid differentiation factor 88; RIPK1, serine/threonine kinase-1; FADD, Fas-associated protein with death domain.

inflammasome. However, the activation of NLRP3 inflammasome by exogenous particles is accompanied by  $K^+$  outflow and  $Ca^{2+}$  inflow, which indicates that lysosomal cleavage may trigger an ion current in the process of activation of the NLRP3 inflammasome, thus activating the NLRP3 inflammasome, so the signal of  $K^+$  and  $Ca^{2+}$  slurry flow is a downstream event of lysosomal rupture (37).

**Non-classical activation pathway of the NLRP3 inflammasome.** In addition to the above classical pathways, more and more scholars have found that there are other activation pathways for the NLRP3 inflammasome. The molecular mechanism of the activation pathway of the non-classical NLRP3 inflammasome was studied and the researchers found that mouse caspase-11 activates the NLRP3 inflammasome by directly recognizing LPS released into the cytoplasm during infection by Gram-negative bacteria. This process is known as the non-classical activation pathway of the NLRP3 inflammasome (38,39). In further research, related researchers found that the secretion of IL-1 $\beta$  was significantly inhibited in mice with caspase-11 gene knockout infected with Gram-negative bacilli. Kayagaki *et al* (39) reasoned that this was because the cells with caspase-11 gene knockout lacked the step of caspase-11 to cut its substrate protein GSDMD. In general, only after this step is completed can the substrate protein form a cavity on the cell membrane. Then it causes  $K^+$  outflow, apoptosis and activation of the NLRP3 inflammasome (the function of human caspase-4/5 is similar to that of mouse

caspase-11) (39). Gram-negative bacteria in the cytoplasm release LPS directly, thus activating caspase-4/5/11. Activated caspase-4/11 can cleave GSDMD, resulting in the formation of cell membrane pores and cell death, but caspase-4/5/11 itself does not have the function of cleaving pro-IL-1 $\beta$  and pro-IL-18. Cell membrane pores formed by GSDMD promote the activation of NLRP3 inflammasome by triggering  $K^+$  outflow, thus inducing the activation of caspase-1 and the mature secretion of IL-1 $\beta$  and IL-18 (40-42) (Fig. 2).

**Alternative activation pathways of the NLRP3 inflammasome.** In addition to the classical and non-classical pathways, there is an alternative activation of the NLRP3 inflammasome. It has been shown that LPS stimulation activates inflammatory bodies in monocytes through its receptor TLR4 signaling pathway. Most scholars find that LPS stimulation causes ATP to be released outside the cells, and then combine with ATP-gated  $K^+$  channel P2X7, resulting in  $K^+$  outflow, which is the key to the activation of the NLRP3 inflammasome (43). Others have confirmed that LPS stimulation activates NLRP3 inflammasome through the TLR4/TIR-domain-containing adaptor-inducing interferon- $\beta$ /serine/threonine kinase-1/Fas-associated protein with death domain/caspase 8 signaling axis, promoting the activation of caspase-1 and the mature secretion of IL-1 $\beta$  and IL-18 (44). However, it is interesting that the alternative pathways have obvious cell and species characteristics, which has only been verified in human and pig monocytes and mouse dendritic cells (43,45) (Fig. 2).

Table I. NLRP3 inflammasome drives respiratory system diseases.

| Disease                        | Related mechanism                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | (Refs.)            |
|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|
| COPD                           | Epithelial cells, macrophages and dendritic cells in the lung, stimulated by harmful particles or gases, activate the NLRP3 inflammasome through the NF- $\kappa$ B signaling pathway or P2X7 receptor pathway, thus promoting the release of inflammatory factors such as IL-1 $\beta$ and IL-18, and leading to inflammatory reaction.                                                                                                                                                                                                                                      | (46-54)            |
| Bronchial asthma               | Airway macrophages, epithelial cells and dendritic cells are stimulated by allergens to activate the NLRP3 inflammasome and then promote the release of IL-1 $\beta$ and IL-18 to induce inflammation.                                                                                                                                                                                                                                                                                                                                                                        | (55-70)            |
| Silicosis                      | SiO <sub>2</sub> can stimulate the assembly of adaptor factors such as NLRP3 and ASC in macrophages, promote the secretion of IL-1 $\beta$ under the mediation of caspase-1 and participate in the occurrence of diseases.                                                                                                                                                                                                                                                                                                                                                    | (71-73)            |
| Bacterial infectious pneumonia | NLRP3 inflammasome mediates the lung injury induced by $\alpha$ -hemolysin, and $\alpha$ -hemolysin activates the processing and secretion of IL-1 $\beta$ in a way similar to pore-forming toxin, which further aggravates the progress of <i>Staphylococcus aureus</i> pneumonia. <i>Streptococcus pneumoniae</i> hemolysin can also interfere with the plasma membrane and cause K <sup>+</sup> outflow, and then activate the NLRP3 inflammasome, trigger the secretion of IL-1 $\beta$ and IL-18, and aggravate the course of <i>Streptococcus pneumoniae</i> pneumonia. | (74-77)<br>(78-80) |

NLRP3, Nod-like receptor family pyrin domain containing 3; COPD, chronic obstructive pulmonary disease; ASC, connexin apoptosis-associated spot-like protein; NF- $\kappa$ B, nuclear factor  $\kappa$ B; P2X7, ATP receptor.

#### 4. NLRP3 inflammasome-related diseases

NLRP3 is an important aseptic inflammatory signal receptor and a key regulator of chronic inflammatory diseases. It was first discovered in 2001 and then further confirmed, researchers have found that the autosomal dominant mutation of the NLRP3 gene leads to a series of inflammatory diseases and the NLRP3 inflammasome has attracted extensive attention in related disease research fields. The NLRP3 inflammasome is subject to research on diseases in almost every system, but there is no doubt that the diseases of the respiratory system, cardiovascular system, digestive system, bone and joint system and central nervous system are the most important diseases.

*NLRP3 inflammasome drives respiratory system diseases.* Various pathogens in the air get in contact with pattern recognition receptors co-expressed by airway epithelial cells, alveolar macrophages or neutrophils in the lung, activating downstream signal transduction and triggering innate immune response. Studies have shown that the early immune response of the lung to harmful stimuli can be mediated by the NLRP3 inflammasome, which promotes the release of inflammatory factors, thus playing a protective role, but excessive inflammatory response will aggravate tissue damage and lead to a series of respiratory diseases. This part summarizes the research on NLRP3 inflammasome in the respiratory system (Table I).

*NLRP3 inflammasome participates in chronic obstructive pulmonary disease (COPD).* COPD is an irreversible chronic progressive lung disease characterized by continuous airflow limitation. The exact cause of COPD has so far remained elusive. Inhaling harmful particles or gases is an important cause of COPD and airway inflammation. These substances act on pulmonary epithelial cells, macrophages and dendritic cells, accelerating the synthesis and release of inflammatory factors and tissue-degrading enzymes, accelerating the

development of inflammation and the destruction of lung structure. Previous studies have confirmed that inhalation of harmful substances directly activates the lung recognition receptor TLR, thus activating the downstream NF- $\kappa$ B signaling pathway, and promotes the transcription of NLRP3 and related inflammatory factors (46). Furthermore, these harmful substances also cause massive death of lung cells, lead to the release of a variety of endogenous dangerous molecules and further promote the activation of the NLRP3 inflammasome. The decrease of extracellular nucleotidase expression and the increase of ATP in patients with COPD further activates the NLRP3 inflammasome through the P2X7 receptor and ATP also promotes the activation of inflammatory cells through the P2X7 receptor, which aggravates the inflammatory reaction in the lung (47,48). At the same time, oxidative stress is another important mechanism of activation of the NLRP3 inflammasome. A large number of studies have found that during the occurrence and aggravation of COPD, the level of ROS in the lung is significantly increased and the infiltration of immune cells in the airway further increases the production of oxygen free radicals and oxidative stress in the lung, which has been confirmed in animal experiments. IL-1 $\beta$  production and confirmed cell infiltration were significantly inhibited in NLRP3 gene knockout mice. In addition, the activity of caspase-1 and the levels of inflammatory factors such as IL-1 $\beta$  and IL-18 in lung tissue of COPD patients was also significantly increased, suggesting that the NLRP3 inflammasome was indeed activated during the occurrence and development of COPD (49,50).

So far, there is little research on the role of NLRP3 inflammasome in the progression of COPD. Certain scholars have found that the specific expression of IL-1 $\beta$  in mouse lung epithelial cells leads to increased infiltration of macrophages and neutrophils in the lung, destruction of elastic fibers in the alveolar septum, fibrosis of the airway wall and dilatation of

small airways at the distal end of the lung (51). In addition, overexpression of IL-18 increased the level of inflammation in the lungs of mice and promoted the formation of emphysema and pulmonary hypertension in mice. However, knocking out IL-1R or IL-18 and intervention with IL-1 $\beta$  neutralizing antibody significantly reduced lung injury and inflammatory reaction in mice (52). Similarly, application of caspase-1-specific inhibitors also reduced infiltration of inflammatory cells in the lung of mice and reduced the level of IL-1 $\beta$  (53). Furthermore, it has been observed that P2X7 receptor-specific inhibitors or knockout of P2X7 can inhibit the activation of caspase-1 and the release of IL-1 $\beta$  in a smoking-induced lung injury model in mice, thus alleviating the symptoms of COPD (54).

*NLRP3 inflammasome involved in bronchial asthma.* Bronchial asthma is a kind of airway hyperreactive chronic inflammation involving numerous cells and its main feature is extensive and variable reversible expiratory airflow restriction (55). It has been shown that asthma pathogens promote macrophages, epithelial cells and dendritic cells to release ATP, activate the NLRP3 inflammasome and enhance airway inflammation. In addition, high levels of ATP and P2X7 receptor were detected in the lungs of asthmatic patients (56). Inhalation of allergens is one of the important causes of asthma. Yazdi *et al* (57) found that allergens promote the production of ROS in the airways and ROS activates the NLRP3 inflammasome, thus increasing the maturity and release of IL-1 $\beta$  and IL-18. An associated study detected an increase in caspase-1 activity and IL-1 $\beta$  levels in a mouse asthma model induced by ovalbumin and alumina, and the IL-1 $\beta$  level was positively correlated with caspase-1 activity (58). In loss-of-function experiments, researchers found that neutralizing lung ATP or applying non-selective purinergic receptor antagonists reduced the infiltration of inflammatory cells and airway hyperresponsiveness in asthmatic mice induced by ovalbumin and alumina, and the application of caspase inhibitors also reduced airway inflammatory reaction in asthmatic mice (56,59). Overexpression of IL-1R antagonist by recombinant adenovirus or direct deletion of IL-1 may also significantly reduce the airway hyperresponsiveness caused by allergens in mice and alleviate inflammation around the airway (60,61). In clinical research, Harada *et al* (62) found that high levels of IL-1 $\beta$  can also be detected in the sputum of asthmatic patients, and it is positively associated with the severity of the disease.

Type 2 T-helper (Th2) cells are closely related to bronchial asthma, which increases airway reactivity of sensitizing eosinophils (63). Knocking out NLRP3 or caspase-1 inhibits the activation of Th2 cells and reduces airway eosinophils and factors related to the activation of Th2 cells. Accordingly, knocking out IL-1 $\beta$  or IL-1R can also significantly inhibit the activation of Th2 cells, which proves that NLRP3 inflammatory corpuscles participate in the occurrence and development of bronchial asthma (64–66). IL-18 has been proved to promote Th2 cells to secrete IL-4, IL-5, IL-9 and IL-13. Lung-specific overexpression of IL-18 increases the infiltration of inflammatory cells such as T cells, eosinophils and neutrophils in the airway of asthmatic mice induced by ovalbumin and enhances airway hyperresponsiveness (67). On the contrary, knocking out IL-18 significantly alleviates asthma symptoms induced by allergens such as ovalbumin in mice (68). Of note, the

NLRP3 inflammasome may not be involved in airway hyperresponsiveness caused by ovalbumin and Allen *et al* (69) also confirmed that knocking out NLRP3 did not affect airway allergic response of mice with four different allergic asthma models. Even Hartwig *et al* (70) found that IL-18 has nothing to do with the occurrence of asthma and can even inhibit the progress of asthma, which also shows that the role of the NLRP3 inflammasome in asthma requires further study.

*Role of NLRP3 inflammasome in silicosis.* Silicosis is one of the most important occupational diseases in the world, which is caused by crystalline silicon dioxide (SiO<sub>2</sub>) (71). In the whole disease development process of silicosis, NLRP3 inflammasome is involved and occupies a large proportion. Studies have shown that SiO<sub>2</sub> can be used as an activating molecule to stimulate the assembly of NLRP3 and ASC in macrophages, promote the secretion of IL-1 $\beta$  under the mediation of caspase-1 and participate in the occurrence of diseases. At the same time, ROS produced by cells is also used as the upstream signal of the NLRP3 signaling pathway to activate the NLRP3 inflammasome. In subsequent animal experiments, Cassel *et al* (72) found that compared with wild-type mice, ASC<sup>-/-</sup> and NLRP3<sup>-/-</sup> mice had less lung inflammation and pulmonary fibrosis, which further confirmed that the NLRP3 inflammasome is involved in the development of silicosis. Jessop *et al* (73) also found that autophagy of macrophages can inhibit the activation of the NLRP3 inflammasome induced by SiO<sub>2</sub> stimulation by chelating or degrading inflammatory corpuscles and cytokines, thus delaying the progress of subsequent diseases.

*NLRP3 inflammasome is involved in the course of bacterial infectious pneumonia.* Community-acquired and hospital-acquired infectious pneumonia are mainly caused by *Staphylococcus aureus* (74). It has been proved that  $\alpha$ -hemolysin is the key virulence factor to induce lung inflammation, which has the function of activating the processing and secretion of IL-1 $\beta$ , and its function of inducing cell necrosis requires the signal transduction of NLRP3 inflammasome (75). Animal experiments have confirmed that NLRP3 inflammasome mediates  $\alpha$ -hemolysin-induced lung injury. Compared with wild-type mice, NLRP3<sup>-/-</sup> mice have no decreased lung compliance and no obvious inflammatory reaction after being infected with pneumonia, which proves that NLRP3 inflammasome plays a role in mediating lung injury caused by pneumonia (76).

*Streptococcus pneumoniae* hemolysin is the main virulence factor of *Streptococcus pneumoniae*, which causes the formation of cavities on the cell surface and leads to cell lysis tissue damage (77). Furthermore, *Streptococcus pneumoniae* hemolysin can also interfere with the plasma membrane and cause K<sup>+</sup> outflow, which further activates the NLRP3 inflammasome, triggering the secretion of IL-1 $\beta$  and IL-18, and leads to a series of downstream inflammatory reactions. It has been shown that IL-1 $\beta$  is necessary to resist pneumococcal infection and the production of IL-1 $\beta$  depends on NLRP3 inflammasome and TLR2; accordingly, the NLRP3 inflammasome is an important mediator of the innate immune response to pneumococcal infection (78,79). van Lieshout *et al* (80) also found that compared with wild-type mice, the levels of cytokines and chemokines expressed by NLRP3<sup>-/-</sup> mice decreased, among which the level of IL-1 $\beta$  decreased significantly and the

Table II. NLRP3 inflammasome and digestive system diseases.

| Disease                                      | Related mechanism                                                                                                                                                                                                                       | (Refs.)   |
|----------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Hp-related stomach diseases                  | Hp infection may lead to the activation of intracellular NLRP3 inflammasome in gastric epithelial cells, mononuclear macrophages and lymphocytes, and then produce a large number of active IL-1 $\beta$ and IL-18 to induce diseases.  | (81-89)   |
| Liver diseases                               | Activation of NLRP3 inflammasome in liver tissue can promote the production of active IL-1 $\beta$ and IL-18, and finally lead to the occurrence and development of liver fibrosis.                                                     | (92-105)  |
| Inflammatory intestinal diseases             | Activation of NLRP3 inflammasome in intestinal mucosa or epithelial cells can promote the production of active IL-1 $\beta$ and IL-18, which leads to intestinal inflammation and hinder the repair mechanism of intestinal epithelium. | (107-125) |
| Acute pancreatitis/acute severe pancreatitis | Activation of NLRP3 inflammasome in acinar cells leads to the massive production of IL-1 $\beta$ and IL-18, which eventually leads to inflammation and injury of the pancreas and peripancreatic tissues.                               | (127-132) |

NLRP3, Nod-like receptor family pyrin domain containing 3; Hp, *Helicobacter pylori*.

animals died within 48 h after being infected with bacteremia, while wild-type mice only showed mild lung inflammation. It shows that NLRP3 plays a beneficial role in pneumonia caused by *Streptococcus pneumoniae* in the early stage (80). In short, the NLRP3 inflammasome is widely involved in the occurrence and development of bacterial infectious pneumonia.

**NLRP3 inflammasome and digestive system diseases.** As a part of the innate immune system, the NLRP3 inflammasome can be activated by various metabolic stimulation signals, among which the main effector molecules, IL-1 $\beta$  and IL-18, play an important role in the occurrence and development of digestive system diseases. As the key link of IL-1 $\beta$ - and IL-18-mediated inflammatory reaction, the NLRP3 inflammasome plays an essential role in the occurrence of digestive system diseases (Table II).

**NLRP3 inflammasome and *Helicobacter pylori* (Hp)-related stomach diseases.** Hp is an aerobic gram-negative bacterium that causes gastric mucosal inflammation in the stomach. The infection rate of Hp was reported to be high and it has been colonized in the gastric epithelial cells of healthy individuals accounting for >50% of the world population (81). Hp infection is closely related to numerous kinds of stomach diseases and persistent Hp infection is related to complications such as chronic gastritis, ulcer and even gastric cancer, among which the relationship between Hp infection and gastric cancer has been studied the most. However, so far, the initiation of the pro-inflammatory signaling cascade in gastric epithelial cells caused by Hp infection has remained elusive and the specific pathogenesis of gastrointestinal diseases caused by Hp infection remains to be further studied (82). However, Pachathundikandi *et al* (83) have confirmed that the activation of the NLRP3 inflammasome and the production of a large number of pro-inflammatory factors, including IL-1 $\beta$ , are the key factors for the occurrence and development of Hp-related gastrointestinal diseases (83). Hp infection induces the production of various cytokines, which promotes the aggregation and infiltration of a series of immune cells, such as monocytes, macrophages and lymphocytes, into the gastric mucosa, thus leading to related gastric diseases. In this process, cytokines

play the role of the initiator and IL-1 $\beta$  is one of the most critical cytokines (84-86). Caspase-1 is the key enzyme for the differentiation and maturation of IL-1 $\beta$ , and IL-1 $\beta$  can transform the precursor of IL-1 $\beta$  into mature IL-1 $\beta$  through its shearing action. However, the production of caspase-1 depends on the activation of the NLRP3 inflammasome. In this process, pro-caspase-1 is cleaved to form caspase-1 with biological activity, which leads to massive secretion of IL-1 $\beta$  and induces diseases (11,12). In a groundbreaking study, Kameoka *et al* (87) found that Hp infection leads to increased IL-1 $\beta$  secretion and this process is achieved through the NLRP3 inflammasome, which initially confirmed the possibility that Hp infection can induce gastric diseases by activating NLRP3 inflammasome. A clinical study on gastric cancer confirmed that Hp induces gastric cancer by activating the NLRP3 inflammasome in the mononuclear phagocyte system and promoting the release of inflammatory cytokines (87). Of course, this process can also be achieved by activating NLRP3 inflammasome in gastric mucosal epithelial cells GES-1 through ROS pathway or Hp toxin-related protein CagA (88). Of note, during the study of peptic ulcer disease induced by Hp infection, Davari *et al* (89) found that the decrease of IL-1 $\beta$  protein expression and the increase of NLRP3 expression promoted Hp to develop digestive tract ulcer. Therefore, it is of great significance to explore the mechanism of activation of the NLRP3 inflammasome in Hp-related stomach diseases to further the understanding of the prevention of Hp-related stomach diseases and even gastric cancer.

**NLRP3 inflammasome and liver diseases.** Liver disease has become a major global health problem. According to relevant surveys, millions of individuals worldwide die of liver disease every year. Liver diseases are mainly divided into nonalcoholic fatty liver disease (NAFLD) and alcohol-related liver disease (ALD), among which NAFLD is the most common chronic liver disease. Both NAFLD and ALD can lead to fibrosis and cirrhosis and increase the risk of hepatocellular carcinoma if they are not properly controlled (90,91). Wree *et al* (92) found that the NLRP3 inflammasome, pro-IL-1 $\beta$ , pro-IL-18 and pro-caspase-1 were significantly increased in the liver tissue of patients with NAFLD. In addition, NAFLD is

often associated with obesity, type 2 diabetes and hyperlipidemia (93). Lee *et al* (94) found that NLRP3 and IL-1 $\beta$  are increased in peripheral blood mononuclear cells and serum of patients with type 2 diabetes. Furthermore, the expression of NLRP3 inflammasome was also increased in the liver tissues of obese patients and mice (95). In a further experimental study, it was found that the NLRP3 inflammasome may play different roles in the process of NAFLD developing into nonalcoholic steatohepatitis (NASH). In the early NAFLD animal model, increased NLRP3 inflammasome components in the liver was only observed at the mRNA level. However, after NAFLD completely developed into NASH, the mRNA and protein levels of NLRP3 inflammasome (caspase-1 and IL-1 $\beta$ ) in the liver tissue and serum of the animal model increased significantly. In the reversal experiment of mice with NLRP3, ASC and caspase-1 gene deficiency, Csak *et al* (96) further confirmed that NLRP3 inflammasome did participate in the occurrence of NAFLD.

Excessive drinking and alcoholism are usually the direct causes of ALD (97). ALD and NAFLD have certain similarities in disease characteristics, both of which develop from liver degeneration to liver fibrosis, and may eventually develop into liver cancer (98). A clinical study showed that the levels of IL-1 $\beta$ , IL-18 and caspase-1 in the liver of patients with ALD were higher than those of healthy controls (99). Furthermore, in animal experiments, compared with the control mice, the protein levels of NLRP3 and IL-1 $\beta$  and caspase-1 activity in the liver of alcohol-fed mice were significantly enhanced, which was consistent with the clinical research conclusions (100). In addition, in the reverse experiment of mice with NLRP3, ASC and caspase-1 gene deletion, it was found that the degree of fat infiltration and damage in the liver of mice was reduced (100,101). Therefore, NLRP3 inflammasome is involved in the pathogenesis of ALD.

Hepatocirrhosis is the ultimate development trend of the above two kinds of liver diseases (102). Boaru *et al* (103) detected a high level of NLRP3 expression in the liver tissue of a mouse liver fibrosis model. In addition, in the mouse model with NLRP3 or ASC gene deficiency, the degree of liver fibrosis was weakened (104). Clinicians also found an increase of IL-1 $\beta$ , IL-18 and active caspase-1 in ascites macrophages of patients with hepatocirrhosis (105). Therefore, NLRP3 inflammasome is involved in the occurrence and development of numerous liver diseases, and effective intervention is likely to be a new strategy to treat these diseases.

*NLRP3 inflammasome and inflammatory intestinal diseases.* Inflammatory bowel disease (IBD) includes Crohn's disease and ulcerative colitis, and its main clinical features are recurrent abdominal pain, diarrhea and even mucus pus and bloody stool. If IBD cannot be effectively treated, it increases the risk of rectal cancer. Previous studies have found that the pathogenesis of IBD mainly includes genetic factors, environmental factors and immune factors, of which immune factors may be the most critical pathogenic factors, and inflammatory factors may play an important role (106). Previous studies have found high expression levels of IL-1 $\beta$  and IL-18 in the intestinal mucosa of patients with Crohn's disease and ulcerative colitis, allowing for the conclusion that the expression level of IL-1 $\beta$  or IL-18 may be related to the susceptibility to IBD (107-109). Further research indicated that NLRP3 was highly expressed

in the early stage of IBD, and then the processing of pro-IL-18 and pro-IL-1 $\beta$  by caspase-1 led to an increase of pro-inflammatory factors IL-1 $\beta$  and IL-18, which eventually led to the aggravation of the course of IBD (110,111). By comparing the secretion of IL-1 $\beta$  in peritoneal macrophages of NLRP3 knockout mice and non-knockout mice, it was confirmed that NLRP3 inflammasome played a role in promoting the progression of the disease in the colitis model induced by dextran sulfate sodium (DSS) (112). Of note, animal studies also found that NLRP3 gene knockout mice are more susceptible to colitis induced by DSS than wild-type mice, and mice with ASC or caspase-1 gene deficiency also have the same susceptibility to colitis (113-115). These gene knockout mice have more serious intestinal pathological changes and higher mortality in the acute and chronic stages of DSS-induced colitis. This shows that the NLRP3 inflammasome may also play a certain role in the process of intestinal inflammatory reaction, which is characterized by the increase of stem cell division at the bottom of the crypt to replace damaged intestinal cells (116). Generally speaking, activation of the NLRP3 inflammasome leads to a large number of secretion traps of IL-1 $\beta$  and IL-18, which hinders the intestinal repair mechanism, increases the permeability of the intestinal epithelium and eventually leads to the deterioration of the disease.

If IBD develops into chronic inflammation, it becomes an important cause of IBD-related intestinal tumors (e.g. colon or rectal cancer) (117). According to reports, IL-1 $\beta$  is not only a proinflammatory factor, but also a cancer-promoting factor (118). A high-cholesterol diet increases the risk of colon cancer. Du *et al* (118) found that cholesterol crystals promote inflammation and tumor development in the intestine of a mouse colon cancer model. Furthermore, cholesterol crystallization can activate the NLRP3 inflammasome, leading to an increase of IL-1 $\beta$  secretion to promote the development of colon cancer. In addition, it was found that knocking out the NLRP3 gene reversed this process, which also preliminarily confirmed that the NLRP3 inflammasome-related pathway did participate in the course of IBD-related tumors (118). However, IL-18, another downstream inflammatory factor of the NLRP3 inflammasome, may have a role in inhibiting the progression of the disease. Studies have found that IL-18 plays an anti-tumor role in several experimental tumor models of sarcoma and melanoma (119-121). IL-18 can not only inhibit tumor angiogenesis, but also promote the repair and regeneration of ulcer epithelium (122,123). It has been indicated that, although IL-18 can repair colon epithelial injury by promoting the proliferation of intestinal cells, it inhibits proliferation in the chronic stage of colitis, so IL-18 may play an important role in preventing and inhibiting IBD-related tumors (124,125). In a word, the NLRP3 inflammasome is closely related to IBD and IBD-related tumors, and further study of its specific mechanism may be helpful in treating these diseases.

*NLRP3 inflammasome and acute pancreatitis (AP)/acute severe pancreatitis (SAP).* AP is one of the most common acute abdominal conditions in the clinic and it is also the most common digestive system disease in patients. As a classic inflammatory disease, patients usually have the main clinical features of elevated serum amylase and lipase. >20% of AP is SAP (126). SAP has an acute onset and rapid development, which easily leads to multiple organ failure, with a mortality

Table III. NLRP3 inflammasome is involved in bone and joint system diseases.

| Disease                          | Related mechanism                                                                                                                                                                                                                                                         | (Refs.)   |
|----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Rheumatoid arthritis             | After synovial tissue is stimulated, the NLRP3 inflammasome can be activated through NF- $\kappa$ B signaling pathway or HIF-1 $\alpha$ -related pathway, thus promoting the production of IL-1 $\beta$ and finally causing the destruction of synovial tissue of joints. | (134-143) |
| Osteoarthritis                   | Activation of the NLRP3 inflammasome in articular chondrocytes or macrophages and monocytes induces the activation and release of IL-1 $\beta$ and IL-18, which eventually leads to degenerative diseases of articular cartilage.                                         | (144-149) |
| Osteoporosis                     | NLRP3 inflammasome in osteoclasts can directly regulate osteoclast activity through proteolysis, and NLRP3 inflammasome may also thicken chondrocyte maturation and osteoblast activity, thus increasing the risk of osteoporosis.                                        | (151-157) |
| Gouty arthritis                  | Stimulators such as uric acid crystals activate NLRP3 inflammasome, which leads to an increase in the expression of pro-inflammatory cytokines IL-1 $\beta$ and IL-18, and finally leads to joint swelling and pain.                                                      | (159-163) |
| Intervertebral disc degeneration | Mitochondrial dysfunction, endoplasmic reticulum stress and ROS damage can activate the NLRP3 inflammasome and finally lead to increased release of IL-1 and other factors, which in turn leads to disc degeneration.                                                     | (165-167) |

NLRP3, Nod-like receptor family pyrin domain containing 3; HIF, hypoxia-inducible factor; ROS, reactive oxygen species.

rate of >20% (127). The essence of pancreatic inflammation is that acinar cells secrete a large number of inflammatory factors after being damaged to trigger the inflammatory process. These inflammatory factors also lead to the recruitment and activation of neutrophils and monocytes, thus releasing oxidants and cytotoxic substances, further damaging pancreatic tissue (127). Various inflammatory factors have been proved to be important indexes to evaluate the severity of AP and IL-1 $\beta$  and IL-18 are among them (128). Different from other inflammatory factors, IL-1 $\beta$  and IL-18 are synthesized as precursor proteins and need to be cleaved to produce their bioactive forms, and it has been speculated that NLRP3 inflammasome may be involved in this process. Fu *et al* (129) found that the inflammatory corpuscles of NLRP3 were obviously activated during AP in mice, and in further experiments, they found that the edema and inflammation of pancreatic tissue in mice were obviously reduced in the absence of caspase-1, ASC or NLRP3. It has also been confirmed that the secretion and maturation of IL-1 $\beta$  in the AP model of mice with NLRP3 gene deficiency are obviously inhibited, which also impairs the further development of the AP inflammatory cascade (129). Studies also found that TLR4 can aggravate the development of AP by mediating the activation of the NLRP3 inflammasome (130,131). Therefore, NLRP3 inflammasome may be involved in the occurrence and development of AP.

Similarly, in SAP, inflammatory reaction of the pancreas and peripancreatic tissues is mainly caused by local proinflammatory factors such as IL-1 $\beta$ , and the production of mature IL-1 $\beta$  is completed by the shearing process of caspase-1, and the biological activity of caspase-1 is obtained on the basis of the formation of NLRP3 inflammasome. In an animal model of SAP, Ren *et al* (132) found that the expression of various components of NLRP3 inflammasome increased, which in turn promoted the activation of caspase-1 and eventually led to the production of proinflammatory factors such as IL-1 $\beta$ , which

caused damage to the pancreas and peripancreatic tissues and participated in the occurrence and development of SAP (132).

*NLRP3 inflammasome is involved in bone and joint system diseases.* With the continuous progress in the research on the structure and function of NLRP3 inflammasome, increasing evidence shows that the NLRP3 inflammasome is a key factor mediating tissue injury in numerous bone and joint diseases, and its pro-inflammatory factor release and cell apoptosis effect can aggravate bone and joint injury. Various studies have confirmed that NLRP3 has an important role in common diseases such as osteoarthritis and certain autoimmune-related orthopedic diseases. The NLRP3/caspase-1/IL-1 $\beta$ /IL-18 axis is a good target for the treatment of bone and joint diseases (Table III).

*Role of NLRP3 inflammasome in rheumatoid arthritis (RA).* RA is an autoimmune disease characterized by joint injury. The initial pathological manifestation is synovitis. With the development of the disease, synovitis can invade cartilage and bone, which may lead to irreversible joint malformation and dysfunction. The pathogenesis of RA is mainly related to rheumatoid factor and anti-citrulline protein antibody (133).

Studies have shown that the NLRP3 inflammasome may have a role in the pathogenesis of RA. NLRP3 and ASC were expressed in myeloid cells, endothelial cells and lymphocytes of patients with RA, but the expression level of NLRP3 did not increase in fibroblast-like synovial cells (134,135), and it also increased in peripheral blood cells of patients with RA (136). Zhang *et al* (137) also found that the expression of NLRP3 in the serum and synovium of mice with collagen-induced arthritis (CIA) was significantly higher than that of the control group and the expression of NLRP3 in the synovium of the articular capsule was positively associated with the severity of CIA/disease imaging score in mice. A further study indicated that the expression of NLRP3 inflammasome in synovium

was obviously inhibited and its clinical symptoms were alleviated after Guo *et al* (138) used NLRP3-specific inhibitors on CIA model mice. The expression of caspase-1 *in vivo* was downregulated and IL-1 $\beta$  in synovium and serum of joints was significantly decreased.

When cells are stimulated to a certain extent, the NF- $\kappa$ B pathway is activated and they can enter the stage of activation of the NLRP3 inflammasome. Therefore, targeting the NF- $\kappa$ B pathway may have potential therapeutic effects on NLRP3-related diseases. A20, TNF- $\alpha$ -inducible protein 3 is a kind of protein involved in the negative feedback regulation of NF- $\kappa$ B signaling and is also considered a susceptible gene of RA. A20 knockout mice show spontaneous erosive arthritis related to the increase of NLRP3 inflammasome and the secretion of IL-1 $\beta$  (139). A20-deficient bone marrow-derived macrophages secreted inflammatory factors and apoptosis related to the overactivation of NLRP3, which indicated that A20 could inhibit RA by weakening the activation of NLRP3 inflammasome (140).

The imbalance of microRNA (miR) in synovial cells and synovial fluid and hypoxia microenvironment in synovium also have an important role in the occurrence and development of RA diseases. Li *et al* (141) found that miR-20a can reduce the production of ROS introduced by thioredoxin binding protein (TXNIP) and the activation of NLRP3 inflammasome by targeting the 3'-UTR of rat TXNIP. In further animal experiments, it was found that the RA rat model could be improved by receiving miR-223 from exosomes, and the related mechanism may be that miR-223 can target the 3'-UTR of NLRP3 and inhibit the expression of NLRP3 (142). The hypoxia environment in the synovium may also induce the activation of NLRP3. In the rat model, synovial succinate accumulates and activates NLRP3 inflammasome by regulating the transcription of hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), and the level of HIF-1 $\alpha$  is increased in fibroblast-like synoviocytes of patients with RA (143). Therefore, the NLRP3 inflammasome can be activated by various upstream signals and its expression tends to increase in patients with RA or animal models, thus participating in the occurrence and development of RA diseases. Therefore, targeting NLRP3 shows a good therapeutic prospect, which effectively inhibits the secretion of downstream inflammatory factors and apoptosis.

**Role of NLRP3 inflammasome in osteoarthritis (OA).** OA is a degenerative joint disease whose main symptom is joint pain. The etiology is still unclear, and the pathological manifestations are mainly deformation and destruction of articular cartilage fibrosis. Numerous inflammatory factors induce local synovitis and cartilage matrix degradation and then promote the progress of knee OA. The expression of NLRP3, ASC and caspase-1 in synovial tissue of patients with knee OA is increased. In early *in vitro* experiments, Han *et al* (144) found that knocking out NLRP3 could weaken inflammation and apoptosis of synovial cells induced by LPS and ATP.

Alkaline calcium phosphate and calcium phosphate crystals usually exist in synovial fluid and tissues of patients with OA and play an important role in the pathogenesis of OA (145). Both alkaline calcium phosphate and calcium phosphate crystals can activate NLRP3 inflammasome, and alkaline calcium phosphate promotes the production of IL-1 $\beta$  by macrophages and monocytes by activating the NLRP3 inflammasome (146).

It is well-known that IL-1 $\beta$  and IL-18 in synovial fluid are positively related to the severity of OA (147). In a mouse OA model experiment, oral selective NLRP3 inflammasome inhibitor obviously improved the inflammation of mouse joint tissue and the inflammatory factors in the synovium tissue of the control mice were obviously reduced, which indicates that targeting NLRP3 can also improve the progress of OA diseases (148).

In the chondrocyte OA model stimulated by IL-1 $\beta$ , Senkyunolide A (SenA) inhibited the pathway of NLRP3 inflammasome and significantly reduced the expression of related proteins, such as NLRP3 and ASC. It was found that SenA inhibits the NLRP3 signaling pathway of chondrocytes in an OA model stimulated by IL-1 $\beta$  and OA mice (148). Further clinical drug research found that metformin, a first-line treatment drug for diabetes, has similar effects, which has attracted much attention for its future prospects in OA treatment. Lordén *et al* (149) performed medial meniscus instability surgery on the knee joint of experimental mice to build a mouse model of OA and the experimental group of mice received metformin intervention. After administration, the researchers found that the expression of NLRP3, caspase-1, IL-1 $\beta$  and other related factors in mouse articular cartilage decreased significantly. In addition, at the subchondral bone level, metformin can inhibit the formation of osteophytes, increase bone density and reduce trabecular separation. Therefore, metformin can inhibit the activation of NLRP3 inflammasome and the focal death of chondrocytes, thereby delaying the progress of OA (149).

As the most common degenerative disease of joints, OA is characterized by degenerative changes of articular cartilage. The characteristics of releasing inflammatory factors and promoting apoptosis of NLRP3 are closely related to the pathological changes of OA such as synovitis and chondrocyte apoptosis, and accordingly, targeting NLRP3 has the potential to delay the progress of OA.

**NLRP3 inflammasome involved in osteoporosis (OP).** OP is a systemic metabolic bone disease characterized by decreased bone mass and destruction of the bone microstructure, which leads to increased bone fragility and easy fracture (150). There are numerous factors leading to OP. According to certain scholars, the NLRP3 inflammasome may play an important role in this process. In a previous study, Alippe *et al* (151) found that NLRP3 deficiency can prevent bone loss caused by ovariectomy (OVX) in mice and play an important role in bone resorption. *In vivo* studies of NLRP3 gene knockout mice showed that NLRP3 participated in bone induction; NLRP3 knockout mice are shorter than normal mice and this growth defect is mainly characterized by growth plate defect and osteopenia of trabecular bone. The researchers speculated that this effect is achieved by regulating the maturation of hypertrophic chondrocytes and the activity of osteoblasts (152). Recently, Jiang *et al* (153) also found that NLRP3 inflammasome can cause dysfunction of osteoclasts by reducing differentiation, and at the same time accelerate the proliferation and differentiation of osteoclasts, which promotes bone absorption and destroys bone formation. NLRP3 inflammasome in myeloid cells indirectly regulates the activity of osteoclasts through systemic inflammation, while NLRP3 inflammasome in osteoclasts directly regulates the activity of osteoclasts

through proteolysis of poly(ADP-ribose) polymerase 1, which is a negative regulator of osteoclast formation. Inhibition of the NLRP3 inflammatory corpuscle pathway promoted osteoblast differentiation and restored bone volume in OVX mice (154,155). The involvement of NLRP3 inflammatory corpuscles in the course of OP has also been preliminarily confirmed in clinical research. Guaraná *et al* (156)'s research on menopausal women with OP showed that NLRP3 inflammatory corpuscles activate pro-inflammatory cytokines IL-1 $\beta$  and IL-18, and the single nucleotide variation caused by NLRP3 inflammasome is closely related to the severity of OP. Therefore, NLRP3 inflammasome not only accelerates bone resorption, but also inhibits bone formation, thus increasing the risk of OP (157). Therefore, inhibiting NLRP3 inflammasome is a promising therapeutic strategy to prevent and control OP.

*Relationship between NLRP3 inflammasome and gouty arthritis (GA).* GA is a disease in which the amount of uric acid in the body increases due to excessive purine metabolism or insufficient uric acid excretion, which leads to the crystallization and accumulation of uric acid in joints, thus leading to joint pain and swelling (158). As the upstream pathway of IL-1 $\beta$  and IL-18, NLRP3 inflammasome is firstly activated by uric acid crystallization and LPS in this process, and the self-activation of caspase-1 is mediated by the activated NLRP3 inflammasome, which leads to the increased expression of pro-inflammatory cytokines IL-1 $\beta$  and IL-18 (159). It is reported that calcium pyrophosphate dihydrate (CPPD) crystals cause pseudo-RA attack and participate in the activation of NLRP3 inflammasome. Zhao *et al* (160) confirmed that the deposition of monosodium urate (MSU) crystals can lead to inflammatory reaction of human macrophages, which is the classic pathogenesis of acute gout. Macrophage phagocytosis of MSU crystals activates NLRP3 inflammasome and then upregulates the expression of IL-1 $\beta$  and IL-18, thus inducing the occurrence of acute GA (160). In addition, inhibiting the activation of NLRP3 inflammasome can prevent acute GA, and therefore, it is thought that the activation of NLRP3 inflammasome is the core process of gout attack (161,162). Blocking the activation of NLRP3/caspase-1/IL-1 $\beta$  pathway induced by ATP has a significant effect on relieving GA inflammation in rats (163). Therefore, inhibiting the activation of NLRP3 inflammasome and promoting the relief of RA inflammation represent a new direction in the treatment of GA.

*NLRP3 inflammasome induces intervertebral disc degeneration (IDD).* IDD is an age-related degenerative disease of the spine, which has a great influence on social economy and human life. It is also one of the main causes of low back pain, which can cause serious nerve damage and disability. The main pathological changes are the decrease of proteoglycan content, height, endplate sclerosis and osteophyte formation, which eventually leads to a decrease of the ability of the intervertebral disc to withstand compression load (164). The pathogenesis of IDD is complex and the efficacy of current treatments is not obvious, and accordingly, it is urgent to find new therapeutic targets. It has been indicated that during the occurrence and development of IDD, NLRP3 inflammasome may be activated, thus mediating the production of a variety of inflammatory cytokines and further promoting the progress of IDD. A study also showed that this process may

depend on NLRP3's ability to perceive change in intracellular homeostasis, the activation of NLRP3 inflammasome, mitochondrial dysfunction related to IDD, endoplasmic reticulum stress and ROS damage (165). At the same time, this study also confirmed that the NLRP3 inflammasome participated in the inflammatory reaction of IDD mice. The researchers found that, compared with normal rat intervertebral disc tissues, the expression of NLRP3, caspase-1 and IL-1 in degenerated intervertebral disc were significantly upregulated and the expression of caspase-1 and IL-1 was similar (165,166). In further research, Zhao *et al* (167) found that the lactic acid content in intervertebral disc tissue increased during IDD, thus stimulating nucleus pulposus cells to drive the activation of NLRP3 inflammatory bodies and increase the release of IL-1 $\beta$ , which further induced the IDD process, which they thought was realized through ROS and NF- $\kappa$ B-related pathways. These findings fully indicate that NLRP3 inflammatory corpuscles may play an important role as a potential target for IDD treatment in the future.

*NLRP3 inflammasome participates in cardiovascular diseases.* The NLRP3 inflammasome is a protein complex that can be activated by crystal or granular PAMPs and ischemic hypoxia risk-related molecular patterns, DAMPs, which promote the secretion of IL-1 $\beta$  and IL-18. Through these mechanisms, it can promote diseases such as atherosclerosis and heart failure. Therefore, NLRP3 inflammasome may have a key role in the physiology and pathology of cardiovascular diseases and play the role of pro-inflammatory mediators. In recent years, it has become one of the focuses of relevant research (Table IV).

*NLRP3 inflammasome and atherosclerosis (AS).* AS is a chronic inflammatory disease and it is also one of the most important causes of death from cardiovascular diseases. The pathogenesis of AS is closely related to inflammatory reaction and lipid accumulation, but the specific pathogenesis of AS has not been fully clarified, and its prevention and treatment are limited (168). In 2010, researchers proposed for the first time that the NLRP3 inflammasome may be involved in the occurrence and development of AS. Duewell *et al* (169) believed that oxidized low-density lipoprotein could promote cholesterol crystallization, and then cholesterol crystallization activated NLRP3 inflammasome AS and endogenous signaling molecules, which eventually led to the increase of IL-1 $\beta$  expression, which was also the reason for the early inflammatory reaction of AS. In subsequent clinical experiments, Paramel Varghese *et al* (170) also found that the expression levels of NLRP3, ASC, IL-1 $\beta$  and IL-18 mRNA in atherosclerotic plaques of patients with AS were significantly higher than those in normal arterial tissues. In the AS model of mice, Usui *et al* (171) found that compared to the control mice, the area of aortic plaque was significantly reduced after caspase-1 knockout, and the number of macrophages and vascular smooth muscle cells in the plaque and the level of plasma inflammatory factors were also reduced (171). At the same time, silencing the expression of the NLRP3 gene inhibited the progress of AS and the expression of inflammatory cytokines, reduced the contents of macrophages and lipids in plaques and promoted the stability of plaques (172). A previous clinical study confirmed that the levels of NLRP3 mRNA,

Table IV. NLRP3 inflammasome participates in cardiovascular diseases.

| Disease                                | Related mechanism                                                                                                                                                                                                                                                            | (Refs.)   |
|----------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Atherosclerosis                        | The activation of NLRP3 inflammasome in arterial tissue leads to the increase of IL-1 $\beta$ expression, which in turn leads to the early inflammatory reaction of AS.                                                                                                      | (169-173) |
| Heart failure                          | The activation of NLRP3 inflammasome in myocardial cells can lead to a large amount of secretion of IL-1 $\beta$ and IL-18, which will lead to cardiac contraction dysfunction and myocardial cell death, which may eventually lead to ventricular remodeling.               | (174-180) |
| Myocardial ischemia-reperfusion injury | The outflow of ROS and K <sup>+</sup> mediates the activation of NLRP3 inflammasome in myocardial fibroblasts or microvascular endothelial cells, which leads to the increase of IL-1 $\beta$ and IL-18, and finally induces myocardial apoptosis and myocardial infarction. | (181-183) |

NLRP3, Nod-like receptor family pyrin domain containing 3. AS, atherosclerosis; ROS, reactive oxygen species.

IL-1 $\beta$  and IL-18 in peripheral blood mononuclear cells of patients with coronary heart disease were higher than those of patients without coronary heart disease and atorvastatin was observed to reduce the levels of IL-1 $\beta$  and IL-18 through the NLRP3 inflammasome to treat AS (173). Therefore, the NLRP3 inflammasome may be a potential target for the prevention and treatment of AS. Further clarifying the role of the NLRP3 inflammasome in AS and developing inhibitors of NLRP3 inflammasome are expected to provide new intervention measures for the prevention and treatment of AS.

*Relationship between NLRP3 inflammasome and heart failure.* Heart failure is a group of clinical syndromes caused by the impairment of ventricular filling and ejection function caused by structural or functional diseases of the heart. In the last decade, great progress has been made in drug treatment of heart failure, but its main pathogenesis has not been fully explained, so most patients still suffer from symptoms such as dyspnea and decreased fatigue tolerance. Previous studies have suggested that the NLRP3 inflammasome has an important role in regulating chronic inflammation and influencing heart failure (174).

Researchers have found that NLRP3, pro-caspase-1 and serum IL-1 $\beta$  in heart failure mice are significantly higher than those in the control group. However, after the NLRP3 gene is knocked out or NLRP3 inhibitor is given, the myocardial inflammatory response and myocardial contraction function of heart failure mice are significantly improved and the heart failure mice can also gain similar effects after applying IL-1 $\beta$  inhibitor (175). In further cell research, Mezzaroma *et al* (176) found that the related components ASC and caspase-1 of the NLRP3 inflammasome in myocardial cells of mice with heart failure were higher than those in the sham-operated group, which further confirmed that NLRP3 inflammasome could mediate the death of myocardial cells and lead to ventricular remodeling, and promote the occurrence of heart failure after myocardial infarction.

IL-1 $\beta$ , an inflammatory factor produced by the activation of NLRP3 inflammasome, can cause myocardial reversible systolic dysfunction, and its mechanism is to induce the activation of IL-18, which leads to cardiac systolic dysfunction (177,178). Clinicians have translated these research

achievements to clinical treatment. For instance, anakinra, an antagonist of IL-1R, can alleviate the inflammatory reaction after myocardial infarction in patients with heart failure (179,180). IL-1 $\beta$  and IL-18 produced by activation of NLRP3 inflammasome are the focus of immunotherapy for heart failure at present. Immunosuppressants, such as anakinra, have shown good application prospects in clinical research, and NLRP3 inflammasome and their products will become new targets of immunotherapy for heart failure in the future.

*Relationship between NLRP3 inflammasome and myocardial ischemia-reperfusion injury.* Myocardial ischemia-reperfusion injury is a common pathophysiological process in ischemic cardiomyopathy, cardiopulmonary bypass and heart transplantation. Its mechanism includes free radical damage, Ca<sup>2+</sup> overload, energy metabolism disorder and inflammatory reaction. Kawaguchi *et al* (181) have suggested that NLRP3 inflammasome may also be involved in this process. The release of IL-1 $\beta$ , the infiltration of inflammatory cells and the expression of inflammatory cytokines in myocardial tissue of mice with myocardial injury and ischemia-reperfusion increased, which was caused by the activation of NLRP3 inflammasome of myocardial fibroblasts mediated by ROS and K<sup>+</sup> outflow (181). After establishing the model of myocardial ischemia in mice, researchers found that the expression of NLRP3, IL-1 $\beta$  and IL-18 mRNA in mouse myocardial fibroblasts increased significantly, while the heart function of NLRP3-related gene knockout mice improved significantly (181-183). In myocardial microvascular endothelial cells of mice with myocardial ischemia, the expression of NLRP3 increased, the activity of caspase-1 increased, and the production of IL-1 $\beta$  and IL-18 increased (183). Cardiac myocyte apoptosis and cardiac function were significantly improved after intracardiac injection of NLRP3 inhibitor (183). Furthermore, Kawaguchi *et al* (181) found that the inflammatory reaction, infarct size and degree of myocardial fibrosis in mice with myocardial ischemia were significantly reduced after silencing ASC or caspase-1 gene expression.

A large number of inflammatory mediators are being released during myocardial ischemia, which mediates the cascade amplification effect of inflammatory reactions. It has

Table V. Relationship between NLRP3 inflammasome and central nervous system diseases.

| Disease                | Related mechanism                                                                                                                                                                                                                 | (Refs.)   |
|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Traumatic brain injury | The specific mechanism remains to be fully elucidated. NLRP3 inflammasome in neurons, astrocytes and microglia was activated in large quantities after brain injury, which induced neuroinflammatory reaction and neuronal death. | (185-188) |
| PD                     | The specific mechanism remains to be fully elucidated. The activation of NLRP3 inflammasome in microglia of patients with PD and PD mouse models may be related to the occurrence and development of PD.                          | (191,192) |
| Alzheimer's disease    | The activation of NLRP3 inflammasome in brain tissue may enhance A $\beta$ aggregation and promote the development of neuroinflammation and Tau lesions.                                                                          | (195-198) |
| MS                     | NLRP3 inflammasome in MS plaque may be activated to promote the production of IL-1 $\beta$ and caspase-1, eventually leading to a series of neuropathy.                                                                           | (200)     |
| Cerebral ischemia      | After cerebral ischemia, NLRP3 inflammasome is activated and pro-inflammatory factors IL-1 $\beta$ and IL-18 are produced to aggravate the neuroinflammatory response.                                                            | (202-206) |

NLRP3, Nod-like receptor family pyrin domain containing 3; PD, Parkinson's disease; MS, multiple sclerosis.

been proved that NLRP3 inflammasome plays an important role in myocardial ischemia. However, its specific signaling pathways and mechanisms require to be further clarified. It has a good research prospect to prevent and treat myocardial ischemia-reperfusion injury with NLRP3 inflammasome as the target.

*Relationship between NLRP3 inflammasome and central nervous system diseases.* In recent years, with the deepening of the research on NLRP3 inflammasome, its role in nervous system diseases has been increasingly studied. Although there have been numerous studies on nervous system diseases and inflammatory factors, research on the relationship between nervous system diseases and NLRP3 inflammatory corpuscles is still in its infancy. It is of great significance to clarify the pathogenesis of central nervous system diseases and improve the therapeutic effect to find out the specific function and relationship between them. Therefore, the role of NLRP3 inflammasome in common nervous system diseases was summarized in the following chapter (Table V).

*NLRP3 inflammasome participates in traumatic brain injury (TBI).* TBI is one of the most serious diseases of the nervous system in the world, which is usually caused by external forces, such as normal brain function damage or pathological brain tissue damage. Due to the irreversible loss of functional neurons and nerve tissue damage, the central nervous system is difficult to repair and regenerate after trauma, resulting in poor prognosis for patients with TBI. To date, the mechanism of TBI has not been thoroughly studied, so although a large number of successful preclinical studies have been used for TBI treatment, they are rarely used in transformation (184).

After TBI, neuroinflammation is a key factor that obviously aggravates brain tissue damage and leads to functional defects. It has been confirmed that inflammatory corpuscles have an important significance in regulating the secondary injury of TBI. A variety of inflammatory corpuscles that damaged brain tissue, particularly NLRP3, were detected in neurons, astrocytes and microglia, which can induce

neuroinflammatory reaction and neuron death, aggravate brain tissue damage and was closely related to the pathogenesis of TBI (185). It has been shown that the gene and protein levels of the NLRP3 inflammasome are obviously upregulated after moderate TBI (186). It was also found that the level of NLRP3 in cerebrospinal fluid increased on the first day after brain injury in infants, decreased the next day and increased again at 3-4 days. Wallisch *et al* (187) indicated that the early increase of NLRP3 may be due to cell necrosis or traumatic dissolution, while the late increase may be due to infection, cell stress or activation of other inflammatory bodies. The NLRP3 inflammasome is activated after brain injury and scholars have found that inhibiting the activation of the NLRP3 inflammasome reduced inflammation and improved neurological function in brain injury model mice. Geng *et al* (188) observed that a high concentration of oxygen can inhibit the activation of the NLRP3 inflammasome and the inflammatory response after traumatic brain injury in mice. It should be noted that hypoxia therapy is a classic treatment for TBI, which indicates that the NLRP3 inflammasome is involved in the occurrence and development of TBI, and its inhibitors are likely to be a new important treatment for TBI.

*Role of NLRP3 inflammasome in Parkinson's disease (PD).* PD is a progressive chronic neurodegenerative disease with a complex pathogenesis, which is closely related to numerous pathogenic factors, such as oxidative stress, neuroinflammation and mitochondrial dysfunction. At present, levodopa, dopamine receptor agonist and anticholinergic drugs are the main drugs used to treat PD in the clinic, but there are no clear treatment methods to improve the pathological progress (189). Studies have shown that the activation of microglia and neuroinflammation may be the key regulatory factors for the loss of dopaminergic neurons in PD. The activation of microglia in the brain and excessive release of proinflammatory mediators can lead to the expression of costimulatory molecules, which leads to neuroinflammation and nerve dysfunction (190). It has been found that the activation of NLRP3 inflammasome and the increase of ASC expression can be observed in microglia

of patients with PD and PD mouse models. In PD mice, daily oral administration of NLRP3 inhibitor had a neuroprotective effect, reducing the loss of dopamine in the striatum and the deformation of dopaminergic substance in the substantia nigra (191,192). These studies show that the NLRP3 inflammasome has an important relationship with the occurrence and development of PD, but at present, only a small number of studies on this topic are available. Further study on the relationship between microglia, NLRP3 inflammasome and PD will lay a foundation for further elucidating its pathogenesis.

**Role of NLRP3 inflammasome in AD.** AD is a chronic and progressive neurodegenerative disease caused by numerous factors. Its main pathological features include amyloid  $\beta$  protein ( $A\beta$ ) deposition, Tau protein hyperphosphorylation and neuronal loss. At present, AD has become a major disease that seriously endangers human health all over the world, but with the current treatment methods, it cannot be cured and these methods have toxic side effects (193,194).

According to Abbott (195), the NLRP3 inflammasome has an important role in the pathogenesis of AD. It can stimulate the innate immune response to trigger the inflammatory response, release the pro-inflammatory factors IL-1 $\beta$  and IL-18, and continue to activate during the occurrence and development of the disease, leading to neurological dysfunction and neuronal apoptosis (195). The NLRP3 inflammasome can regulate the activation of caspase-1 and increase the expression of IL-1 $\beta$  and IL-18 in AD brain tissue, which is related to the occurrence and development of diseases (196). The NLRP3 inflammasome has been proved to be co-located with amyloid plaques and its level is significantly increased in the brains of patients with AD. Activation of the NLRP3 inflammasome can enhance the aggregation of  $A\beta$  by weakening the phagocytosis of  $A\beta$  by microglia, and promote the development of neuroinflammation and Tau lesions. It has been reported in the literature that the NLRP3 inflammasome was significantly activated after  $A\beta$  was added to cultured astrocytes, and it was confirmed by experiments that the phosphorylation level and pathological features of Tau decreased significantly after  $A\beta$  was injected into the brain of ASC- or NLRP3-deficient mice, indicating that  $A\beta$ -induced Tau lesions were dependent on the NLRP3 inflammasome (197). Of note, certain researchers found that loss of NLRP3 and the subsequent activation of inflammatory corpuscles did not significantly affect the distribution of microglia in  $A\beta$ -related pathological reactions. It was indicated that the NLRP3 inflammasome did not play a significant role in forming microglia activation or driving  $A\beta$ -induced neurodegeneration (198). Therefore, although its role in the pathogenesis of AD is controversial, targeting the NLRP3 inflammasome is likely to become a potential treatment for AD.

**Role of the NLRP3 inflammasome in multiple sclerosis (MS).** MS is an autoimmune disease of the central nervous system, which is characterized by inflammatory cell infiltration, neuronal degeneration, axonal injury and reactive glial hyperplasia. The causes and mechanisms of the disease have not been fully clarified, and it is generally thought that it is the result of multiple factors, such as genetic susceptibility, environment, viral infection and immunity (199).

Inoue *et al* (200) found that peripheral immune cells could enter the nervous system through the damaged blood-brain

barrier during the pathological process of MS. IL-1 $\beta$  and caspase-1 are present in MS plaques and the expression of these proteins in peripheral blood mononuclear cells of patients with MS is also increased. Experimental autoimmune encephalomyelitis (EAE) is an ideal animal model of MS and numerous studies have confirmed that the NLRP3 inflammasome has an important role in EAE. In mice with silenced expression of the NLRP3 gene, the severity of pathological changes was reduced, the proliferation of astrocytes and the infiltration of inflammatory cells were reduced, and the symptoms were improved (200). The above research indicates that the neuroprotective effect of inhibiting inflammasome activation of NLRP3 in MS may provide a new breakthrough for the effective treatment of MS, and exploring drugs and methods to inhibit inflammasome activation of NLRP3 will provide new hope for the effective treatment of MS.

**Role of NLRP3 inflammasome in cerebral ischemia.** Cerebral ischemia is a destructive cerebrovascular disease, which is highly associated with neurological dysfunction, cognitive dysfunction and severe brain injury. The mechanism of its occurrence and development has been proved to be closely related to neuroinflammation, which is the main factor of secondary brain damage after cerebral ischemia (201).

At present, the clinical efficacy of treating cerebral ischemia is limited and its pathogenesis needs further discussion. As an important mediator of neuroinflammatory response after cerebral ischemia, NLRP3 mediates nerve cell injury and neuroinflammatory response, and linker ASC and effector protein caspase-1 are important components of the NLRP3 inflammatory complex. It has been shown that the expression levels of ASC and caspase-1 increase significantly after cerebral ischemia, which plays an important role in regulating the maturation and differentiation of IL-1 $\beta$  and IL-18 (202). Therefore, Wang *et al* (203) preliminarily concluded that the NLRP3 inflammasome produced proinflammatory factors IL-1 $\beta$  and IL-18 after cerebral ischemia. In animal experiments, researchers found that NLRP3-selective inhibitor MCC950 blocked the activation of NLRP3 inflammasome that can relieve cerebral ischemia-reperfusion injury (203), and previous studies have confirmed that TLR4 is an important mediator of neuroinflammatory cascade reaction in the central nervous system, which activates the NF- $\kappa$ B pathway to promote the transcription of NLRP3 components, and further regulates the release of downstream inflammatory mediators through inflammatory reaction. Subsequent studies further confirmed that cerebral ischemia can induce high-level phosphorylation of TLR4, NLRP3 and NF- $\kappa$ B (204,205). These findings indicate that NLRP3 inflammasome plays an important role in the pathogenesis of cerebral ischemia, and inhibiting the activation of NLRP3 inflammasome may provide new ideas for the treatment of cerebral ischemia (206).

## 5. Study of the NLRP3 inflammasome in clinical treatment

Abnormal activation of NLRP3 inflammasome will drive the occurrence and development of inflammatory diseases in numerous systems, so the NLRP3 inflammasome provides important targets for the treatment of these inflammatory diseases, and inhibiting the activity of the NLRP3 inflammasome is an important strategy to alleviate these inflammatory

diseases. Scientists have found numerous biological inhibitors that can prevent the activation of NLRP3, and found that direct NLRP3 protein inhibitors have obvious advantages and good therapeutic potential compared with other types of inhibitors. However, there are no clinical drugs specifically targeting the NLRP3 inflammasome at present. Although related inhibitors have entered the clinical trial stage, their safety and effectiveness need to be further verified and there is still a lot of work to be done before their clinical application. Certain studies have made great breakthroughs in gene therapy. MiRNA and gene editing have become important in the study of this disease. A series of research results have proved that gene therapy can improve the course of this disease, but these methods have not yet entered the clinical trial stage. Therefore, it is of far-reaching significance to find an NLRP3 inflammasome inhibitor or gene editing method that can effectively treat NLRP3-related diseases with high efficiency, specificity and safety.

**NLRP3 inflammasome inhibitors.** Components of the complex signaling cascade during the activation of the NLRP3 inflammasome are being targeted to inhibit NLRP3 inflammasome. According to current development strategies, these inhibitory effects can be mainly divided into indirect inhibitors, direct inhibitors and protein inhibitors related to NLRP3 inflammasome (Table VI).

**Indirect inhibitors.** Indirect inhibitors mainly inhibit the activation of NLRP3 inflammasome in three ways: Inhibiting the protein expression of NLRP3 inflammasome, blocking the upstream signaling pathway of the NLRP3 inflammasome and inhibiting the post-translational modification of regulatory proteins of NLRP3. For instance, auranofin inhibits the differentiation and maturation of NLRP3 and pro-IL-1 $\beta$  by inhibiting NF- $\kappa$ B activation, and inhibitors such as FC11A-2 inhibit the expression of NLRP3, ASC, caspase-1 and pro-IL-1 $\beta$ .  $\beta$ -Hydroxybutyrate, celastrol and glyburide inhibit the activation of NLRP3 inflammasome by inhibiting K<sup>+</sup> channels. Epigallocatechin-3-gallate (EGCG) and Licochalcone A inhibit the production of ROS in the upstream signaling of NLRP3 inflammasome activation, and EGCG can also inhibit mitochondrial DNA synthesis (207-211). These indirect inhibitors have been proven to have significant therapeutic effects in specific NLRP3 inflammasome-related disease models in previous studies, but the defects are also obvious: i) The mechanism of most indirect inhibitors is unclear; ii) the signaling pathways inhibited by numerous inhibitors lack specificity, and these signaling pathways may not be unique to NLRP3 inflammasome, which may easily lead to immunosuppression and even infection; iii) the upstream signaling pathway network of the NLRP3 inflammasome is complex and it is difficult to effectively inhibit the activation process of NLRP3 inflammasome only by inhibiting one of the signaling processes; iv) this type of inhibitor is only in the experimental stage of disease models and has not been applied in the clinic.

**Direct inhibitors.** Direct inhibitors of NLRP3 inflammasome have the advantages of clear mechanisms of action and specific inhibition of NLRP3 inflammasome activation. For instance, in the study of GA, Yang *et al* (212) found that provitamin A can directly combine with the PYD structure of NLRP3, inhibit the activation of NLRP3 inflammasome

and directly reduce the expression level of IL-1 $\beta$  secreted by synovial cells isolated from patients with GA. CY-09 also inhibits the ATPase activity of NLRP3, which has also been proven to have the effect of alleviating GA in mice (213). There are many such direct inhibitors. For instance, 3,4-methylenedioxy- $\beta$ -nitrostyrene can directly bind to the LRR and NACHT domains of NLRP3 and then inhibit the activation of the NLRP3 inflammasome by inhibiting the ATPase activity of NLRP3 (214). Bay11-7082 can alkylate the ATPase active region of NLRP3, inhibit the NF- $\kappa$ B signal and further inhibit the activation of NLRP3 inflammasome (215,216). Although direct inhibitors of NLRP3 have obvious advantages and show good therapeutic potential, and many inhibitors directly targeting NLRP3 protein have been identified, there are still no therapeutic drugs that specifically target NLRP3 inflammasome in the clinic. Although a few inhibitors have been promoted to clinical trials of inflammatory diseases related to NLRP3, their clinical efficacy and safety still need to be further verified, which is still a long time from their potential clinical application.

**Protein inhibitors of other components of NLRP3 inflammasome.** Protein inhibitors of other components of NLRP3 inflammasome include ASC inhibitors, caspase-1 inhibitors and IL-1 $\beta$ /IL-18 inhibitors. Caffeine acid phenethyl ester (CAPE) is the only reported ASC inhibitor at present. CAPE binds directly to ASC and then inhibits the activation of NLRP3 inflammasome by blocking the interaction between NLRP3 and ASC (217). VX-740 is a typical inhibitor of caspase-1, which impairs the activity of caspase-1 by covalently modifying the cysteine residue at the active site of caspase-1, thus inhibiting the activation of the NLRP3 inflammasome, and has shown good anti-inflammatory effects in the first and second clinical trials for patients with RA. However, both ASC and caspase-1 are component proteins needed for the activation of other inflammatory corpuscles, and the lack of specificity may lead to immunosuppression, which will eventually increase the risk of infection (218). IL-1 $\beta$  inhibitor is the only strategy that can be used to treat NLRP3 inflammasome-related diseases. Its three related biological agents, anakinra, IL-1 $\beta$ -neutralizing antibody cankinumab and soluble inducible receptor rilonacept, have been approved by the Food and Drug Administration, and previous studies have confirmed that it can effectively reduce the inflammatory response of joint, bone and muscle diseases, hereditary systemic autoinflammatory diseases and systemic and local inflammatory diseases (219). The treatment with IL-18 antagonist is similar to that with IL-1 $\beta$  antagonist, but it has not been used in clinical treatment. Although anti-IL-1 $\beta$  therapy has been proven to be applicable in the clinic, the related side effects are quite serious: i) IL-1 $\beta$  is not the specific downstream pathway of NLRP3 inflammatory corpuscles and other cytokines such as IL-18 may be involved in the occurrence and development of inflammatory diseases. ii) IL-1 $\beta$  can be produced by other inflammatory corpuscles or independent of inflammatory corpuscles. Blocking IL-1 $\beta$  signaling may increase the risk of infection. Studies have confirmed that certain infections are even fatal. iii) The currently available biological agents targeting IL-1 $\beta$  have a weak ability to penetrate the blood-brain barrier, which leads to the related treatment methods possibly not being suitable for central nervous system diseases (220). In

Table VI. Classical inhibitors of the NLRP3 inflammasome.

| A, Indirect inhibitors                                          |                                                                                                                                                                                     |           |
|-----------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Name                                                            | Mechanism of action                                                                                                                                                                 | (Refs.)   |
| Auranofin                                                       | Inhibition of the differentiation and maturation of NLRP3 and pro-IL-1 $\beta$ by inhibiting NF- $\kappa$ B activation.                                                             | (207)     |
| FC11A-2                                                         | Inhibition of the expression of NLRP3, ASC, caspase-1 and pro-IL-1 $\beta$ .                                                                                                        | (207,208) |
| $\beta$ -Hydroxybutyrate                                        | Inhibition of activation of NLRP3 inflammatory corpuscles is achieved by inhibiting K <sup>+</sup> channels.                                                                        | (207-211) |
| Celastrol                                                       |                                                                                                                                                                                     |           |
| Glyburide                                                       |                                                                                                                                                                                     |           |
| Epigallocatechin-3-gallate                                      | Inhibition of the production of ROS in mitochondria, which is the upstream signal activated by NLRP3 inflammatory corpuscles, and inhibition of the synthesis of mitochondrial DNA. | (207)     |
| Licochalcone A                                                  | Inhibition of the upstream signal mitochondrial ROS production of NLRP3 inflammatory corpuscle activation.                                                                          | (207)     |
| B, Direct inhibitors                                            |                                                                                                                                                                                     |           |
| Name                                                            | Mechanism of action                                                                                                                                                                 | (Refs.)   |
| Provitamin A                                                    | Direct binding to the PYD structure of NLRP3 to inhibit the activation of NLRP3 inflammatory bodies.                                                                                | (212)     |
| CY-09                                                           | Inhibition of ATPase activity of NLRP3.                                                                                                                                             | (213)     |
| MNS                                                             | Direct binding to LRR and NACHT domains of NLRP3 and subsequent inhibition of ATPase activity of NLRP3.                                                                             | (214)     |
| Bay11-7082                                                      | Alkylation modification of the ATPase active region of NLRP3 can be carried out and NF- $\kappa$ B signaling can be inhibited.                                                      | (215,216) |
| C, Protein inhibitors of other components of NLRP3 inflammasome |                                                                                                                                                                                     |           |
| Name                                                            | Mechanism of action                                                                                                                                                                 | (Refs.)   |
| ASC inhibitor CAPE                                              | Direct binding of ASC and blocking of the interaction between NLRP3 and ASC.                                                                                                        | (217)     |
| Caspase-1 inhibitor VX-740                                      | The activity of caspase-1 is inhibited by covalent modification of a cysteine residue at the active site of caspase-1.                                                              | (218)     |
| IL-1 $\beta$ inhibitors                                         |                                                                                                                                                                                     |           |
| Anakinra                                                        | Antagonist of IL-1 $\beta$ receptor.                                                                                                                                                | (219)     |
| Cankinumab                                                      | IL-1 $\beta$ -neutralizing antibody.                                                                                                                                                | (219)     |
| Rilonacept                                                      | An IL-1 trapping agent, which connects two molecules of IL-1 $\beta$ receptors with an immunoglobulin Fc segment.                                                                   | (219)     |

NLRP3, Nod-like receptor family pyrin domain containing 3; ROS, reactive oxygen species; CAPE, caffeic acid phenethyl ester; ASC, Apoptosis associated spot-like protein.

short, simply blocking inflammatory cytokine signals may not be an effective or specific treatment.

**Research on targeted gene therapy.** Most inhibitors only inhibit the upstream signaling of the NLRP3 inflammasome, but not NLRP3 itself, which limits the effectiveness of inhibitors and may lead to unexpected side effects. Therefore, gene editing is probably an effective measure for NLRP3 and numerous scientists have performed large amounts of research on it.

**Role of miRNA in the regulation of NLRP3 inflammasome.** Studies have proved that miRNAs regulate inflammatory corpuscles after transcription. They can inhibit or increase the expression of inflammatory genes by binding to their mRNA targets. In the literature, it is known that different miRNAs regulate different inflammatory corpuscles and NLRP3 is one of the star members (Table VII).

MiR-233-3p is the first human miRNA that has been proven to regulate NLRP3. Related studies found that NLRP3 contained the conserved binding site of miR-223-3p

Table VII. List of miRNAs known to target NLRP3 inflammasome genes and associated diseases.

| miRNA       | Target gene | Disease                                                                        | (Refs.)   |
|-------------|-------------|--------------------------------------------------------------------------------|-----------|
| miR-223     | NLRP3       | Inflammatory bowel diseases, respiratory diseases and hepatocellular carcinoma | (221-223) |
| miR-133a    | NLRP3       | Inflammatory diseases                                                          | (224)     |
| miR-22      | NLRP3       | Gastric cancer                                                                 | (225)     |
| miR-30e     | NLRP3       | PD                                                                             | (226)     |
| miR-7       | NLRP3       | PD                                                                             | (227)     |
| miR-146a-5p | NLRP3       | MS                                                                             | (228)     |
| miR-20b-5p  | NLRP3       | MS                                                                             | (229)     |
| miR-495-3p  | NLRP3       | Cardiovascular disease                                                         | (230)     |

miRNA/miR, microRNA; NLRP3, Nod-like receptor family pyrin domain containing 3; PD, Parkinson's disease; MS, multiple sclerosis.

in its 3'-UTR. The binding between this conserved region and miR-223-3p leads to decreased NLRP3 activity and mutation of this region leads to the complete loss of apoptosis regulation mediated by miR-223 (221-223). In the process of studying the role of miRNA in NLRP3 inflammasome, Bandyopadhyay *et al* (224) overexpressed and inhibited miR-133a-1 in differentiated THP1 cells and found that overexpression of miR-133a-1 increased the levels of caspase-1 and IL-1 $\beta$ . They demonstrated that miR-133a-1 did not change the basic expression of individual components of the NLRP3 complex but could regulate IL-1 $\beta$  (224). Other scholars identified the constitutive expression of miR-22 in gastric mucosa and found that miR-22 directly targeted NLRP3 at the transcription level, which weakened the carcinogenic effect of NLRP3 *in vitro* and *in vivo*. As mentioned above, NLRP3 inflammatory corpuscles play an important role in the process of Hp infection, and Hp infection significantly inhibits the expression of miR-22, which directly inhibits the biological effect of miR-22, leading to the activation of NLRP3 inflammatory corpuscles, thus promoting cell proliferation, aggravating the course of disease and even leading to the transformation into gastric cancer (225).

Other miRNAs can affect the NLRP3 inflammasome. For instance, Li *et al* (226) found that NLRP3 showed a conserved binding site for miR-30e in its 3'-UTR, indicating that there is a connection between miR-30e and neuroinflammation mediated by NLRP3 inflammatory corpuscles in the pathogenesis of PD. Animal experiments have confirmed that in the induced PD mouse model, miR-30e can improve neuronal damage by negatively regulating the expression of NLRP3 and inhibiting the activation of NLRP3 inflammatory bodies (226). MiR-7 and neuroinflammation mediated by the NLRP3 inflammasome are also directly related to the pathogenesis of PD, which directly regulates the expression of  $\alpha$ -Synuclein in dopaminergic neurons through post-transcriptional regulation, which is related to the pathophysiology of PD (227). During the research of multiple sclerosis, Boxberger *et al* (228) and Tezcan *et al* (229) also found that miR-146a-5p and miR-20b-5p can also target and regulate NLRP3 inflammasome to control the progress of the disease. MiR-495-3p may regulate the progression of cardiovascular diseases by targeting NLRP3 (230).

*Gene editing targeting NLRP3 inflammasome.* As the third-generation genome editing tool, clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas 9) can specifically and effectively destroy or repair pathogenic genes through a single guide (g)RNA-directed Cas 9 nuclease. To date, T cells modified by CRISPR/Cas 9 *in vitro* have entered the clinical trials stage for the treatment of metastatic non-small cell lung cancer (231). Adenovirus-associated or adeno-associated virus-mediated CRISPR/Cas9 delivery has been used to treat diseases such as hypercholesterolemia and Duchenne muscular dystrophy (232,233). Jiang *et al* (213) used CRISPR/Cas9 to directly destroy the key molecule NLRP3 at the genomic level and the results showed that it completely inhibits the activation of NLRP3 while avoiding the potential risk of inhibiting the off-target pathway of anti-inflammatory biological agents and inhibitors. In a mouse model experiment, Xu *et al* (234) encapsulated mCas9 and gNLRP3 in cationic lipid-assisted nanoparticle (CLAN) and then delivered Cas9 mRNA (mCas9)/gNLRP3 to macrophages, which prevented septic shock and peritonitis, and improved diabetes-related inflammation and insulin resistance by destroying NLRP3 in macrophages. To date, only a small number of studies on gene editing of NLRP3 have been published, but scholars have successfully verified that CLAN carrying mCas9/gNLRP3 can alleviate acute and chronic inflammatory diseases, which is undoubtedly expected to be a potential treatment for NLRP3-dependent inflammatory diseases.

## 6. Discussion

With the development of research on the structure and function of the NLRP3 inflammasome, more and more evidence shows that the NLRP3 inflammasome is a key molecule mediating tissue damage in numerous systemic diseases, and their pro-inflammatory factor release and cell death effects can aggravate the corresponding tissue damage. As mentioned above, NLRP3 plays an important role in common diseases of the respiratory system, cardiovascular system, digestive system, bone joint system and central nervous system. The NLRP3/caspase-1/IL-1 $\beta$ /IL-18 axis is a good target for treating these diseases.

Small molecular inhibitors against NLRP3 inflammasome have shown certain therapeutic prospects. Among the known indirect inhibitors, direct inhibitors and other protein inhibitors of NLRP3 inflammasome (ASC, caspase-1, IL-1 $\beta$ /IL-18), only IL-1 $\beta$  inhibitors can be used in clinical treatment and others are still in the experimental stage. In addition to specific small molecules, the effective components of classical drugs and plant extracts have also been verified to inhibit the activation of NLRP3 nonspecifically. Targeting NLRP3 through exosomes and miRNA or certain gene editing methods may also provide more ideas for related research.

However, research on NLRP3 inflammasome in the field of diseases is still in its infancy and the in-depth mechanism of activation and assembly of NLRP3 inflammasome and its mediation of cell death requires further clarification. The defects of various inhibitors, such as low specificity, likeliness to produce immunosuppression and possible infection, need further improvement. Therefore, the role of the NLRP3 inflammasome in the occurrence and development of numerous systemic inflammatory diseases has been confirmed. However, there is still a long way to go for its clinical treatment and transformation. Furthermore, although gene editing or non-coding RNA have successfully confirmed the feasibility of the new targeted therapy of NLRP3 in basic research, they are only of great significance for the diagnosis of diseases in clinical activities from the perspective of epigenetics and are still rare in clinical application. How to improve patients' symptoms and delay the progression of the disease as much as possible while ensuring biological safety requires further experimental models and clinical research to confirm. Further elucidation of the mechanisms of the NLRP3 inflammasome will lay the foundation for fully understanding the occurrence and development of related diseases and proposing specific targeted therapies with NLRP3 inflammasome inhibitors and NLRP3 gene intervention as the core.

It is worth noting that natural small molecular compounds of traditional Chinese medicine, such as polyphenols, flavonoids and alkaloids, have attracted the wide attention of scholars because of their rich sources, complex and diverse structures, high biocompatibility, few adverse reactions and incomparable biochemical valence diversity of synthetic compounds. Although the specific mechanism of action remains to be clarified, it has been preliminarily confirmed that dihydromyricetin (235) and amygdalin (236) can inhibit the NF- $\kappa$ B signaling pathway in the start-up stage; oxyphyllin (237) and piperine (238) can block the interaction between NEK7 and NLRP3; tanshinone I (239) can block the interaction between ASC and NLRP3; chrysotin (240) and silybin (241) can inhibit the activation of caspase-1. Related research is still in the early stage and more evidence is needed in the future to clarify the specific molecular mechanisms, as well as to evaluate the bioavailability, effectiveness and safety of the compounds, so as to determine whether they are suitable for treating related diseases and beneficial to improve clinical applications.

There have been numerous studies and summaries on the NLRP3 inflammasome and various systemic diseases in the past, but the relevant results are scattered and there is no systematic summary. There is still no comprehensive summary and analysis of the NLRP3 inflammasome and the

whole clinical disease research. The role of NLRP3 in various systemic diseases is becoming increasingly significant. How to prepare related inhibitors or targeting methods with strong efficacy, long action time and high specificity has undoubtedly become the focus of current clinical research. In this paper, the diseases of various systems and the current treatment methods were comprehensively analyzed, so as to attract the attention of clinical workers in this field and provide a reference for follow-up researchers.

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Not applicable.

## Authors' contributions

JL and NX designed the study; HW wrote the manuscript; WS and YL prepared the figures; and LM edited the manuscript. All authors have read and approved the manuscript. Data authentication is not applicable.

## Ethics approval and consent to participate

Not applicable.

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## Competing interests

The authors declare that they have no competing interests.

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