sNASP mutation aggravates to the TLR4-mediated inflammation in SLE by TAK1 pathway

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Abstract

Genetic factors play an important role in the pathogenesis of systemic lupus erythematosus, and abnormal TLR signaling pathways are closely related to the onset of SLE. In previous studies, we found that the mutant somatic nuclear autoantigenic sperm protein (sNASP) gene in the mouse lupus susceptibility locus Sle2 can promote the development of lupus model mice, but the mechanism is still unclear. Here, we stimulated mouse peritoneal macrophages with different concentrations of LPS. The results showed that sNASP gene mutations can promote the response of the TLR4-TAK1 signaling pathway, but have no significant effect on the TLR4-TBK1 signaling pathway. sNASP mutations enhanced TLR4-mediated NF-KB and MAPK activation and IL-6, TNF secretion in murine peritoneal macrophages. Collectively, our study revealed the impact of sNASP gene mutation on the sensitivity of TLR4 receptors in mouse peritoneal macrophages and shed light on potential mechanisms underlying inflammation in autoimmune diseases.

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Abstract

Genetic factors play an important role in the pathogenesis of systemic lupus erythematosus, and abnormal TLR signaling pathways are closely related to the onset of SLE. In previous studies, we found that the mutant somatic nuclear autoantigenic sperm protein (sNASP) gene in the mouse lupus susceptibility locus Sle2 can promote the development of lupus model mice, but the mechanism is still unclear. Here, we stimulated mouse peritoneal macrophages with different concentrations of LPS. The results showed that sNASP gene mutations can promote the response of the TLR4-TAK1 signaling pathway, but have no significant effect on the TLR4-TBK1 signaling pathway. sNASP mutations enhanced TLR4-mediated NF-KB and MAPK activation and IL-6, TNF secretion in murine peritoneal macrophages. Collectively, our study revealed the impact of sNASP gene mutation on the sensitivity of TLR4 receptors in mouse peritoneal macrophages and shed light on potential mechanisms underlying inflammation in autoimmune diseases.
Introduction

Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease characterized by the production of autoantibodies and subsequent inflammation, that affects multiple organs (1, 2). The etiology of SLE involves a complex interplay between genetic and environmental factors. While genome-wide association studies (GWAS) have successfully identified more than 100 susceptibility gene loci associated with SLE (3), the specific genes responsible for its clinical pathogenicity remain to be elucidated.

Mouse models have significantly contributed to our understanding of the pathogenesis of systemic lupus erythematosus (SLE) (4). In the NZM2410 model, three susceptibility loci associated with lupus, namely Sle1, Sle2, and Sle3, have been identified (5). In a previous study, we utilized whole exome sequencing (WES) to identify a variant of somatic nuclear autoantigenic sperm protein (sNASP) from the Sle2 locus. This variant harbored two mutations in exon 10, specifically 841G>A and 844C>T in the sNASP cDNA sequence, resulting in the substitution of two consecutive amino acid residues (V281I and L282F) within the sNASP protein. Based on these findings, we hypothesized that the mutated sNASP represents a pathogenic gene at the Sle2 locus. To validate this hypothesis, we introduced the mutated sNASP into B6.lpr mice, generating B6.[?]sNASP.lpr mice. In comparison to the control B6.lpr mice, the B6.[?]sNASP.lpr mice exhibited enlarged spleen and lymph nodes, elevated total counts of T and B cells, increased activation and effector T cells, heightened levels of autoantibodies, and significantly enhanced inflammation in the kidneys and lungs (6). Furthermore, we introduced the sNASP mutation into Sle1.Yaa mice, which demonstrated higher proportions of CD3+ T cells and activated CD19+CD86+ B cells in the spleen and lymph nodes compared to Sle1.Yaa mice (7).

Numerous studies have highlighted the role of sNASP, a histone chaperone protein, in the storage and transportation of histones, particularly histones H1, H3, and H4. sNASP is involved in regulating the dynamic balance of nucleosome formation, chromatin assembly, and disassembly, thereby influencing the conformation and accessibility of chromatin (8, 9). Importantly, sNASP has the ability to bind to TRAF6 in unstimulated macrophages and prevent its own ubiquitination and subsequent degradation, thus exerting a negative regulatory effect on the Toll-like receptor (TLR) signaling pathway (10). Recent studies have observed elevated mRNA expression levels of TLR4, TLR7, and TLR9 in SLE patients compared to healthy individuals (11, 12). In vitro experiments have shown that mutations in TLR7 lead to increased production of inflammatory factors and elevated autoantibody titers (13), suggesting a crucial role for TLRs in the pathogenesis of SLE.

The impact of sNASP gene mutation on Toll-like receptor (TLR) sensitivity remains poorly understood. In this study, we employed various concentrations of lipopolysaccharide (LPS) to stimulate mouse peritoneal macrophages, aiming to elucidate the effects of sNASP gene mutations on the TLR4 signaling pathway and the subsequent production of inflammatory cytokines. Our research findings are expected to offer novel insights into the mechanistic role of sNASP mutation in promoting systemic lupus erythematosus (SLE) in mice.

Material and Methods

Mice

B6.lpr were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). sNASP mutant B6.WT (B6. [?]sNASP) mice was prepared by Guangzhou Saiye Biotechnology Co. The B6.[?]sNASP.lpr line was derived by breeding male B6. [?]sNASP mice to female B6.lpr mice and subsequent intercrossing of progeny. B6.WT mice provided by the Experimental Animal Center of Weifang Medical University. Female mice aged 3 months were used in this experiment. All animals were cared for under experimental protocols approved by the Weifang Medical University Animal Care Committee and housed in a specific pathogen-free (SPF) facility.

Cell culture

Mouse primary peritoneal macrophages were obtained by peritoneal lavage using PBS with 3% fetal calf
serum. Samples were then centrifuged and reconstituted into fresh culture media. Cells were next transferred to plate and incubated for 3 hours. After incubation, media was changed to remove free-floating cells. Three hours later, nonadherent cells were removed, and the adherent monolayer cells were used as peritoneal macrophages (14). The cells were cultured at 37°C under 5% CO2 in RPMI 1640 supplemented with 12% FBS.

Reagents and Abs

LPS (Escherichia coli, 0111: B4) was from Sigma-Aldrich (St. Louis, MO). The final concentrations of agonists were used as follows: 100ng/ml, 10ng/ml, 1ng/ml. The Abs specific to anti–p-TAK1 (Ser439) (catalog number:P01458), anti–MAP3K7 (catalog number:BM5328), and anti–NAK/TBK1 (catalog number:A00261-1) were from Boster Technology (Wuhan, China); anti–p-JNK (catalog number:AF1762), anti-JNK (catalog number:AF1048), anti–p-p38 (catalog number:AF5887),anti-p38 (catalog number:AF1111), anti–IKB-α (catalog number:AF1282), anti-IRF3 (catalog number:AF2485) and an HRP-conjugated secondary Abs (catalog number:A0208) were from Beyotime Technology (Shanghai, China). Anti-TBK1/NAK (Ser172) (catalog number:#5483), and p-IKB-α (Ser32) (catalog number:#2859) was from Cell Signaling Technology (Beverly, MA). Anti-p-IRF3 (catalog number: bs-9278R), were from Bioss Technology. Anti-NASP (sc-398379) and anti-TRAF6 (sc-8409) was from Santa Cruz Biotechnology (Santa Cruz, CA).

Western blot

For immunoblot analysis, cells were lysed with Servicebio (Wuhan, China) RIPA Lysis Buffer (catalog no.G2002) supplemented with a protease inhibitor mixture (catalog no.G2007; Servicebio), and then the protein concentrations of the intermixture were measured with a bicinchoninic acid assay (catalog no.P0012; Beyotime). Equal amounts of extracts were separated by SDS-PAGE and then were transferred onto polyvinylidene fluoride membranes for immunoblot analysis.

Real-time RT-PCR

RNA was extracted from cells using the RNAex pro Reagent (Code No: AG21101-S, Accurate Biotechnology, Hunan, China) and converted into cDNA by reverse transcription using the Evo M-MLV RT Premix Kit (Code No:AG11706, Accurate Biotechnology, Hunan, China). The sequences of primers were (forward) 5’-CAGGCCTGCTATGTCTC-3’ and (reverse) 5’-CGATCACCCCCGAAGTTTAGT-3’ for TNF-α, (forward) 5’-TGTGCAATGGGCAATTCTGAT-3’ and (reverse) 5’-GGTACTCCAGAACCAGAGG-3’ for IL-6 and (forward) 5’-TCTTTGCAGCTCCTCGTTGCGGTA-3’ and (reverse) 5’-GTCCTTCTGACCATTCCCCACCAC-3’ for β-actin.

ELISA

The peritoneal macrophages were stimulated with LPS (1ng/ml, 10ng/ml, 100ng/ml, 0h/0.5h/1h/2h), and cell culture supernatants were assayed for IL-6 (catalog number 88-7064) and TNF-α (catalog number 88-7324) by ELISA kits (Invitrogen Biotech, USA) following the manufacturer’s directions.

Statistical analysis

Results are expressed as means ± SEM. Data were analyzed using an independent samples t-test using Origin 2021 and SPSS 26 software. A p value <0.05 was considered significant. Error bars depict SEM.

Result

sNASP mutation promotes TLR4-induced inflammatory cytokines expression
FIGURE 1. sNASP mutation promotes LPS-induced proinflammatory cytokine production. The expression of mRNA (A-C and G-I) and protein (D-F and J-K) of IL-6 and TNF-α were measured by RT-PCR and ELISA in peritoneal macrophages, compared between B6.lpr and B6.sNASP.lpr mice. Following 0 h/0.5 h/1 h/2 h stimulation with (A and D) LPS (1ng/ml), (B and E) LPS (10ng/ml), (C and F) LPS (100ng/ml). Data was shown as means ± SEM (n=3) of one representative experiment. *P <0.05, **P <0.01.
FIGURE 2. sNASP mutation promotes LPS-induced proinflammatory cytokine production. The expression of mRNA (A-C and G-I) and protein (D-F and J-K) of IL-6 and TNF-α were measured by RT-PCR and ELISA in peritoneal macrophages, compared between B6.WT and B6.sNASP mice. Following 0 h/0.5 h/1 h/2 h stimulation with (A and D) LPS (1ng/ml), (B and E) LPS(10ng/ml), (C and F) LPS(100ng/ml). Data was shown as means ± SEM (n=3) of one representative experiment. *P <0.05, **P <0.01. 

The activation of the Toll-like receptor 4 (TLR4) signaling pathway ultimately leads to the release of inflammatory cytokines (15, 16). Excessive expression of inflammatory factors exacerbates the inflammatory response (17). In order to examine the potential inhibitory function of sNASP and whether the mutation of the sNASP gene could enhance the sensitivity of the TLR4 receptor, resulting in the expression of inflammatory cytokines, we assessed the levels of IL-6 and TNF-α proteins and mRNA in mouse peritoneal macrophages after stimulation with different concentrations of lipopolysaccharide (LPS) using ELISA and RT-PCR. Following various levels of LPS stimulation, both the mRNA and protein levels of IL-6 in peritoneal macrophages from B6.sNASP.lpr mice were higher compared to those from B6.lpr mice (Figure 1A-F). Similarly, the levels of TNF-α mRNA and protein were also increased (Figure 1G-L). Additionally, the ex-
pression levels of IL-6 and TNF-α mRNAs and proteins in peritoneal macrophages from B6.[]sNASP mice were significantly higher than those from B6.WT mice following different concentrations of LPS stimulation (Figure 2A-L). Taken together, these findings suggest that mutations in the sNASP gene can impact the sensitivity of the TLR4 receptor, leading to increased expression levels of IL-6 and TNF-α genes and proteins in mouse peritoneal macrophages.

*sNASP mutation downregulates protein expression of TRAF6*

[FIGURE 3. *sNASP* mutation downregulates the protein of TRAF6 expression. The expression of TRAF6 was measured by Western blot in peritoneal macrophages and compared among B6.lpr - B6.[]sNASP.lpr - B6.WT and B6.[]sNASP mice, and mouse β-actin was used as control. Following 0 h/0.5 h/1 h/2 h stimulation with (A and D) LPS (1ng/ml), (B and E) LPS (10ng/ml), (C and F) LPS (100ng/ml). *P <0.05, **P <0.01.

Lipopolysaccharide (LPS), a specific agonist for Toll-like receptor 4 (TLR4), effectively activates sNASP, leading to its dissociation from the TRAF6 complex and subsequent ubiquitination and degradation of TRAF6. This activation triggers downstream signaling pathways and induces the release of inflammatory factors from the cells (10). To assess the expression of sNASP and TRAF6, we initially stimulated peritoneal macrophages obtained from B6.lpr mice, B6.[[]]NASP.lpr mice, B6.WT mice, and B6.[[]]NASP mice with different concentrations of LPS (1 ng/ml, 10 ng/ml, 100 ng/ml), and quantified the levels of sNASP and TRAF6 proteins. Interestingly, we did not observe any significant differences in the protein levels of sNASP in peritoneal macrophages among B6.[[]]NASP.lpr, B6.lpr, and B6.[[]]NASP mice compared to the B6.WT control (Figure 3A-F). However, the expression of TRAF6 following LPS stimulation showed a significant reduction. This reduction in TRAF6 expression may indicate a change in the sensitivity of the TLR4 receptor, particularly evident at 1 hour post LPS stimulation. These findings suggest that the sNASP mutation does not affect the quantity of sNASP, but may influence its function, potentially contributing to TRAF6 degradation and subsequent activation of the downstream signaling pathway.
σΝΑΣΠ μυτατιον προμοτες ΤΛΡ4-ινδυςεδ ΝΦ-κΒ ανδ ΜΑΠΚ αςτιvατιον

FIGURE 4. sNASP mutation promotes TLR4-induced NF-κB and MAPK activation of the pathway. The expression of phosphorylated and total proteins were measured by Western blotting in peritoneal macrophages and compared between B6.lpr and B6.[]\(^{sNASP}\).lpr mice, and mouse β-actin was used as control. Following 0 h/0.5 h/1 h/2 h stimulation with (A) LPS (1ng/ml), (B) LPS(10ng/ml), (C)LPS(100ng/ml). *P <0.05, **P <0.01.

The activation of TLR4 involves two downstream signaling pathways, namely the TLR4-TAK1 pathway and the TLR4-TBK1 pathway. Here, mouse models (B6.lpr, B6.[]\(^{sNASP}\).lpr, B6.WT, and B6.[]\(^{sNASP}\)) and Western blot were used to investigate the protein dynamics alterations involved in TLR4-TAK1 pathway that is potentially under the modulation by TRAF6. Mouse peritoneal macrophages were stimulated with 1 ng/ml LPS for durations of 0.5, 1, and 2 hours. The levels of p-TAK1 and p-p38 in peritoneal macrophages of B6.[]\(^{sNASP}\).lpr mice were higher than those in B6.lpr mice only after 2 hours of stimulation (Figure 4A). In the groups stimulated with 10 ng/ml and 100 ng/ml LPS, the levels of p-TAK1, p-p65, and p-p38 in peritoneal macrophages of B6.[]\(^{sNASP}\).lpr mice were significantly higher than those in B6.lpr mice at 0.5 hours after LPS stimulation. There was no significant change in p-JNK protein (Figure 4B, 4C). These findings indicate that mutations in the sNASP gene increase the protein expression levels of the TLR4-TAK1 signaling pathway in mouse peritoneal macrophages compared to B6.lpr mice. In summary, the mutation in
the sNASP gene enhances the sensitivity of the TLR4 receptor in B6.lpr mice, resulting in the activation of NF-κB and MAPK pathways.

FIGURE 5. sNASP mutation promotes TLR4-induced NF-κB and MAPK activation of the pathway. The expression of phosphorylated and total proteins were measured by Western blotting in peritoneal macrophages and compared between B6.WT and B6.[?]sNASP mice, and mouse β-actin was used as control. Following 0 h/0.5 h/1 h/2 h stimulation with (A) LPS (1ng/ml), (B) LPS(10ng/ml), (C)LPS(100ng/ml). *P <0.05, **P <0.01.

As Fas ligands have been shown to promote a TLR-dependent model of lupus-like inflammation (18), we sought to rule out the effect of Fas deficiency on TLR4-TAK1 pathway. The expression of p-TAK1, p-p65, p-JNK, p-p38, and p-IκBα in peritoneal macrophages from both B6.WT mice and B6.[?]NASP mice following stimulation with different concentrations of LPS under identical conditions were examined to test the hypothetical effects of Fas deficiency. After being stimulated with 1 ng/ml LPS for 2 hours, the expression
levels of p-TAK1, p-p65, p-JNK, and p-p38 proteins in peritoneal macrophages from B6.[?]NASP mice were higher compared to those from B6.WT mice (Figure 5A). Furthermore, the levels of p-TAK1, p-p65, and p-p38 in peritoneal macrophages from B6.[?]NASP mice were significantly higher than those from B6.WT mice at 0.5 hours following stimulation with 10 ng/ml and 100 ng/ml LPS (Figure 5B, 5C). Additionally, the expression level of p-JNK protein was also higher in B6.[?]NASP mice compared to B6.WT mice at 0.5 hours after stimulation with 10 ng/ml LPS, but there was no difference observed at 100 ng/ml LPS. Overall, these observations suggest that mutations in the sNASP gene indeed lead to increased protein expression levels of the TLR4-TAK1 signaling pathway in mouse peritoneal macrophages, thereby enhancing the sensitivity of the TLR4 receptor through the TLR4-TAK1 signaling pathway in response to LPS stimulation. Notably, a comparison between Figure 2 and Figure 3 reveals distinct variations in p-p65 and p-TAK1 alterations at the lowest LPS concentration, implying an influence of the lpr mutation. In conclusion, the sNASP gene mutation in B6.WT mice genuinely improves the sensitivity of the TLR4 receptor compared to lpr mice, resulting in the activation of the NF-κB and MAPK pathways independent of Fas.

sNASP mutation does not impact TLR4-induced IRF3 activation
FIGURE 6. sNASP mutation has no influence on TLR4-induced IRF3 activation of the pathway. The expression of phosphorylated and total proteins were measured by Western blotting in peritoneal macrophages and compared between (A-C) B6.lpr and B6.[?]sNASP.lpr mice, (D-F) B6.WT and B6.[?]sNASP mice, and mouse β-actin was used as control. Following 0 h/0.5 h/1 h/2 h stimulation with (A and D) LPS (1 ng/ml), (B and E) LPS (10 ng/ml), (C and F) LPS (100 ng/ml). *P < 0.05, **P < 0.01.

To investigate the impact of mutation on the TLR4-TBK1 signaling pathway, we examined the protein expression levels of this pathway in mouse peritoneal macrophages after LPS stimulation using western blot analysis. Comparing B6.lpr mice with B6.[?]sNASP.lpr, B6.WT, and B6.[?]sNASP mice, the expression levels of TBK1 and phosphorylated IRF3 proteins in the TLR4-TBK1 signaling pathway did not show significant differences among the groups when stimulated with various concentrations of LPS (Figure 6A-F). These findings indicate that there were no notable changes in protein levels within the TLR4-TBK1 signaling pathway following LPS stimulation between the B6.[?]sNASP.lpr and B6.lpr groups, as well as the
B6.sNASP and B6.WT groups. Thus, different LPS stimulation did not significantly affect the sensitivity of the TLR4-TBK1 signaling pathway following sNASP gene mutation, suggesting that the activation of TLR4 by LPS does not drive the IRF3 signaling pathway. In conclusion, these results suggest that the mutation in sNASP may induce proinflammatory response and enhance the sensitivity of TLR4 through its downstream signaling (Figure 7).

FIGURE 7. Model of TLR4 signaling regulated by sNASP. sNASP mutation promotes LPS-induced TLR4-TAK1 activation in peritoneal macrophages and has no influence on TLR4-TBK1, resulting in the activation of NF-κB and MAPK signaling pathways and expression of proinflammatory cytokines.

Discussion

The NZB/WF1, MRL/lpr, and BXSB/Yaa mice are commonly used as animal models for systemic lupus erythematosus (SLE) due to their natural development of immunopathological symptoms that resemble those observed in SLE patients (19). The clinical relevance of these animal models suggests similarities in the underlying pathogenesis and genetic basis of the disease (20). Three lupus-susceptible loci, known as Sle1, Sle2, and Sle3, have been identified in these mouse strains and are associated with autoimmune diseases (5). In the B6.Sle2 congenic strain, the Sle2 locus has been found to regulate B cell hyperactivity and expansion.
of B1a cells, although it does not cause clinical disease (21). Lupus susceptibility genes, such as Skint6, have been identified from the Sle2c1 locus of the NZM2410 strain, and their mutations contribute to autoimmune disease by producing a truncated Skint6 peptide that binds to Skint6 receptors on lymphocytes (22, 23). Recently, we discovered a variant of sNASP within the Sle2c1 locus, which, when combined with the lpr mutation in the Fas gene, enhances autoimmunity, resulting in more severe lupus nephritis and significantly increased lymphadenopathy in the B6.lpr strain (24).

Genetic sequencing of Exon in the Sle2 interval revealed a substitution in the sNASP gene where two consecutive amino acid residues were replaced. NASP contains two isoforms, a longer testis-specific tNASP and a shorter somatic sNASP. Traditionally, sNASP is crucial in assembling chromosomes and is vital in the final stages of DNA replication and folding chromosomes. It binds to H1, and H4, participates in histone transport, and promotes cell proliferation (25, 26). sNASP also maintains histone H3K9me1 to regulate chromatin accessibility (6). A recent study showed that sNASP can maintain homeostasis of the innate immunity as a negative regulator of TLRs signaling in macrophage. Following LPS stimulation, the phosphorylated sNASP protein dissociates from TRAF6 and is subsequently auto-ubiquitinated to activate the downstream TLRs signaling to trigger the transcription of inflammatory cytokines (10). To test whether the mutation of sNASP gene changes the sensitivity of the TLR4 receptor, resulting in the difference between sNASP mutation groups and control groups in the activation level of TLRs signaling, we detected the expression of proteins of the TLR4 signaling pathways and the expression levels of the mRNA and protein of the pro-inflammatory cytokines at both the mRNA and protein levels and enhanced the TLR4 signaling pathways.

Cytokine dysregulation aggravates immune dysfunction, leading to autoimmune diseases such as SLE (27). Compared to B6.lpr, the proteins of IL-6 and TNF-α in macrophage culture supernatant in B6.[?]sNASP.lpr mice were significantly higher, and the level of the mRNA was also increased with LPS stimulation. The same results were observed in B6.[?]sNASP and B6.WT mice, suggesting that the mutation in sNASP gene increase the expression levels of IL-6 and TNF-α genes and proteins in mouse peritoneal macrophages. To investigate how the mechanism increases the transcription of the pro-inflammatory cytokines, we measured the protein in TLR4 signaling pathways following the different stimulation LPS. TLR4, a Toll-like receptor, is associated with two parallel downstream signaling pathways. The first pathway, known as the TLR4-TBK1 pathway, is MyD88-dependent and involves the molecules TRAF3 and TBK1 (28, 29). The second pathway, called the TLR4-TAK1 pathway, is MyD88-independent and operates through the molecules TRAF6 and TAK1. These two pathways play crucial roles in transmitting signals downstream of TLR4 activation. Studies have shown that after stimulation of mouse peritoneal macrophages with 1 ng/ml LPS for 0.5 hour, 1 hour and 2 hours, the levels of p-TAK1 and p-p38 in peritoneal macrophages of B6.[?]sNASP.lpr mice were higher than those of B6.lpr mice only after stimulation for 2 hours. In the 10 ng/ml and 100 ng/ml LPS stimulation groups, the levels of p-TAK1, p-p65 and p-p38 in peritoneal macrophages of B6.[?]sNASP.lpr mice were significantly higher than those of B6.lpr mice at 0.5 h after LPS stimulation. Additionally, the expression levels of p-TAK1, p-p65, p-JNK and p-p38 protein in peritoneal macrophages of B6.[?]sNASP mice were higher than those of B6.WT mice after stimulated with 1 ng/ml LPS for 2 hours; The levels of p-TAK1, p-p65, p-JNK and p-p38 in peritoneal macrophages of B6.[?]sNASP mice were significantly higher than those of B6.WT mice at 0.5 h after stimulation with 10 ng/ml and 100 ng/ml LPS. These results indicated that the mutation in sNASP gene enhances the sensitivity of the TLR4 receptor in mouse peritoneal macrophages by the TLR4-TAK1 signaling pathway. Furthermore, compared with B6.[?]sNASP.lpr, B6.WT and B6.[?]sNASP mouse peritoneal macrophages, the expression levels of TBK1 and IRF3 phosphorylated proteins in TLR4-TBK1 signal pathway were not significantly different. This study suggested that mutation of sNASP gene has no significant effect on TLR4-TBK1 signaling pathway in mouse peritoneal macrophages.

In conclusion, a variant of sNASP enhances the sensitivity of the TLR4 to aggravate the signaling pathway and increases the release of inflammatory cytokines. Despite the current knowledge of sNASP, further investigations are needed to elucidate whether the mutant sNASP exerts similar effects on other Toll-like receptors (TLRs) and whether its involvement in histone acetylation modification and DNA methylation,
which may contribute to the SLE progression (24, 30). These additional investigations will provide further insights into the broader impact of the mutant sNASP and its role in modulating immune responses associated with SLE.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no competing interests.

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