NK-1R deficiency alleviates psoriasis by reducing the inflammatory response of dendritic cells

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Research Article

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Abstract

**BACKGROUND:** Abnormal neuroimmune regulation is involved in the occurrence and development of psoriasis. The elevated levels of neurotransmitters in the skin locally promote the activation of immune cells by binding to their corresponding receptors, leading to the inflammation of psoriasis lesions. As an important neurotransmitter receptor, Neurokinin-1 Receptors (NK-1R) can bind to a variety of ligands to intensify the activation of immune cells and maintain the inflammatory response in psoriasis. However, its specific role in psoriasis needs to be further elucidated.

**METHODS:** Immunohistochemistry was used to detect the expression levels of Substance P (SP) and NK-1R in human skin lesions. Mice with imiquimod (IMQ)-induced psoriasis were treated with NK-1R agonist or inhibitor, and NK-1R knockout (KO) mice were treated with IMQ to establish a psoriatic lesion model. The severity of skin lesions was scored using the Psoriasis Area Severity Index (PASI). The epidermal thickness of the mice was measured by hematoxylin–eosin staining. The levels of SP and NK-1R in the skin lesions were detected by fluorescence immunohistochemistry. The expression of inflammatory factors in the skin lesions and peripheral blood was detected using Luminex assay. Western blotting was used to investigate the expression levels of Akt/PKB (Protein kinase B), p-Akt, Rho family GTPase 3 (RND3), and vascular endothelial growth factor (VEGF). Flow cytometry was used to detect the number of dendritic cells (DCs), macrophages, T lymphocytes, and so on. The expression of CD80, CD86, and (major histocompatibility complex II, MHC II) molecules on CD11c+ DCs in ears, skin lesions, and spleens were also examined by flow cytometry. Bone marrow–derived DCs were cultured from the bone marrow of wild-type (WT) and NK-1R KO mice. Luminex assay was used to detect the cytokines secreted by DCs.

**RESULTS:** The expression levels of SP and NK-1R increased in human skin lesions. NK-1R agonist aggravated IMQ-induced psoriasis-like skin lesions in mice, while NK-1R inhibitor could alleviate skin lesions. NK-1R KO mice showed alleviated skin lesions, reduced epidermal thickness, decreased expression of proliferating cell nuclear antigen and CD3+, decreased levels of inflammatory cytokines in the skin and peripheral blood, decreased protein expression levels of SP, NK-1R, and p-Akt, RND3, and VEGF in the skin lesions. Further, the expression level of MHC II on CD11c+ DCs in ears decreased in the ears of NK-1R KO mice, the expression levels of CD80 and CD86 on CD11c+ DCs in the skin lesions decreased, and the expression levels of CD80 and MHC II on CD11c+ DCs were decreased in the spleen. The secretion of cytokines IL-1β, IL-12p70, IL-23, and tumor necrosis factor-α decreased in NK-1R KO mice when bone marrow–derived DCs were cultured in vitro.

**CONCLUSIONS:** NK-1R deficiency alleviated psoriasis by reducing the inflammatory response of DCs, which was also associated with SP/NK-1R/Akt and RND3/VEGF pathways. The results also suggested that NK-1R might be a key molecular target of psoriasis.

Introduction
Psoriasis is a chronic inflammatory skin disease, affecting approximately 125 million people globally [1]. It is considered as an autoimmune disease because it is mediated by the interactions of the immune system, inflammatory mediators, psoriasis-related susceptible genes, autoantigens, and environmental factors [2]. Various treatments have emerged in the last few years, although the application of vitamin D derivatives, retinoic acid, immunosuppressive agents, and biological agents has improved the treatment and management of psoriasis to some extent. Multiple side effects of drugs significantly affect the patient's compliance and the improvement in clinical efficacy. Hence, safe and effective treatments are urgently needed to address the aforementioned issues and hence develop drug regimens accordingly [3, 4].

Evidence indicates that psoriasis is involved in neuroimmune regulation [5]. Recent studies have reported that peripheral sensory nerves can be activated by many factors and can release some neuropeptides or neurotransmitters that can control the function of local cells, such as DCs, T cells, keratinocytes, and mast cells, to exacerbate the local inflammation; in turn, these activated cells secrete more cytokines and chemokines to increase the activation of nerve fibers [6, 7]. Clinical studies reported that the number of nerve fibers and the expression levels of neuropeptides as well as neurotransmitters were higher in psoriasis skin lesions, blocking the nerves and hence relieving the skin lesions [8]. Many studies showed that the substance P (SP)/neurokinin-1 receptor (NK-1R) system was involved in psoriasis, and the NK-1R might be a crucial target for psoriasis treatment [9]. Our previous studies also revealed that NK-1R antagonists partially abrogated increased epidermal thickness and the level of neurotransmitters [10]. We used imiquimod (IMQ) to induce psoriasis-like lesions in an NK-1R knockout (KO) mice to assess the disease severity and the changes in immune cell function to explore the role of NK-1R in the psoriasis pathogenesis, and further investigated the possible mechanism. We believed that NK-1R might be a potential pharmacological target for psoriasis therapy.

**Materials And Methods**

2.1 Animal model

2.1.1 Animals

Male and female C57BL/6 WT and NK-1R KO mice (8–12 weeks, 20–22 g) were purchased from Beijing View Solid Biotech Co. Ltd (Beijing, China). The protein-coding region of the target gene Tacr1 was sheared by CRISPR technology. The mouse-fertilized oocytes were repaired using NHEJ DNA, resulting in fragment deletion in the protein-coding region and inactivating the tacr1 protein, thereby achieving the purpose of Tacr1 gene knockout. The latest Spcas91.1 was used to cut genomic DNA, reducing the off-target effects.

All mice were kept under pathogen-free conditions. All animal studies were conducted according to the National Institutes of Health Guidelines on Laboratory Research and approved by the animal care and use committee of the Beijing Institute of Traditional Chinese Medicine (Beijing, China).
2.1.2 IMQ-induced psoriasis-like skin inflammation

All mice were shaved on the back skin, and specimens of size 2 × 2 cm² were obtained. WT and NK-1R KO mice were assigned randomly to groups and treated with Vaseline or 5% IMQ on the back skin and both sides of the right ear for 7 days (n = 8 per group): (1) WT/control: WT mice were treated with Vaseline (Lanlianfeitian Petrochemical Co. Ltd., China); (2) WT/IMQ: WT mice were treated with IMQ (Mingxinlidi Laboratory, China); (3) KO/control: NK-1R KO mice were treated with Vaseline; and (4) KO/IMQ: NK-1R KO mice were treated with IMQ. The application dose of IMQ was 62.5 mg/day on the back skin and 12.5 mg/day on the right ear; the application dose of Vaseline was 0.4 mL/day on the back skin and 0.1 mL/day on the right ear.

The skin lesions were imaged, and the severity of the lesions was graded using the Psoriasis Area Severity Index (PASI): The scaling, erythema, and thickness were scored separately as 0, not present; 1, slight; 2, moderate; 3, marked; and 4, extremely marked. The total scores were summed up and expressed the severity of the lesion inflammation.

2.1.3 NK-1R agonist and antagonist treatment

Netupitant (Cat. No. S4654; Selleck, USA) and Substance P TFA (Cat. No. HY-P0201A; MCE, USA) intragastric or intraperitoneal injections were administered to mice daily for 7 days, and phosphate-buffered saline was used for negative control. Further, netupitant (20 mg/kg) was administered intragastrically, and TFA (2mM/kg) was administered intragastrically and 1mM/kg intraperitoneally.

2.3 Histological analysis, immunochemical analysis, and immunofluorescence

After the treatment of mice as described in Section 2.2.2, all mice were sacrificed by administering an overdose of pentobarbital sodium, and the skin lesions were excised from the back and fixed in 10% formalin. The tissue sections (5 µm) were cut from the paraffin-embedded sections and stained with hematoxylin and eosin (H&E) for pathological observation. The staining was observed under a light microscope (Olympus, Tokyo, Japan). The epidermal thickness was measured using an image analysis system (Image-Pro Plus 6.0 software; Media Cybernetics, Inc., MD, USA).

For immunochemical and immunofluorescence staining, the sections were stained with antibodies against proliferating cell nuclear antigen (Cat. No. ab15497; Abcam, Cambridge, UK), SP (Cat. No. ab 7340; Abcam), and NK-1R (Cat. No. DF 7405; Affinity, USA). The slides were observed using Zeiss Axio Imager (Germany), and the positively stained cells were detected using Image-Pro Plus 6.0 software (Media Cybernetics, Inc.).

2.4 Flow cytometric analysis

The skin, ear, and spleen samples from each group were obtained to analyze the number and function of the immune cells. The spleen samples were minced through a 70-µm mesh to obtain single-cell suspensions. Single cells were first circled with forward scatter-height/forward scatter-area (FSC-H/FSC-
A) to remove the adhesions, the cell population was again circled using forward scatter-area/side scatter-area (FSC-A/SSC-A) to remove the left debris, and the CD45+ cells were circled using CD45/SSC-A.

2.5 Cell culture

Bone marrow–derived dendritic cells (BMDCs) were generated based on previous findings [11]. Mice were sacrificed by administering an overdose of pentobarbital sodium, and the bone marrow cells were obtained from the femurs and tibias and then cultured in Roswell Park Memorial Institute (RPMI) 1640 (Invitrogen, USA) containing 100 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF) and 10 ng/mL IL-4. The cells were incubated in an incubator at 37°C and in the presence of 5% CO₂. On day 3, the floating cells were removed and the culture medium was changed every other day. On day 7, immature DCs were collected and stimulated with SP.

2.6 Luminex test

The skin and peripheral blood were incubated with a buffer containing collagenase IV, hyaluronidase, and DNase-I to obtain cell suspensions, and the serum samples and tissue supernatants were collected and tested using Luminex assay. The concentration of cytokines was measured using the ProcartaPlex Multiplex Immunoassay (Invitrogen) following the manufacturer's protocols.

2.7 Western blotting

The skin samples were lysed and subjected to 10% Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The membranes were blocked using 5% skimmed milk at room temperature for 1 h and incubated with the following antibodies overnight at 4°C: NK-1R (1:1000), SP (1:500), Akt (1:500), phospho-Akt (1:1000), NF-κB p65 (1:1000), phospho-NF-κB p65 (1:1000), β-actin (1:2500), RND (1:1000), Stat3 (1:500), phospho-Stat3 (1:500), vascular endothelial growth factor (VEGF) (1:500), and GAPDH (1:5000). After washing with Tris-buffered saline supplemented with Tween 20, the membranes were incubated with specific secondary antibodies for 1 h in the dark. Immunofluorescence was assessed using an Odyssey infrared imaging system (LI-COR; Biosciences, NE, USA).

2.8 Statistical analysis

All experiments were conducted at least three times. All quantitative data were reported as means ± standard deviation. One-way analysis of variance was used to analyze the statistical significance of differences between groups. A P value <0.05 indicated a statistically significant difference.

Results

3.1 NK-1R expression was elevated in patients with psoriasis, and NK-1R agonist aggravated IMQ-induced psoriatic skin lesions in mice, while NK-1R inhibitors improved skin lesions.
Studies reported abnormal neuroimmune modulation in psoriasis and increased expression of neurotransmitters such as SP, NKA, and corresponding neurotransmitter receptors in psoriatic skin lesions. Immunofluorescence assay was used to detect the expression of the neurotransmitter SP and its receptor NK-1R in psoriatic lesions. The results showed that the expression of SP and NK-1R in psoriatic lesions increased compared with that in healthy lesions, which was consistent with previous studies (Fig. 1). We further observed the effects of NK-1R agonists and inhibitors on the skin lesions in IMQ-induced psoriasis-like lesions in mice. The results showed that NK-1R agonists aggravated the skin lesions in mice; especially, the PASI score increased significantly (Fig. 2A and 2B). After the application of the NK-1R inhibitor, the skin lesions of mice were alleviated, especially after intraperitoneal injection of NK-1R inhibitor, showing a decrease in PASI score and epidermal thickness (Fig. 2C and 2D).

### 3.2 IMQ-induced psoriasiform dermatitis was alleviated in NK-1R KO mice

IMQ was applied on the back skin and right ear of mice continuously for 7 days to compare psoriasiform dermatitis between NK-1R KO and WT control mice. We found that the manifestations of psoriasiform dermatitis in mice were less severe in the KO/IMQ group (Fig. 3A). The PASI scores, infiltration, and erythema, as well as the total scores, decreased in the KO/IMQ group (Fig. 3B). We also compared the epidermal hyperproliferation and thickening conditions. H&E staining showed hyperkeratosis and acanthosis after treatment with IMQ; the epidermal thickness of back skin and ears were measured and found to be reduced in NK-1R KO mice compared with WT control mice (Fig. 3C, 3D, 3G, and 3H). We also examined the number of CD3+ T lymphocytes in the dermis. The immunohistochemical results showed that the number of CD3+ lymphocytes increased in the dermis of both WT and KO mice after IMQ application; however, the number of CD3+ cells was decreased in KO mice than in WT mice after IMQ application (Fig. 3E and 3F).

### 3.3 Expression levels of SP and NK-1R reduced in IMQ-induced NK-1R KO mice compared with WT mice

SP is a tachykinin peptide whose receptor is NK-1R. The interaction between them triggers intracellular signaling in some immune cells, which affects the function of immune cells. Clinical studies reported that the expression levels of SP were higher in the psoriasiform skin than in the healthy skin. In this study, we compared the expression levels of SP and NK-1R between WT and NK-1R KO mice. Immunohistochemistry (IHC) results showed the immunoreactivity of SP on nerve fibers after IMQ treatment, which extended from the epidermis to the dermis, but the number of SP-positive fibers decreased in NK-1R KO mice, which appeared as discontinuity of SP-positive nerve fibers (Fig. 4A). The expression level of SP was also detected using Western blotting. The results showed that the expression level of SP decreased after IMQ treatment in NK-1R KO mice compared with WT mice (Fig. 4C). We also detected the expression level of NK-1R and found that NK-1R expression significantly increased after IMQ treatment in the WT/IMQ group, but only slightly increased in the KO/IMQ group, which was consistent with Western blot results (Fig. 4B and 4D).

### 3.4 NK-1R KO mice showed decreased production of cytokines in both skin and peripheral blood after IMQ treatment
It was believed that inflammatory cytokines played a crucial role in the pathogenesis of psoriasis, and biologics against cytokines, such as TNF-α, IL-12, IL-17, and IL-23, were recommended as the first-line treatment of moderate-to-severe plaque psoriasis [1]. Therefore, the expression levels of some cytokines associated with the development of psoriasiform lesions were determined in this study. We found that the secretion of some cytokines, such as interferon (INF)-γ, IL-12p70, IL-1β, IL-4, TNF-α, GM-CSF, IL-17A, IL-23, IL-13, and IL-22, in the supernatants of skin tissue increased in the WT/IMQ group compared with the WT group, while only the secretion of IL-1β, TNF-α, GM-CSF, and IL-13 was upregulated in the KO/IMQ group compared with the KO group. It was observed that the expression levels of INF-γ, IL-12p70, IL-4, IL-17A, and IL-23 did not increase after IMQ treatment in NK-1R KO mice. Furthermore, the expression levels of the aforementioned cytokines were significantly higher in the WT/IMQ group than in the KO/IMQ group (Fig. 5A–5J).

Besides, we also determined the secretion levels of cytokines in serum. In serum, the expression levels of IL-13, IL-22, IL-23, IL-1β, TNF-α, IL-27, IL-9, IL-4, MIP-1α, MIP-1β, MIP-2, and IL-1α significantly increased in WT mice after IMQ treatment, while only the secretion of IL-13 and TNF-α was upregulated in the KO/IMQ group compared with the KO group (Fig. 5H–5S).

3.5 SP/NK-1R and RND3/VEGF pathways were involved in reducing psoriatic lesions in NK-1R KO mice

Next, we wanted to explore the mechanisms underlying the reduction of inflammation in skin lesions of NK-1R KO mice. Studies showed that the binding of NK-1R to SP triggered guanosine triphosphate (GTP) to Guanosine diphosphate (GDP) conversion on its Gα subunit, which dissociated Gαq from Gβγ and subsequently activated downstream effector molecules, such as phospholipase C (PLC) and Phosphoinositide-3 kinase (PI3K). PLC could directly activate Raf by activating protein kinase C (PKC), leading to the activation of mitogen-activated protein kinase (MAPK) pathway, and could also activate the MAPK pathway through kinase protein Shc. PI3K could activate Akt, leading to the activation of the nuclear factor kappa-B (NF-κB) pathway and the synthesis of inflammatory cytokines [12]. Therefore, we examined the expression levels of Akt, p-Akt, NF-κB, and p-NF-κB in mouse skin lesions. The expression level of p-Akt was upregulated after IMQ treatment in WT mice, and no changes were observed in NK-1R KO mice (Fig. 6A–6C).

We further examined the expression level of VEGF, which is important in psoriasis. The result showed that the level of VEGF was higher after IMQ treatment in WT mice, while no change was observed after IMQ treatment in KO mice. We also determined the content of RND3, the upstream of VEGF, we found that the expression level of RND3 was higher after IMQ treatment in WT mice, while it was almost the same after IMQ treatment in KO mice (Fig. 6D–6F). These results revealed that RND3/VEGF pathway was involved in reducing psoriatic lesions in NK-1R KO mice.

3.6 Function of DCs was restrained in IMQ-induced NK-1R KO mice

We examined the cell types in the ears and skin by flow cytometric analysis to investigate why psoriasiform dermatitis was alleviated in NK-1R KO mice. This study found that the number of CD11c+...
DCs in the ear decreased after IMQ treatment, and was lower in KO/IMQ mice compared with WT/IMQ mice (Fig. 7A). The number of CD11c+ DCs in the skin also decreased after IMQ treatment, but no differences were observed between WT/IMQ and KO/IMQ mice (Fig. 8A). Furthermore, we analyzed the expression levels of I-A/I-E, CD80, and CD86, which represented the function of CD11c+ DCs. The result showed that the expression level of I-A/I-E in the ear was decreased in KO/IMQ mice compared with WT/IMQ mice; CD86 was upregulated after IMQ treatment, while no significant differences were observed in the expression level of CD80 (Fig. 7B–7D). However, the results were not the same in the skin. We found that the expression level of I-A/I-E decreased after IMQ treatment in both WT and NI-1R KO mice. Furthermore, KO/IMQ mice showed a lower expression level of I-A/I-E than WT/IMQ mice. The expression levels of CD80 and CD86 were upregulated after IMQ treatment in both WT and NK-1R KO mice but were downregulated in KO/IMQ mice compared with WT/IMQ mice (Fig. 8B–8D). We next analyzed the number of CD11b+F4/80+ macrophages and CD11b+F4/80- cells in the ears and skin. The number of macrophages and CD11b+F4/80- cells increased in both WT and NK-1R KO mice after IMQ treatment, the number of macrophages was almost the same in WT/IMQ and KO/IMQ mice, and the number of CD11b+F4/80- cells decreased in KO/IMQ mice compared with WT/IMQ mice (Fig. 7E).

We also investigated the numbers of CD3+ T cells, CD3+TCRγδ+ T cells, CD3+CD4+ cells, and CD3+CD8+ cells. The results indicated that the number of CD3+ T cells decreased after IMQ treatment in the ears in NK-1R KO mice, and the number of CD3+TCRγδ+ T cells decreased in both WT and NK-1R KO mice (Fig. 7F and 7G). However, the number of CD3+ T cells in the skin increased only in WT mice after IMQ treatment. The number of CD3+TCRγδ+ T cells increased in the skin of both WT and NK-1R KO mice and was even higher in KO/IMQ mice compared with WT/IMQ mice (Fig. 8F and 8G). We found that, in the ear, the number of CD3+CD4+ and CD3+CD8+ cells was unchanged after IMQ treatment (Fig. 7H and 7I), while in the skin, only the number of CD3+CD8+ cells increased in WT mice after IMQ treatment (Fig. 8H and 8I).

We also detected the expression levels of immune cells in spleens. The results showed that only the expression levels of CD80 and CD11c+ MHC II were lower in the KO/IMQ group than in the IMQ group (Fig. 9A–9D). These results suggested that the inhibition of CD11c+ DC function rather than the reduction in their numbers might result in the reduction of skin lesions in NK-1R KO mice.

3.7 Inhibition of NK-1R reduced the proinflammatory function of BMDCs

We used mouse BMDCs and stimulated cells with Toll-like receptor (TLR) 7/8 agonist R848 to observe the changes in cytokines secreted by mouse BMDCs so as to further verify the effect of NK-1R on the function of DCs. The results showed that IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12P70, IL-13, IL-22, IL-27, IL-23, and TNF-α were secreted by BMDCs of WT mice after R848 stimulation. However, the secretion of only IL-2, IL-4, IL-5, IL-6, IL-10, IL-12P70, and TNF-α increased in BMDCs of NK-1R KO mice after R848 stimulation. The secretion of IL-2, IL-4, IL-5, IL-6, IL-10, IL-12P70, and TNF-α in BMDCs of KO mice was significantly lower than that in WT mice, suggesting that NK-1R affected the proinflammatory function of R848 in DCs (Fig. 10A–10M).
Discussion

Although the pathogenesis of psoriasis has not been fully elucidated, it is confirmed that the disease is closely related to the immune system. A variety of immune cells, such as DCs and T lymphocytes, are involved in the occurrence of psoriasis [13]. At the same time, psoriasis is a typical psychosomatic disease [14]. Accumulating clinical evidence shows that mental stimulation and stress are important factors in the onset and aggravation of psoriasis. The anxiety of patients with psoriasis accounts for 73.4%, and depression accounts for 84.9%. Also, 30%–40% of patients have anxiety and tension during the onset or aggravation of the disease, and 71% of patients’ symptoms aggravate after experiencing stressful events [15, 16]. Psoriasis is a chronic condition characterized by extensive skin lesions and a significant amount of desquamation, which aggravate patients’ psychological symptoms, forming a vicious circle and resulting in severe and long-lasting disease. Controlling bad emotions and improving mental and psychological states have certain therapeutic effects, and combined psychotherapy is better than single therapy [17].

The nervous system plays an important role in the pathogenesis of psoriasis. Clinical studies have found that patients have an abnormal neuroendocrine function, and the expression levels of serotonin, calcitonin gene–related peptide, nerve growth factor, vasoactive intestinal peptide, SP, adenosine, glucagon-like peptide, somatostatin, pituitary adenylate cyclase polypeptide, and their receptors are changed. These changes can affect the activity of the patients’ immune system, inducing neurogenic inflammation [8]. The number of neurofilaments in the lesions, the density of nerve distribution, and the secretion of neurotransmitters such as SP, calcitonin gene–related peptide (CGRP), and nerve growth factor (NGF) increase in patients with psoriasis compared with healthy individuals [18]. The results of this study revealed that the expression levels of SP and its receptor NK-1R increased in the skin lesions of patients. Psoriasis lesions and local inflammation were alleviated after local nerve transection in clinical patients and IMQ-treated mice (5). After local nerve injury caused due to reasons such as anesthesia, nerve resection, and cerebrovascular accident, patients with psoriasis show improvement in local skin lesions, while other parts of the skin lesions do not change. The skin lesions also begin to aggravate in some patients with the recovery of local sensory nerves [5]. Lidocaine and scopolamine can improve the clinical symptoms of patients with psoriasis through their nerve-blocking effects [19]. Animal experiments also found that the psoriatic skin lesions of mice with spontaneous psoriasis improved after skin nerve dissection. The inflammation of psoriatic lesions induced by IMQ could be improved by the injection of neurotransmitter antagonist or drug reducing the activity of local nerve fibers [20]. All these findings indicated that blocking the inflammatory response triggered by local neurotransmitters was a potential step in treating psoriasis.

When people with a genetic predisposition to psoriasis are exposed to higher levels of psychosocial stress, their autonomic responses are enhanced, triggering local neurogenic inflammation and leading to the release of neuropeptides from unmyelinated sensory nerve fibers in the skin, such as SP and NGF [21]. SP is one of the important neuropeptides and a key mediator connecting the brain and hair
It has the highest affinity with NK-1R, which plays an important role in the pathogenesis of psoriasis [22].

NK-1R is involved in signal transduction in afferent neurons. The expression levels of SP and NK-1R are found to increase in psoriatic lesions [22], which are associated with pruritus, chronic stress, and depression in patients. The results of this study were consistent with the previous findings. Furthermore, SP-related inhibitors were tested in phase II clinical trials as therapeutic drugs [23]. The relationship between NK-1R and the severity of skin lesions was further confirmed by animal experiments. The NK-1R agonist increased the PASI score of skin lesions, while the NK-1R inhibitor improved the skin lesions in mice with IMQ-induced psoriasis. We compared the response of WT and NK-1R KO mice to IMQ treatment to further explore the specific mechanism. The results showed that NK-1R KO mice had alleviated skin lesions and reduced levels of inflammatory cytokines and neurotransmitter SP in the skin lesions. We also found that the expression level of p-Akt, which was the key protein on the SP/NK-1R pathway, decreased in KO/IMQ mice compared with WT/IMQ mice. Besides, psoriasis is characterized by increased dermal vascularity, indicating that aberrant angiogenesis is associated with the pathogenesis of psoriasis [24]. RND3, as the upstream of VEGF, has been reported to act as a novel proangiogenic factor. Xiaojing Yue confirmed that RND3(+/-) hearts showed significantly impaired angiogenesis and decreased HIF1α and VEGFA expression [25]. In this study, we found that the knockdown of NK-1R did not increase the expression of RND3 and VEGF even after the application of IMQ, suggesting that NK-1R affected the angiogenic pathway and was associated with the reduction in the number of skin lesions in psoriasis.

The number and function of immune cells in skin lesions were detected by flow cytometry to further investigate which immune cells were affected by NK-1R. We found that surface markers such as CD80 and CD86 were different between groups. After IMQ treatment, the expression levels of CD80 and CD86 in the skin lesions increased in WT mice but not in NK-1R KO mice, although the number of CD11c+ DCs was higher in KO/control mice. Based on this and our previous findings, we believed that the reduction in skin lesions in NK-1R KO mice was associated with limited local DC function. Recent studies have shown that DCs play an important role in psoriasis as they are recognized as the upstream component of local inflammation and also function as antigen-presenting cells. DCs are activated by some factors and then stimulate naive CD4+ T cells to differentiate Th1, Th17, and Th22 and secrete cytokines such as IL-17 and IL-22 [26]. The distribution of skin nerves increases in the mouse model of psoriasis, and local neurotransmitters such as SP and NKA promote the accumulation of DCs, which is related to epidermal thickening [27]. In the skin lesions of mice with IMQ-induced psoriasis, IL-23-producing dermal DCs were in close contact with local sensory nerve fiber pain receptors. Eliminating sensory nerve fibers or inhibiting nerve fibers blocked the release of IL-23 from dermal DCs [28]. One day after nerve removal from KC-Tie2 mice with psoriasis, the number of local CD11c+ DCs decreased by 40%; 7 days later, acanthosis improved by 30%. Restoring local SP and other neurotransmitter signals could reverse the improvement in acanthosis [29]. Under conditions of normal innervation, the use of SP inhibitors could also lead to a reduction in the number of local CD11c+ DCs and improvement in skin lesions [20]. Our previous study found that, compared with normal mice, IMQ-treated mice with anxiety had skin lesions,
accompanied by an increase in the levels of SP and IL-23. Further, the PASI score of skin lesions in mice with anxiety was higher, which was related to the increased levels of SP, NK-1R, and IL-23 in skin lesions. *In vitro* experiments showed that SP combined with TLR 7/8 agonist could promote DCs to secrete more IL-23 than TLR 7/8 agonist alone, suggesting that SP exacerbated the activation of DCs [10].

In psoriasis, high concentrations of SP can increase the sensitivity of DCs and promote the aggregation and activation of DCs, initiating the inflammatory response of psoriasis; however, SP exerts its effects mainly by binding to NK-1R [11]. *In vitro* experiments showed that SP could increase the expression of MHC II, costimulatory molecules, adhesion molecules, and CCR7 on the surface of DCs by activating NK-1R, thus enhancing the ability of DCs to secrete IL-12 and triggering Th1-type inflammatory response. At the same time, it also promoted the secretion of IL-1β, IL-6, and TNF-α, upregulated the production of IL-23, promoted the differentiation of Th17 cells, and triggered the Th17 inflammatory response [30-33]. In this study, we clarified that NK-1R was essential for the activation of DCs in psoriasis. The results showed that bone marrow–derived DCs from NK-1R KO mice had a significant decrease in proinflammatory function after R848 stimulation compared with WT mice, as manifested by the decreased secretion of major inflammatory factors such as IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-22, IL-27, IL-23, and TNF-α.

**Conclusion**

Based on the results of *in vivo* and *in vitro* experiments, we believed that NK-1R deficiency alleviated psoriasis by reducing the inflammatory response of DCs, which was associated with SP/NK-1R/Akt and RND3/VEGF pathways. The results also suggested that NK-1R might be a key molecular target of psoriasis.

**Declarations**

**Ethics approval and consent to participate**

All animal experiments were approved by the National Institutes of Health Guidelines on Laboratory Research and approved by the animal care and use committee of the Beijing Institute of Traditional Chinese Medicine (Beijing, China).

**Consent for publication**

Not applicable

**Availability of data and materials**

Data will be made available on request.

**Competing interests**
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Authors’ contributions**

All authors have contributed significantly to this work. YW and JXZ conceived and designed the experiments; JNG and YZW performed most of the experiments; LZ contributed to animal model establishment; TYW contributed to data collection and analyzed the data; YJM and TTD provided experimental support, YW wrote the manuscript; JXZ and PL revised and edited the manuscript. All authors read and approved the final manuscript.

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**References**


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**Figures**
Figure 1

Substance P and NK-1R were upexpressed in psoriasis lesions. (A) Immunohistochemical staining of SP in skin lesions. (B) Immunohistochemical staining of NK-1R in skin lesions. Scale bar = 20μm.
Figure 2

NK-1R agonists Substance P TFA aggravates Imiquimod-induced psoriasis like lesions and NK-1R inhibitor Netupitant improves imiquimod-induced psoriasis like mice skin lesions. (A) Images of mice skin lesions on day 7. (B) PASI scores (scale sores, infiltration scores, erythema scores, and total scores) of each group. (c) Images of mice skin lesions on day 7. (d) PASI scores (scale sores, infiltration scores, erythema scores, and total scores) of each group. (n = 8/group).

Figure 3

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NK-1R deficiency alleviates Imiquimod-Induced psoriasis-like skin lesions. (A) Images of mice skin lesions on day 7. (B) PASI scores (scale sores, infiltration scores, erythema scores, and total scores) of each group. (C) H&E staining of lesioned skin tissue. Scale bar = 50 µm. (D) Epidermal thickness of mice skin lesions. (E F) Immunohistochemical staining of CD3+ cells in skin lesions. (G) H&E staining of lesioned ear tissue. Scale bar = 50 µm. (H) Epidermal thickness of mice ear lesions. Data show represent mean ± SD (n = 6/group). #P < 0.05, ##P < 0.01, ###P < 0.001.

**Figure 4**

NK-1R KO mice showed lower expression levels of SP and NK-1R after IMQ treatment. (A) Immunohistochemical staining of SP in skin lesions. Scale bar = 100 µm. (B) Immunohistochemical staining of NK-1R in skin lesions. Scale bar = 50 µm. n = 4/group (C) The protein expression levels of SP in mice skin. (D) The protein expression levels of NK-1R in mice skin. Data show represent mean ± SD (n = 3/group). *P < 0.05, **P < 0.01. The gels and blots are cropped and the original blots/gels are presented in Supplementary Figure 1-4.
Figure 5

NK-1R KO mice showed lower production of cytokines both in skin and peripheral blood after IMQ treatment. (A-J) Quantification of cytokines in the supernatants of mouse skins. H-S Quantification of cytokines in the peripheral blood of mice. Data show represent mean ± SD (n = 4/group). *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 6

SP/NK-1R and RND3/VEGF pathways are involved in the reduction of psoriatic lesions in NK-1R KO mice. (A-C) The protein expression levels of AKT and p-AKT in mice skin. (D-F) The protein expression levels of RND3 and VEGF in mice skin. Data show represent mean ± SD (n = 4/group). *P < 0.05, **P < 0.01, ***P < 0.001. The gels and blots are cropped and the original blots/gels are presented in Supplementary Figure 5-11.
Figure 7

NK-1R regulates the expression of I-A/I-E and the ratio of CD3+ cells in the ears of IMQ induced psoriasis-like model.

Flow cytometric analysis of (A) the ratio of CD11c+ cells, (B) the expression of CD80, (C) the expression of CD86, (D) the expression of I-A/I-E, (E) the ratio CD11b+F4/80+ and CD11b+F4/80- cells, (F) the ratio
of CD3+ cells, (G) the ratio of CD3+TCRgd+ cells, (H) the ratio of CD3+CD4+ cells, (I) the ratio of CD3+CD8+ cells in mouse ears. Data show represent mean ± SD (n = 4/group). *P < 0.05, **P < 0.01, ***P < 0.001.
NK-1R regulates the expression of I-A/I-E, CD80, CD86 and the ratio of CD11b+F4/80- in the skin of IMQ induced psoriasis-like model. Flow cytometric analysis of (A) the ratio of CD11c+ cells, (B) the expression of CD80, (C) the expression of CD86, (D) the expression of I-A/I-E, (E) the ratio CD11b+F4/80+ and CD11b+F4/80- cells, (F) the ratio of CD3+ cells, (G) the ratio of CD3+TCRgd+ cells, (H) the ratio of CD3+CD4+ cells, (I) the ratio of CD3+CD8+ cells in mouse skin. Data show represent mean ± SD (n = 4/group). *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 9

NK-1R regulates the ratio of CD11c+ MHC II+ and the expression of CD80 in the spleen of IMQ induced psoriasis-like model. Flow cytometric analysis of (A) the ratio of CD11c+ I-A/I-E+ cells, (B) the ratio of CD11c+ MHC II+ cells, (C) CD11b+F4/80+ cells, (D) the expression of CD80, (E) the expression of CD86, (F) the ratio of CD3+ cells,(G) the ratio of CD3+ TCRgd+ cells,(H) the ratio of CD3+CD4+ cells, (I) the ratio of CD3+CD8+ cells in mouse spleens. Data show represent mean ± SD (n = 4/group). *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 10

NK-1R deficiency inhibits proinflammatory function of bone marrow derived dendritic cells. (A- M) Quantification of cytokines in the supernatants of BMDCs. (A) IL-1β secretion, (B) IL-2 secretion, (C) IL-4 secretion, (D) IL-5 secretion, (E) IL-6 secretion, (F) IL-9 secretion, (G) IL-10 secretion, (H) IL-12p70 secretion, (I) IL-13 secretion, (J) IL-22 secretion, (K) IL-23 secretion, (L) IL-27 secretion, (M) TNF-α secretion. Data show represent mean ± SD (n = 4/group). *P < 0.05, **P < 0.01, ***P < 0.001.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- gelsblotsdescription.docx
- FigS1SP.tif
- FigS2SPinternalcontrol.tif
- FigS3NK1R.tif
- FigS4NK1Rinternalcontrol.tif
- FigS5pAKT.tif
- FigS6AKTM.tif
- FigS7AKTpAKTinternalcontrol.tif
- FigS8RND3internalcontrol.tif
- FigS9RND3.tif
- FigS10VEGF.tif
- FigS11VEGFinternalcontrol.tif