Accepted Manuscript

Submission Date: 2023-11-28
Accepted Date: 2024-04-03
Accepted Manuscript online: 2024-04-08

Experimental and Clinical Endocrinology & Diabetes

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DOI: 10.1055/a-2301-3970

Please cite this article as: Su X, Chen W, Fu Y et al. Protective role of MerTK in diabetic peripheral neuropathy via inhibition of the NF-κb signaling pathway. Experimental and Clinical Endocrinology & Diabetes 2024. doi: 10.1055/a-2301-3970

Conflict of Interest: The authors declare that they have no conflict of interest.

This study was supported by National Natural Science Foundation of China (http://dx.doi.org/10.13039/501100001809), 81860105, the Yunnan Key Laboratory of Innovative Application of Traditional Chinese Medicine, 202105AG070032, the Yunnan Health Training Project of High Level Talents, YNWR-QNBJ-2020-236, the Special Joint Program of Yunnan Province, 202001AY070001-159

Abstract:
Diabetic peripheral neuropathy impacts patient quality of life. Increased Mer tyrosine kinase expression has been demonstrated in such patients, yet its mechanism remains unclear. This study established type 2 diabetes mellitus and diabetic peripheral neuropathy models in Sprague Dawley rats via low-dose streptozotocin and a high-fat diet. Mer tyrosine kinase-specific inhibitors were administered by gavage once daily for 2 weeks. Sciatic nerve conduction velocity and nerve structure were measured. The levels of Mer tyrosine kinase, nuclear factor kappa-light-chain-enhancer of activated B cells, tumor necrosis factor-alpha, interleukin-1 beta, and relevant biochemical indexes were detected. The study revealed Mer tyrosine kinase upregulation in type 2 diabetes mellitus and more so in diabetic peripheral neuropathy groups. Inhibiting Mer tyrosine kinase led to reduced nerve conduction velocity and further deterioration of sciatic nerve structure, as evidenced by structural morphology. Concurrently, serum levels of total cholesterol, glycated hemoglobin, and triglyceride significantly rose. Moreover, nuclear factor kappa-light-chain-enhancer of activated B cells levels increased in both serum and nerve tissue, alongside a significant rise in tumor necrosis factor-alpha and interleukin-1 beta expressions. Mer tyrosine kinase was found to bind to inhibitor of kappa B kinase beta in Schwann cells, establishing inhibitor of kappa B kinase beta as a precursor to nuclear factor kappa-light-chain-enhancer of activated B cells activation. Inhibition of Mer tyrosine kinase exacerbates neuropathy, indicating its protective role in diabetic peripheral neuropathy by suppressing the nuclear factor kappa-light-chain-enhancer of activated B cells pathway, highlighting a potential new target for its diagnosis and treatment.

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Protective role of MerTK in diabetic peripheral neuropathy via inhibition of the NF-κb signaling pathway

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Abstract

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tyrosine kinase upregulation in type 2 diabetes mellitus and more so in diabetic peripheral neuropathy groups. Inhibiting Mer tyrosine kinase led to reduced nerve conduction velocity and further deterioration of sciatic nerve structure, as evidenced by structural morphology. Concurrently, serum levels of total cholesterol, glycated hemoglobin, and triglyceride significantly rose. Moreover, nuclear factor kappa-light-chain-enhancer of activated B cells levels increased in both serum and nerve tissue, alongside a significant rise in tumor necrosis factor-alpha and interleukin-1 beta expressions. Mer tyrosine kinase was found to bind to inhibitor of kappa B kinase beta in Schwann cells, establishing inhibitor of kappa B kinase beta as a precursor to nuclear factor kappa-light-chain-enhancer of activated B cells activation. Inhibition of Mer tyrosine kinase exacerbates neuropathy, indicating its protective role in diabetic peripheral neuropathy by suppressing the nuclear factor kappa-light-chain-enhancer of activated B cells pathway, highlighting a potential new target for its diagnosis and treatment.

Key words: diabetic peripheral neuropathy; MerTK; NF-κb signaling pathway; inflammatory factors; protective effect

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1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder with increasing incidence in developing countries, representing a global health concern. Its prevalence is estimated to reach 642 million by 2040 [1,2]. Patients with diabetes usually present a series of macrovascular and microvascular complications, such as ischemic heart disease, nephropathy, retinopathy, and neuropathy [3,4]. In addition to disease duration and glucose control, genetic background may influence diabetic complications [5]. Diabetic neuropathy (DN) is a syndrome characterized by central and peripheral nervous system lesions caused by chronic hyperglycemia; notably, it is one of the most common diabetic target organ damages that can occur in both patients with type 1 DM and those with type 2 DM (T2DM) [6]. Moreover, diabetic peripheral neuropathy (DPN) is a major type of DN with an incidence of 58.5% in patients with diabetes and a high mortality rate, which severely impairs the quality of life of patients [7,8]. However, the clinical onset of DPN is often severe, and disease development is relatively long [9]. The early diagnosis of DPN is challenging. Thus, studying the pathogenesis and identifying early biomarkers of DPN will contribute to the effective prediction and intervention of DPN progression.

In our previous studies, we reported that Mer Tyrosine Kinase (MerTK) levels were significantly increased both in the peripheral blood of patients with DPN and in the sciatic nerve of early DPN mice, suggesting that changes in MerTK gene expression may be a potential biomarker for the early diagnosis of DPN [10]. Specifically, MerTK is a member of the MER/AXL/TYRO3 tyrosine receptor kinase family, is involved in cell phagocytosis, and encodes a transmembrane protein that is distributed on the surface of various specific and non-specific phagocytic cells, such as retinal pigment epithelial cells, macrophages, and dendritic cells [11]. Recently, studies have suggested that Nuclear Factor kappa-light-chain-

enhancer of activated B cells (NF-κb) was implicated in the pathophysiological mechanism of DPN [12,13]. NF-κb is an important precursor of proinflammatory factor and a heterodimer composed of p65 (RelA) and p50 [14]. The formation of DPN is commonly attributed to endothelial cell activation and inflammatory gene induction, regulated centrally by the transcription factor NF-κb [15,16]. Previous studies have found that the MER/AXL/TYRO3 family was associated with the NF-κb pathway [17], and MerTK could alleviate staphylococcal lipophosphate-induced inflammatory response by blocking NF-κb activation [18]. Therefore, we speculate that MerTK may influence the development of DPN by regulating the NF-κb signaling pathway.

To simulate the disease characteristics of T2DM progressing to DPN, a high-sugar and -fat diet combined with a low-dose streptozotocin (STZ) injection was used to create a T2DM animal model in Sprague Dawley (SD) rats. The DPN model was constructed by continuously feeding a high-sugar and high-fat diet for 8 weeks. We focused on the administration of MerTK-specific inhibitors MRX-2843, the conduction velocity and structural changes of the sciatic nerve, and the levels of MerTK, NF-κb, Tumor Necrosis Factor (TNF)-α, interleukin (IL)-1β, and relevant biochemical indexes. Our study aimed to determine the regulatory role of MerTK in the NF-κb signaling pathway and its effect on the pathogenesis of DPN to provide a new biomarker for the early prediction of DPN as well as an effective therapeutic target for the treatment of DPN.

2. Materials and methods

2.1 Animal studies

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All experiments were performed with the approval of the Animal Experimentation Committee of Kunming Medical University and in accordance with the institutional regulations (No. kmmu20221595). Overall, 70 8-week-old SD rats (310–350 g) from Kunming Medical University's Animal Center (No. 2019-0004) were divided into 5 groups, with 14 rats per group. The control (CON) group received an ordinary diet and 0.2 mL of 0.9% normal saline (NS) by gavage daily. The CON+ MRX-2843 group was similarly fed but received a 0.2 mL 65 mg/kg MRX-2843 suspension daily for 2 weeks. The T2DM group was fed a high-fat and -sugar diet for 6 weeks, then injected with a 1.5 mL solution of STZ (35 mg/kg) to induce diabetes. Blood glucose levels were measured on days 3, 7, and 14 post-STZ administration; levels ≥ 16.7 mmol/L indicated successful T2DM modeling. Rats then received an ordinary diet and 0.2 mL 0.9% NS gavage daily. For the DPN group, post-T2DM establishment, rats were switched to a high-fat and -sugar diet for 8 weeks, followed by an ordinary diet and daily 0.2 mL 0.9% NS gavage. In the DPN+ MRX-2843 group, post-DPN establishment, a 65-mg/kg MRX-2843 suspension was administered daily for 2 weeks.

2.2 Detection of sciatic nerve conduction velocity (NCV)

Based on the body weight and status of the rats, 0.9% pentobarbital sodium (30 mg/kg) was injected intraperitoneally, the sciatic nerve was isolated and the NCV was detected. The double-stimulation needle electrode was placed at the left sciatic notch, and the recording electrode was placed between the second toe of the right plantar. The reference electrode was placed between the stimulation electrode and the recording electrode, approximately 1 cm away from the recording electrode. The intensity of the stimulation was gradually increased

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from a small value, and the measured electromiography was recorded over time. NCV was indicated according to the ratio of latency to conduction distance of compound action potential, calculated by dividing the distance between the stimulation electrode and the recording electrode by the difference obtained from the proximal latency minus the distal latency and expressed in meters per second. The Biological Function Experimental System was provided by the Biological Function Laboratory of the Kunming Medical University. Sciatic NCV measurement under <40 m/s indicated successful DPN modeling [19].

2.3 Detection of blood biochemical indexes

Blood was collected from the hearts of rats after execution, separated at 2795 G for 15 min at 4 ℃, and the upper plasma was obtained after stratification. Blood glucose, triglyceride (TG), and total cholesterol (TC) levels were measured using a biochemical analyzer (BS-240VET, Shenzhen Mairead Company). Insulin (INS) and glycated hemoglobin (GHB) levels were measured using enzyme-linked immunosorbent assay (ELISA) kits (INS: Elabscience Item, No. E-EL-R2466; GHB: Mlbio Item, No. ml059468).

2.4 ELISA

The levels of MerTK, NF-κb (P65), TNF-α, and IL-1β in the serum were determined by the double antibody sandwich method. Purified rat antibodies were coated onto each microwell plate to prepare solid-phase antibodies, added to the corresponding coated microwell, and combined with horseradish peroxidase (HRP)-labeled antibodies. The antibody-antigen-enzyme-labeled antibody complex was formed, and tetramethylbenzidine (TMB) substrate

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was added for color development after thorough washing. TMB was transformed into blue under the catalysis of the HRP enzyme and into yellow under the action of an acid. Absorbance (OD value) was measured at 450 nm using a microplate reader to calculate the concentrations of MerTK, NF-κb (P65), TNF-α, and IL-1β in samples via standard curves from ELISA kits (MerTK: Thermo Fisher, EHMER; NF-κb(P65): Elabscience, E-EL-R0674; TNF-α: Elabscience, E-EL-R2856; IL-1β: Elabscience, E-EL-R0012).

2.5 Detection of sciatic nerve histopathology

The sciatic nerve was fixed in 10% buffered formaldehyde. The fixed tissues were soaked in alcohol for dehydration and placed in dimethylbenzene. The tissue was soaked and permeated in paraffin, and the paraffin-embedded tissue was sliced into about 5-μm-thick slices. The tissue was baked at 60 °C in an incubator for 30 min. The tissues were then hydrated. Hematoxylin-eosin (HE) staining was performed using hematoxylin for 10–20 min. After rinsing with water, the solution was placed in a weakly alkaline aqueous solution and turned blue until blue appeared. Next, 85% alcohol was added for 3–5 min, followed by staining with eosin for 3–5 min. The toluidine blue (TB)-stained section was incubated in 1% toluidine blue aqueous solution at 50 ℃, then at 56 ℃ for 20 min. After rinsing, samples were immersed in 70% alcohol for 1 min, dehydrated, sealed, and examined under a light microscope.

2.6 Observation by electron microscopy

Sciatic nerve tissue, approximately 1 mm thick, was treated with 4% glutaraldehyde for pre-

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fixation and 1% acid for post fixation. After rinsing with PBS, the sections were dehydrated with acetone, embedded in epoxy resin, sliced using an ultrathin slicer, stained with uranium acetate and lead citrate solution in a box, and observed and photographed using a transmission electron microscope (JEM-1400).

2.7 Immunohistochemistry

The sciatic nerve was fixed in 10% buffered formaldehyde, dehydrated using an ethanol gradient, embedded in paraffin, and sliced. The sections were immersed in citrate buffer (PH 6.0) for repair and then cleaned with PBS. The slices were then treated with a buffer-blocking solution (5% goat serum). The blocking solution of goat serum was added, and the first antibody of MerTK (1:100, Affinity, USA) and NF-κb (1:100, Affinity, USA) was added. The glass slides were placed in a 4 °C refrigerator overnight. After rinsing with PBS, the sections were incubated with secondary antibodies, including goat anti-rabbit IgG (H+L) HRP (1:200, Affinity, USA) and goat anti-mouse IgG (H+L) HRP (1:200, Affinity, USA). DAB chromogen solution was added to terminate the staining. Finally, the experimental results were observed under an optical microscope, and the brown or brown-yellow particles in the cell membrane or cytoplasm were considered protein expression. Four fields from each section were randomly selected under a microscope.

2.8 Immunofluorescence staining

The sciatic nerve was fixed in 10% buffered formaldehyde, dehydrated using an ethanol gradient, embedded in paraffin, and sliced. The sections were then immersed in a citrate

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buffer (PH 6.0) for repair. Goat serum was added to the blocking solution, and the primary antibody against MerTK (Affinity: DF4785) was added. The glass slides were put in a wet box and placed in a 4 °C refrigerator overnight. After rinsing with PBS buffer, the slices were added with the second antibodies of goat anti-rabbit IgG (H+L) HRP (1:200, Affinity, USA) and treated at 37 ℃ for 30 min. After washing with PBS three times, DAPI was dropped and incubated at room temperature for 10 min. After rinsing with PBS, the slices were sealed with anti-fluorescence attenuating tablets. Finally, images were captured and observed by fluorescence microscopy.

2.9 Western blotting analysis

Total protein was extracted using RIPA buffer (Beyotime Biotechnology, China), isolated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to a polyvinylidene fluoride membrane (Millipore ISEQ00010). Subsequently, the membranes were incubated with primary antibody (1:1000 for anti-MerTK，Abcam, ab52981; 1:1000 for anti-NF-κb, Proteintech, 80979-1-RR; 1:3000 for anti-TNF-α, Proteintech, 60291-1-Ig; 1:3000 for anti-β-actin, Proteintech, 81115-1-RR) and kept at 4 ℃ overnight. Detection was performed using a horseradish peroxidase secondary antibody (goat anti-mouse IgG[H+L] HRP, 1:3000, Affinity, USA) after incubation at room temperature for 2h. Subsequently, protein expression was visualized using an ECL kit and an X-ray film. Membranes displayed luminescence using a Bio-Rad luminescence imaging system.

2.10 Immunoprecipitation (co-ip) assays

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The interaction of endogenous MerTK and Ikbkb was detected by the co-ip assays using RSC96 cells. RSC96 cells were lysed and centrifuged. The input protein was retained, and MerTK, Ikbkb, and IgG primary antibodies were added to the resulting product. After overnight incubation at 4 °C, 5 μL Protein A and Protein G were added and then incubated, washed, and centrifuged. Following incubation, washing, centrifugation, resuspension in SDS buffer, and denaturation, the final product was obtained, and finally, the interaction between MerTK and Ikbkb was detected by immunoblotting. An immunoprecipitation kit was purchased from Shanghai Ebixin Technology Co.

2.11 Statistical analysis

All experiments were performed at least three times. Data are expressed as the mean ± SD. A normality test was performed to determine normal distribution. Student t-test was used to compare the means between two groups, while the data among multiple groups were analyzed using one-way analysis of variance followed by the "Tukey or Dunnet post hoc" test. All statistical analyses were performed using SPSS 22.0 software. Statistical significance was defined as a P-value <0.05.

3. Results

3.1 Model of DPN rats was established successfully, and the NCV was decreased after inhibiting MerTK

Rats fed a high-fat and -sugar diet combined with STZ injections exhibited typical symptoms of T2DM, such as hyperglycemia, polyuria, polydipsia, polyphagia, and weight loss. The DPN rat model was established by continuously feeding rats high-fat and -sugar diets and

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was confirmed by blood glucose detection and electrophysiological experiments. After 8 weeks of continued high-fat and -sugar feeding, diabetic rats exhibited significantly lower body weights and higher blood glucose levels than did non-diabetic rats. MRX-2843 had no significant influence on the body weight or blood glucose levels in the inhibitor group **(Figure 1A, B)**. Moreover, sciatic NCV was significantly reduced in the DPN group, and NCV was significantly lower in the DPN+MRX-2843 group than in the DPN group **(Figure 1C)**.

3.2 Changes of MerTK expression in each group

MerTK expression was detected in the longitudinal section of the sciatic nerve using immunofluorescence **(Figure 2)**, immunohistochemistry **(Figure 3)**, and western blotting **(Figure 4a),** and in the serum of rats using ELISA **(Figure 4b,c)**. The results showed that MerTK expression was upregulated in both the T2DM and DPN groups and increased significantly in the DPN group than in the CON and T2DM groups. After the MerTK inhibitor MRX-2843 was administered, MerTK expression decreased significantly in the CON +MRX-2843 and DPN +MRX-2843 groups, indicating that the MerTK inhibitor effectively inhibited MerTK.

3.3 Changes of INS, TC, GHB, and TG in each group

INS, TC, GHB, and TG levels were notably higher in the DPN group than in the CON group, indicating the involvement of glucose and lipid metabolism disturbances and insulin resistance in DPN's development. Inhibiting MerTK expression significantly raised TC, GHB, and TG serum levels, exacerbating these metabolic disorders **(Figure 5)**.

3.4 Changes in structural morphology in the longitudinal sections of the sciatic nerve

After HE staining, the sciatic nerves in the CON group showed that the medullated nerve

fibers were evenly distributed, continuous, complete, neat, and orderly, and the myelin sheaths were regular in shape and densely arranged. In the DPN group, the nerve fibers were arranged sparsely and chaotically, with irregular morphology and unclear borders. Some axons were shrunken or lost, which was accompanied by demyelination and myelinolysis. The structure of the medullated nerve fibers was disordered in cross-section, with axonal swelling accompanied by axon shrinkage and partial demyelination. TB staining revealed that the nerve fibers in the CON group were dense, the individual fibers were full, and the thickness of the myelin sheath was uniform. In the DPN group, the nerve fibers were loose, the myelin sheath was thin, and the axons were small and centrally located. After MerTK inhibition, the structure of the nerve fibers was further disrupted, and the lesion worsened **(Figure 6a)**.

Under an electron microscope, the sciatic nerve in the CON group exhibited evenly arranged medullated nerve fibers, the structure of the myelin sheath was intact and dense, and the morphology of Shewan's cells showed no obvious abnormalities. In the DPN group, the shape of myelinated nerve fibers was irregular in cross-section, the myelin sheath was loose and foveolar, some of the myelin sheaths were deformed and vacuolated, and microtubules and microfilaments in axons were disturbed or had disappeared. The nuclei of Shewan cells had an irregular morphology with dilated perinuclear gaps, and the nuclear membranes were partially dissolved and ruptured. After MerTK inhibition, the looseness of the nerve fibers was aggravated, axons were reduced, and lesions worsened **(Figure 6b)**.

3.5 Expression of NF-κb and downstream inflammatory factors was upregulated when MerTK was inhibited

The expression of NF-κb (P65) was determined via immunohistochemistry **(Figure 7)**, western blotting **(Figure 8)**, and ELISA **(Figure 9a)**. The inflammatory factors TNF-α and IL-1β were detected via western blotting **(Figure 8)** and ELISA **(Figure 9 (b and c),**

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respectively). The results indicated upregulated expressions of P65, TNF-α, and IL-1β in the DPN group, with further significant increases following MerTK inhibition, suggesting MerTK's protective role in DPN by inhibiting the NF-κb pathway.

3.6 MerTK bonded to Ikbkb in Schwann cells

Immunoprecipitation assays demonstrated specific binding between MerTK and Ikbkb antibodies to their respective immunoprecipitates in RSC96 cells **(Figure 10A, B)**. Western blot analysis revealed an increase in Ikbkb expression concurrent with a decrease in MerTK expression ($P < 0.05$) **(Figure 10C, D)**. Immunofluorescence double staining indicated enhanced fluorescence of Ikbkb (red) and P65 (green) following MerTK-siRNA3 interference **(Figure 10E)**, highlighting MerTK's endogenous binding to Ikbkb and its regulatory role in the NF-κb signaling pathway.

4. Discussion

In this study, the T2DM model was established using high-sugar and -fat diets for 6 weeks combined with low-dose STZ injection in SD rats (35 mg/kg), which was considered a successful model when the blood glucose level was greater than 16.7 mmol/L. Based on the T2DM model, the rats were fed with high-sugar and -fat diets continuously for 8 weeks, and the DPN model was confirmed by detecting the blood biochemical index, sciatic nerve conduction velocity (NCV <40 m/s), and the morphology of the nerve structure, which is easy to generate and widely available for exploring behavioral or pharmacological tests associated with long-term diabetic complications as well as for studying the pathogenesis of diabetic complications.

MerTK is expressed in both immune and non-immune cells and is involved in inflammation, metabolism, and vascular homeostasis [20]. Previous studies have indicated that MerTK protects against liver fibrosis and cancer [21-23]. The expression of MerTK in

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the sciatic nerve and serum of rats was detected via immunohistochemistry, immunofluorescence, western blotting, and ELISA methods in our study, which showed that MerTK was upregulated in the T2DM and DPN groups, and the expression of MerTK was significantly increased in the DPN group than in the CON and T2DM groups, which is consistent with the results of our previous study [10]. After administering MerTK-specific inhibitors, sciatic nerve conduction velocity decreased, and structural disruption was observed, indicating DPN exacerbation upon MerTK inhibition and its protective role in DPN development. MerTK, a crucial tyrosine kinase, significantly influences PI3K/AKT and NF-κB pathway regulation and apoptotic cell-induced monocyte-derived dendritic cell inhibition [24, 25]. In addition, MerTK has been reported to affect organ function during inflammatory processes or any other injury, such as pathogen invasion [26].

In clinical studies, the levels of inflammatory markers have been shown to predict the onset and progression of diabetic complications [27]. NF-κb is a protein complex that controls transcribed DNA, cytokine production, and cell survival; it is present in almost all animal cell types and is involved in cellular responses to stimuli. Moreover, it plays a key role in regulating the immune response to infections [28，29]. Previous studies have shown that the NF-κb pathway-mediated inflammatory response played a central role in the pathogenesis of diabetes-related complications, including peripheral neuropathy. NF-κb activation was involved in the insulin resistance and apoptosis of pancreatic β-cells [30]. TNF-α and IL-1β, the NF-κb-mediated downstream inflammatory factors, play a central role in the inflammatory response, including immediate, acute, and chronic inflammation [31]. Our studies showed NF-κb (P65), TNF-α, and IL-1β expressions were higher in the DPN group, further increasing with MerTK inhibition, indicating MerTK's protective effect in DPN by inhibiting the NF-κb pathway. Additionally, MerTK influences metabolism-related indices by programming macrophages metabolically and epigenetically [24,32]. Here, the levels of TG, TC, and GHB increased significantly in the serum after MerTK inhibition,

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indicating the aggravation of blood glucose and lipid metabolism disorders.

To elucidate the MerTK-NF-κb interaction, we identified Ikbkb (IKKβ, IKK2), a catalytic subunit of the Iκb kinase complex and key NF-κb pathway regulator, from the National Centre for Biotechnology Information. This complex swiftly activates NF-κb, orchestrating target gene expression [33]. Diabetes induces Iκb phosphorylation and degradation, allowing P50 and P65 to signal the nucleus and activate genes that regulate DPN-associated pathophysiological changes [34]. We verified MerTK's endogenous interaction with Ikbkb in Ceibomb cells via immunoprecipitation. Interfering with MerTK increased Ikbkb expression, significantly enhancing Ikbkb and P65 immunofluorescence ($P<0.05$); this indicates that MerTK silencing upregulates Ikbkb, activating the NF-κb pathway and P65 expression, thus suggesting MerTK's role in inflammation through NF-κb signaling.

In summary, this study found that MerTK increased significantly in the DPN rats, neuropathy was aggravated, and inflammatory factors were upregulated when MerTK was inhibited. MerTK played a protective role in the progression of DPN by inhibiting the NF-κb pathway. Our findings provide a valuable reference for studies on other diabetic complications owing to their similar pathogenesis and suggest that MerTK may be an early diagnostic marker and a new target for the treatment of DPN.

Acknowledgments

We thank Prof. Di Lu and Prof. Lechun Lv for their valuable discussions and technical assistance. We thank the scientific and technological achievements of the incubation center of Kunming Medical University for providing the experimental platform.

Authors contributions

Danfeng Lan, Qiuping Yang, and Yan Zhao constructed the concept of the study. Wenting Chen and Yidan Fu performed the experiments and wrote the manuscript. Bian Wu and Fugang Mao analyzed the results. Danfeng Lan and Qiuping Yang revised the manuscript. All

the authors have read and approved the publication of the manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (81860105), the Special Joint Program of Yunnan Province (202001AY070001-159, 202401AY070001-356040), the Yunnan Youth Top Talent Project of High-Level Talents (YNWR-QNBJ-2020-236), the Yunnan Health Training Project of High-Level Talents Medical Reserve Talent Project (H-2019036), the Kunming University of Science and Technology, the First People's Hospital of Yunnan Province Joint Special Project on Medical Research (KUST- KH2022013Y), and the Yunnan Key Laboratory of Innovative Application of Traditional Chinese Medicine (202105AG070032).

Conflict of Interest

The authors declare no conflict of interest.

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Figure legends

Figure 1. Basic parameters of rats in each group. (A) Changes in body weight in each group at weeks 2, 4, 6, 8, 10, 12, 14, and 16. There were no significant changes in the body weight of the rats after MerTK inhibition. (B) Changes in blood glucose levels in each group. (C) Changes in sciatic NCV in each group at week 18. * $p < 0.05$. Group counts were as follows: CON: 14; CON+MRX-2843: 14; T2DM: 10; DPN: 12; DPN+MRX-2843: 10. All rats were aged 96 weeks.

Figure 2. Immunofluorescence detection of MerTK expression in each group. Confocal images of MerTK (green) and DAPI (blue) staining of the nucleus. Scale bar = 500 μm (first column). Scale bar = 50 μm (second, third, and fourth columns). Rat counts per group were: CON: 14, CON+MRX-2843: 14, T2DM: 10, DPN: 12, DPN+MRX-2843: 10, with a uniform

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age of 96 weeks. Sciatic nerves served as the study tissue.

Figure 3. Immunohistochemical detection of MerTK expression in each group. MerTK was expressed in all the groups and was significantly upregulated in the DPN group. Scale bars = 50 μm. Rat counts per group were: CON: 14, CON+MRX-2843: 14, T2DM: 10, DPN: 12, DPN+MRX-2843: 10, with all rats aged 96 weeks. The sciatic nerve was the tissue of interest.

Figure 4. Western blotting and ELISA detection of MerTK expression in each group. (a) Immunoblotting images of MerTK are representative of each group. MerTK protein levels were detected in different groups using western blotting. Gene expression was normalized to the β-actin levels in each sample. (b) Serum MerTK levels were measured using ELISA. (c) Phosphorylated MerTK (p-MerTK) serum levels were also assessed via ELISA. *$p < 0.05$; ns = no statistical difference. Rat numbers were: CON: 14, CON+MRX-2843: 14, T2DM: 10, DPN: 12, DPN+MRX-2843: 10, all aged 96 weeks.

Figure 5. Levels of INS, TC, GHB, and TG in the serum. (a) INS levels in each group. (b) TC levels in each group. (C) GHB levels in each group. (d) TG levels in each group. * $p < 0.05$; ns, no statistical difference. Rat group counts were: CON: 14, CON+MRX-2843: 14, T2DM: 10, DPN: 12, DPN+MRX-2843: 10. Rats in all groups were 96 weeks old.

Figure 6. Structural changes of the sciatic nerve by the observation of light and electron microscope. (a) The structure of the sciatic nerve was observed under light microscopes after the HE and TB staining respectively (scale bar = 50 μm). (b) Structure of the sciatic nerve was observed under an electron microscope at magnification of 4000x,8000x and 20000x. The rat group counts were: CON: 14, CON+MRX-2843: 14, T2DM: 10, DPN: 12, DPN+MRX-2843: 10, with each group's rats aged 96 weeks.

Figure 7. Immunohistochemical detection of NF-κb (P65) expression level in each group. P65 was expressed in all groups, and the expression of P65 was upregulated in the DPN group and further upregulated after MerTK inhibition. Scale bar = 50 μm. Rat numbers per

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group were: CON: 14, CON+MRX-2843: 14, T2DM: 10, DPN: 12, and DPN+MRX-2843: 10, all aged 96 weeks. The sciatic nerve was selected as the study tissue.

Figure 8. Western blotting detection of NF-κb (P65) and TNF-α expression level in each group. Immunoblotting images of P65 and TNF-α are representative of each group. The levels of proteins for P65 and TNF-α were detected in different groups by western blotting. Gene expression was normalized to the β-actin levels in each sample. * $p < 0.05$; ns, no statistical difference. Group rat numbers were: CON: 14, CON+MRX-2843: 14, T2DM: 10, DPN: 12, and DPN+MRX-2843: 10, with an age of 96 weeks for all rats. The sciatic nerve was the chosen tissue.

Figure 9. Levels of P65, TNF-α, and IL-1β in the serum detected by ELISA. (a) P65 levels in each group. (b) TNF-α levels in each group. (c) IL-1β levels in each group. * $p < 0.05$; ns, no statistical difference. Rat counts for each group were: CON: 14, CON+MRX-2843: 14, T2DM: 10, DPN: 12, and DPN+MRX-2843: 10. All groups' rats were aged 96 weeks.

Figure 10. MerTK and Ikbkb interaction in Schwann cells via immunoprecipitation assay. (A) Ikbkb immunoprecipitates were probed with MerTK antibody. (B) MerTK immunoprecipitates were probed with Ikbkb antibody. "Input" signifies the positive control, while "IgG" marks the negative control. (C) Ikbkb expression post-treatment with three MerTK siRNAs and a negative control agent (NC) was determined by western blot. (D) Protein levels of Ikbkb across groups. (E) Ikbkb and P65 expression post-MerTK-siRNA3 treatment was observed through immunofluorescence (blue fluorescence: nuclei; red fluorescence: Ikbkb; green fluorescence: P65).

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