NeuroMolecular Medicine (2025) 27:23
https://doi.org/10.1007/s12017-025-08845-1

RESEARCH

Folic Acid Promotes Peripheral Nerve Injury Repair via Regulating DNM3-AKT Pathway Through Mediating Methionine Cycle Metabolism

Weibo Kang[1] · Yanli Zhang[2] · Wei Cui[1] · Hua Meng[1] · Duo Zhang[1]

Received: 22 January 2025 / Accepted: 19 March 2025

Abstract

Emerging evidence suggests that folic acid (FA) supports nerve repair, but its beneficial effects in peripheral nerve injury (PNI) remains unclear. This study aims to investigate protective effects of FA against PNI and the underlying molecular mechanisms. High-performance liquid chromatography–tandem mass spectrometry was utilized for precise quantification of metabolites. A sciatic nerve crush injury model was established in rats, followed by assessments of cell proliferation, apoptosis, and motor function using CCK-8 assays, flow cytometry, and the balance beam test, respectively. Neuromorphological observations, electromyography, and ELISA were conducted to evaluate structural, electrophysiological, and biochemical parameters. In vitro, FA restored methionine cycle balance in Schwann cells and neurons disrupted by enzyme inhibition, improving cell viability, reducing apoptosis, and preserving cellular structure. In vivo, FA supplementation restored S-adenosylmethionine and homocysteine levels in a methionine metabolism disorder model and enhanced motor function, neural morphology, neuron survival, and electrophysiological recovery after PNI. Epigenetic analyses revealed that FA modulated DNA methylation and histone modifications of the DNM3 promoter, influencing gene expression. Furthermore, FA facilitated nerve repair via the DNM3-AKT pathway, regulating apoptosis, autophagy, and oxidative stress-related enzymes. These findings highlight FA's potential in promoting nerve repair through metabolic and epigenetic mechanisms.

Keywords Folic acid · Peripheral nerve injury · Methionine cycle metabolism · DNM3 · Epigenetic modification

Introduction

Peripheral nerve injuries are common in clinical practice, causing sensory, motor, and autonomic impairments that significantly affect patients' quality of life and socioeconomic status (Hussain et al., 2020; Lopes et al., 2022). While peripheral nerves have some regenerative ability, spontaneous repair is often incomplete, leading to poor recovery and complications like muscle atrophy and chronic pain (An et al., 2024; Wang et al., 2019). Drugs such as growth factors and neurotrophic factors can promote nerve regeneration (Huang et al., 2024; Vijayavenkataraman, 2020). However, their efficacy is limited, and side effects like allergic reactions and liver or kidney damage may occur, underscoring the need for effective treatments with clearer mechanisms of action (Bolandghamat & Behnam-Rassouli, 2020; Vijayavenkataraman, 2020).

Folic acid (FA), a vital nutrient in one-carbon metabolism, has gained attention for its role in neurological diseases. Although it does not directly repair nerve tissue, FA supports neuron growth by facilitating DNA synthesis and cell division (Soury et al., 2021). It has been shown to promote neural stem cell proliferation and differentiation through the MAPK pathway and enhance nerve growth factor (NGF) expression (Kang et al., 2019; Poulose et al., 2017). FA deficiency can hinder nervous system development, impair neural stem cell activity, and adversely affect nerve regeneration (Chen et al., 2012; Reynolds, 2002, 2006). Furthermore, it is linked to neural tube defects and cognitive impairment, while supplementation can improve neurodevelopment and neurodegeneration outcomes (Finnell

Weibo Kang and Yanli Zhang have contributed equally to this work.

✉ Weibo Kang
kangweibo@bjtth.org

[1] Department of Orthopedic Surgery, Beijing Tiantan Hospital, Capital Medical University, No. 119 South Fourth Ring Road, Beijing 100070, China

[2] College of Veterinary Medicine/College of Biomedicine and Health, Huazhong Agricultural University, Wuhan 430070, Hubei, China

Published online: 31 March 2025

et al., 2002; Iskandar et al., 2004; Piedrahita et al., 1999; Wallingford et al., 2013). Studies also suggest FA promotes functional recovery in peripheral nerve injury (PNI) by regulating Schwann cell activity and neurotrophic factor secretion (Kang et al., 2019). However, its precise molecular mechanisms remain unclear.

Dynamin 3 (DNM3), a GTPase crucial for membrane transport and cytoskeleton remodeling, plays a significant role in axon growth and synaptic plasticity (Yang et al., 2017). Upregulation of DNM3 has been observed after nerve trauma, such as spinal cord injury, and its knockout can reduce axonal degeneration and inflammation, improving neurological function (Brown et al., 2021; Yang et al., 2017). Bioinformatics analyses suggest epigenetic regulation of DNM3 due to CpG island-rich promoter regions (Shi et al., 2020). However, DNM3's role in PNI and its relationship with FA remains largely unexplored.

In-depth studies on how FA promotes peripheral nerve repair could provide new theoretical insights and intervention strategies for treating these injuries. This study aims to investigate the effects of FA in promoting the repair of PNI and the underlying mechanisms.

Materials and Methods

Cell Culture

Under sterile conditions, the dorsal root nerve (containing sensory Schwann cells) and the muscular branch of the femoral nerve (containing motor Schwann cells) were obtained from 5-day-old neonatal SD mice. Additionally, the dorsal root ganglion (sensory neurons) and the anterior horn of the spinal cord (motor neurons) were collected from 14-day-old embryonic SD mice. These tissues were finely minced and placed into centrifuge tubes. A solution containing 0.125% trypsin and 0.03% type IV collagenase (Sigma) was added, and the mixture was digested for 30–40 min. The digested samples were centrifuged at 1500 rpm for 5 min. The resulting precipitates were resuspended in DMEM/F12 (Gibco) medium to create a cell suspension, which was then inoculated into Petri dishes. The cells were incubated in a humidified incubator with 5% CO_2 at 37 °C.

High-Performance Liquid Chromatography–Tandem Mass Spectrometry (UPLC–MS/MS)

The S-adenosylmethionine (SAM) standard product, with a purity of ≥ 97.5%, was purchased from MACKLIN Company, China, while the S-adenosyl-l-homocysteine (SAH) standard product, with a purity of ≥ 98.0%, was obtained from Cayman Company, USA. Methanol, HAc, and NH_4Ac were chromatographically pure, and HCl was analytically pure, all procured from Tedia Company, USA. A standard stock solution of SAM and SAH at a concentration of 2.0 g/L was prepared using 0.1 mol/L HCl. A mixed standard solution containing 200 μg/mL SAM and 20 μg/mL SAH was then prepared using the initial mobile phase. Water was obtained using a Milli-Q ultrapure water system (Millipore Company, USA). The homocysteine (Hcy) standard product, with a purity of ≥ 95%, was purchased from Sigma-Aldrich Company, USA.

Chromatographic conditions: CORTES UPLC-C_{18}+ column (100 mm × 2.1 mm, 1.6 μm; American Waters Company); Mobile phase A: 5.0 mmol/L NH_4Ac and 0.4% HAc solution; Mobile phase B: methanol; the elution procedure was 0–1 min, 98% A; 1–3 min, 25% A; 3–4 min, 98% A; 4–6 min, 98% A. Column temperature 40 ℃; Flow rate 0.35 mL/min; Injection volume 2.0 μL.

Mass spectrum conditions: Multi-response monitoring (MRM) mode scanning; Electrospray ionization mode (ESI+); CUR: 206 kPa; Collision Gas (CAD): Medium; Ion Spray Voltage (IS): 5500 V; Temperature (TEM): 500 ℃; GS1: 384 kPa; GS2: 384 kPa.

DNM3 Overexpression or Interference Technique

Total RNA was extracted from cultured Schwann cells and neurons using suitable commercial kits. Specific siRNA or RNA interference techniques were employed to knock down genes encoding key enzymes of the methionine cycle, such as Methionine Synthase and Methionine Adenosyltransferase. By introducing siRNA into Schwann cells and neurons, the activity of these critical enzymes was effectively inhibited, simulating disruptions in methionine cycle metabolism.

Sciatic Nerve Crush Injury

Animal studies were approved by Animal Welfare Ethics Committee of Beijing Neurosurgery Institute (#2124210). An incision was made along the long axis of the femur of the rats. The biceps femoris and superficial gluteal muscles were separated to expose the sciatic nerve at the mid-thigh level. The surrounding connective tissue was carefully removed. The sciatic nerve was crushed twice using flat tweezers, each crush lasting 30 s. The site of the crush injury was marked on the muscle surface with sutures. The muscle and skin layers were then closed with 6.0 sutures.

Cell-Counting Kit-8 (CCK-8) Assay

The proliferation of transfected cells was evaluated using the CCK-8 assay. Cells were seeded into 96-well plates at a density of 5000 cells/well, with five replicates per condition. The culture was maintained for 4 days. Every 24 h, 10

μL of CCK-8 solution (BOSTER, USA) was added to each well, followed by a 2-h incubation. Absorbance was then measured at 450 nm using a microplate reader (SpectraMax M5, USA).

Flow Cytometry

Apoptosis was analyzed using flow cytometry. Transformed cells were cultured to the desired density, harvested, and stained with an apoptosis detection kit (Living, 556547, China). First, 300 μL of 1 × Binding Buffer was added to suspend the cells. The cells were then stained with 5 μL of Annexin V-FITC and 5 μL of PI. Finally, the samples were analyzed using flow cytometry.

Analysis of DNM3 Methylation by qPCR

The tissue sample for testing was collected for DNA extraction and subsequently treated with bisulfite. The extracted genomic DNA was removed, and a bisulfite conversion mixture was prepared by adding 90 μL of Bisulfite Mix, 10 μL of buffer DP, the corresponding DNA samples, and ddH_2O into a 200 μL centrifuge tube, resulting in a total volume of 120 μL. Following system preparation, bisulfite conversion was performed using a real-time fluorescence quantitative PCR instrument (SLAN-96S, Shanghai Hongshi). The PCR reaction conditions were set as follows: 95 °C for 10 min, 64 °C for 60 min, followed by storage at 4 °C. The entire process took approximately one hour. After bisulfite treatment, DNA was purified using a centrifuge column, washed multiple times, and dried. Finally, 100 μL of EB eluent was added to collect the DNA solution for subsequent qPCR analysis. The PCR procedure was carried out on the SLAN-96S instrument. A final melting curve analysis was performed at the end of the process.

Co-immunoprecipitation

Antibodies targeting histone H3 and H4 were prepared and conjugated to magnetic beads, forming antibody–magnetic bead complexes. The antibody-magnetic bead complexes were then mixed with a DNA-fragmented sample and incubated overnight to allow specific binding of the antibodies to the target histones. Unbound antibodies and non-specifically bound histones were removed through a series of washing steps. After the final wash, the magnetic beads were lysed using an SDS-containing buffer to release the bound histones.

Western blot

The transfected cells were lysed with RIPA buffer (Abcam) containing a protease inhibitor mixture (Abcam). Total protein was separated using a 10% SurePAGE gel (GenScript) and transferred to a 0.45 μm polyvinylidene fluoride (PVDF) membrane (Millipore). The membrane was then blocked with 5% non-fat milk in TBST (APPLYGEN) for 1 h. Next, the membrane was incubated overnight at 4 °C with the primary antibody. Afterward, the secondary antibody was applied and incubated at room temperature for 1 h. Protein bands were visualized using the ECL substrate kit (Abcam).

Motor Function Assessment

The balance beam test was used to assess the motor coordination and integration abilities of rats at 7, 14, and 28 days after the nerve injury model. A 170 cm long, 2 cm wide square wooden rod was placed horizontally 70 cm above the ground, and the rats were allowed to walk along the balance beam. The scoring criteria were as follows: 0 points: Maintains steady balance; 1 point: Completely grasps one end of the balance beam; 2 points: Grips the balance beam, but one limb slips; 3 points: Grips the balance beam, but both limbs fall or the rat rotates around the beam for ≥ 60 s; 4 points: Attempts to maintain balance but slips for 40–60 s; 5 points: Attempts to maintain balance but slips for 20–40 s; 6 points: Attempts to maintain balance but slips within 20 s; The balance beam test serves as an indicator of the rats' coordination and integration abilities.

Neuromorphological Observation

Rats were anesthetized with a subcutaneous injection of 4% pentobarbital sodium at a dose of 35 mg/kg body weight. Once complete anesthesia was achieved, an incision was made between the right biceps femoris and semimembranosus muscles to expose the sciatic nerve, which was then separated bluntly between the two muscles. A 1.5–2 cm segment of the sciatic nerve was excised and fixed in a 12% formalin solution. Hematoxylin and eosin (HE) staining was performed to observe morphological changes in the sciatic nerve. Nissl staining was used to count neurons in frozen sections of the lumbar (L5) dorsal root ganglion. To identify large-diameter neurons, 20-μm serial sections were labeled with NeuroTrace fluorescent Nissl Green dye (1:200 dilution), and DAPI was used to label the nuclei for assessing the survival of nerve cells.

Electromyography (EMG)

EMG was used to assess the electrophysiological activity of the nervous system. After the final administration, rats from each group were anesthetized via intraperitoneal injection of 10% chloral hydrate (0.35 g/kg). A stimulating double-needle electrode was placed at the right sciatic notch, while the

recording electrode was positioned between the second and third toes of the right hind paw. The reference electrode was placed between the stimulating and recording electrodes, approximately 1 cm from the recording electrode. Following stimulation with a single-pulse square wave (pulse width 0.1 ms, stimulation intensity 1.5 times the threshold, and a stimulation interval of > 5 s), the electromyographic signals were recorded. The sciatic nerve conduction velocity (MNCV) was calculated using the formula: MNCV = Distance/Latency, where "Distance" is the distance between the stimulation and recording electrodes, and "Latency" is the time delay in the recorded response.

ELISA

The antibody was diluted to the desired concentration using a coating buffer and then added to the reaction wells. The plates were incubated at 4 °C overnight. The following day, the solution in the wells was discarded, and the wells were washed three times with washing buffer, each wash lasting 3 min. Afterward, the sample was added to the coated wells and incubated at 37 °C for 1 h, followed by washing. Next, a freshly diluted enzyme-labeled secondary antibody was added, and the plates were incubated at 37 °C for 0.5 to 1 h, followed by another wash. A freshly prepared TMB substrate solution was then added, and the plates were incubated at 37 °C for 10 to 30 min. Finally, 2 M sulfuric acid was added to terminate the reaction. The optical density (OD) of each well was measured at 450 nm, and the concentration of the target substance in the sample was calculated using the standard curve.

Data Analysis

Data were analyzed using one-way analysis of variance (ANOVA) with a post hoc test, with GraphPad Prism 9 software. Experiments were repeated at least in triplicate. The data are presented as mean ± SEM.

Results

Methionine Circulation Metabolism In Vitro

A methionine cycle metabolism disorder model was established in Schwann cells and neurons in vitro by inhibiting or knocking out key enzyme genes of the methionine cycle using specific inhibitors or RNA interference technology. To investigate the regulatory effects of FA on methionine cycle metabolism, cells were treated with FA at varying doses (1–10 μM), and levels of metabolites, including SAM and Hcy, were measured using UPLC–MS/MS.

The results demonstrated that, compared to the control group, SAM levels significantly decreased while Hcy levels increased in the disorder model group. However, following FA supplementation, both SAM and Hcy levels were restored (Fig. 1A, B).

To further evaluate whether FA can promote PNI repair through methionine cycle regulation, cell viability was assessed using the CCK-8 assay. Results indicated reduced cell viability in the disorder model group, which was restored following FA treatment (Fig. 1C). Additionally, flow cytometry revealed an increase in the proportion of apoptotic cells in the disorder model group, which was significantly reduced after FA supplementation (Fig. 1D, E). Morphological observations confirmed that FA intervention improved neural cell structure.

Methionine Circulation Metabolism In Vivo

To assess the regulatory effects of FA on methionine cycle metabolism in vivo, the rats were administered 0.8 mg/kg FA daily for 4 weeks. The results revealed that, compared to the control group, SAM levels significantly decreased while Hcy levels increased in the disorder model group. However, following FA supplementation, the levels of both SAM and Hcy were restored (Fig. 2A, B).

To further investigate whether FA promotes the repair of PNI via regulation of methionine cycle metabolism, a PNI was induced in the methionine metabolism disorder model. FA treatment was administered concurrently, and its effects on motor function, neural morphology, and electrophysiological recovery post-injury were evaluated.

The findings showed that, compared to the PNI + MI group, the FA − PNI + MI group exhibited improved motor coordination, restored neural morphology, increased neuron counts, enhanced survival of nerve cells, and faster sciatic nerve conduction velocities (Fig. 2C–F).

DNM3 Epigenetic Modification

To investigate whether FA promotes the repair of PNI by influencing the epigenetic modification of DNM3, sciatic nerve tissues were extracted from rats. The methylation level of DNM3 promoter in sciatic nerve tissue of PNI rats was quantitatively analyzed by qPCR. The results showed that after FA supplementation, the methylation level of DNM3 promoter region in rat sciatic nerve tissue was significantly reduced, indicating that FA could attenuate the inhibitory effect of methylation on DNM3 by reducing the methylation level of DNM3 promoter region, and then promoted its expression (Fig. 3A).

Subsequently, chromatin immunoprecipitation (ChIP) was performed to examine histone modifications, such as changes in histones H3 and H4, within the DNM3 promoter

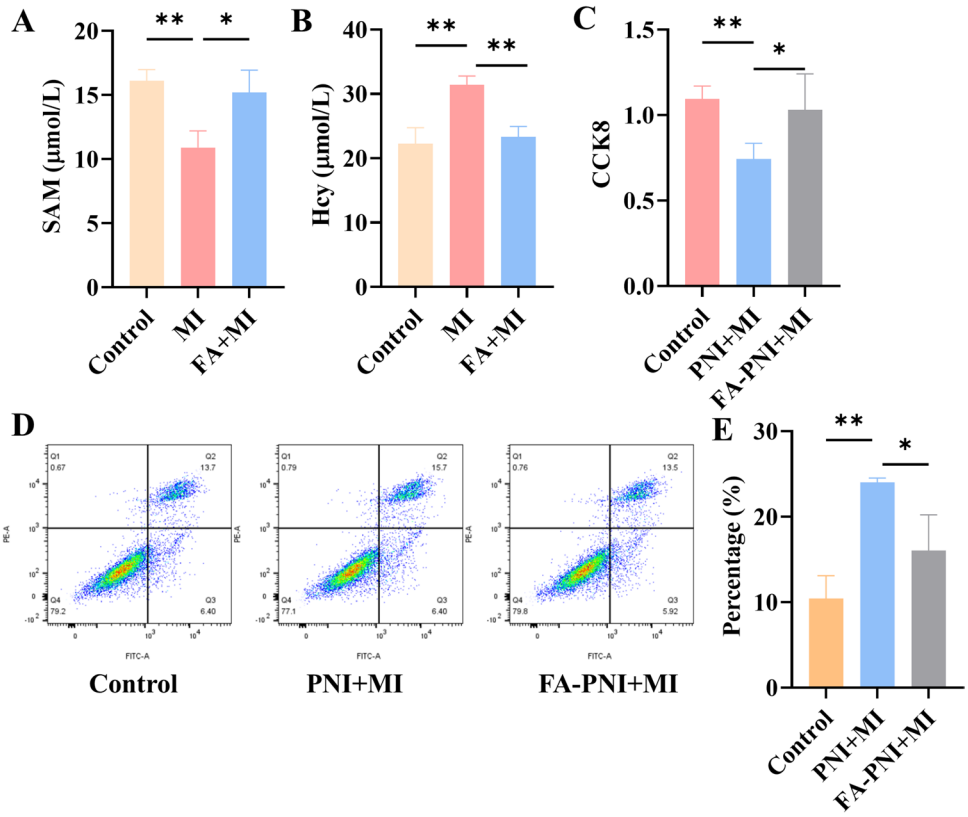

Fig. 1 In vitro experiments demonstrate that folic acid (FA) regulates methionine cycle metabolism and plays a crucial role in the repair of peripheral nerve injury (PNI). **A** UPLC–MS/MS analysis of SAM metabolite levels. **B** UPLC–MS/MS analysis of Hcy metabolite levels. **C** Cell viability measured using CCK-8 assay. **D** and **E** Apoptosis detection via flow cytometry. MI group was subjected to methionine cycle metabolism disorder interference. PNI + MI group was subjected to peripheral nerve injury in methionine cycle metabolism interference model. Statistical significance is indicated as $*p < 0.05$ and $**p < 0.01$ compared with the control group

region. The results demonstrated that histone modifications were also involved in the transcriptional regulation of DNM3 (Fig. 3B, C). These findings suggest that FA may enhance the repair of PNI by modulating the epigenetic landscape of DNM3 through mechanisms involving both DNA methylation and histone modification.

Effects of FA Against PNI

To further explore the mechanism by which FA promotes the repair of PNI, DNM3 adenovirus was locally injected into PNI rats. This was achieved using DNM3 adenovirus-mediated overexpression or RNA interference technology in vitro-cultured Schwann cells and neurons. The interaction between DNM3 and AKT was examined both in vivo and in vitro through immunoprecipitation assays. The results demonstrated that DNM3 interacts with AKT (Fig. 4A, B).

Subsequently, Western blot analysis was performed to assess the expression levels of apoptosis-related proteins BCL-2, Bax, Caspase-3, and autophagy-related proteins LC3 and Beclin-1, which are downstream of AKT. Compared to the control group, the DNM3-nerve injury group exhibited upregulation of BCL-2, LC3, and Beclin-1 proteins. Following the downregulation of Bax and Caspase-3 genes, the expression levels of BCL-2, LC3, Beclin-1, Bax, and Caspase-3 were restored after FA treatment (Fig. 4C, D).

Finally, the activities of oxidative stress-related enzymes, including glutathione peroxidase (GPx) and superoxide dismutase (SOD), were measured using ELISA. The results showed that the activities of GPx and SOD were elevated in the DNM3-nerve injury group, and these activities were restored to normal levels following FA treatment (Fig. 4E, F).

In summary, the in vitro and in vivo studies suggest that FA promotes PNI repair through the DNM3-AKT pathway, modulating cell fate and oxidative stress responses.

Discussion

This study systematically elucidates how FA promotes PNI repair by modulating the DNM3-AKT pathway through methionine cycle regulation and epigenetic modification. The establishment of methionine cycle interference models in Schwann cells, neurons, and rats using specific inhibitors or gene knockdown provides a valuable experimental platform, simulating pathological disruptions and enabling the study of their impact on nerve cell behavior. In vitro, disrupting the methionine cycle in Schwann cells and neurons—the primary components of the peripheral nervous system—reveals changes in their biological activity, while in vivo rat models, with physiological similarities to humans, enhance clinical relevance.

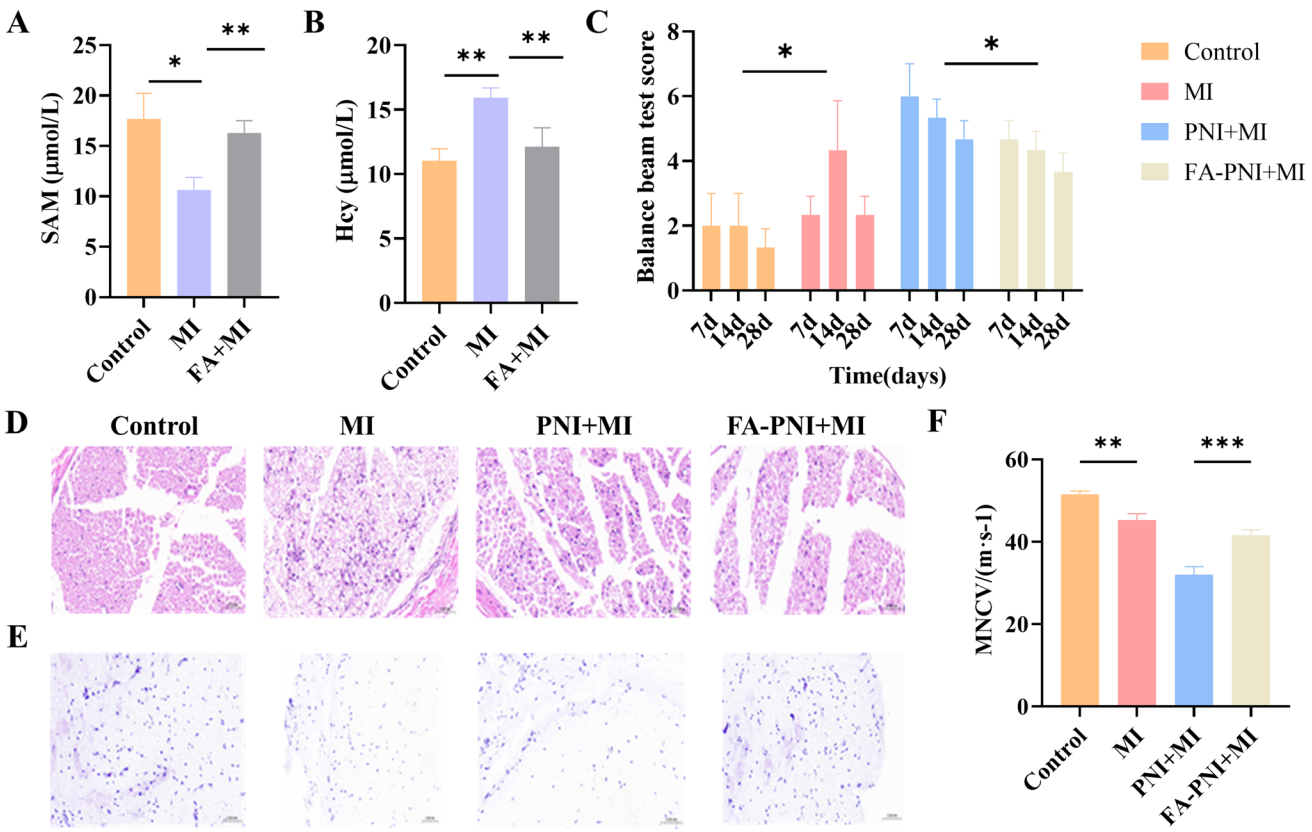

Fig. 2 In vivo experiments demonstrate that folic acid (FA) regulates methionine cycle metabolism and plays a crucial role in the repair of peripheral nerve injury (PNI). **A** UPLC–MS/MS analysis of SAM metabolite levels. **B** UPLC–MS/MS analysis of Hcy metabolite levels. **C** Balance beam test to assess the motor coordination and integration ability of rats. **D** HE staining to observe morphological changes in brain tissue. **E** Nissl staining to count neurons and evaluate the survival of nerve cells. **F** Electromyography (EMG) to assess the electrophysiological activity of the nervous system. MI group was subjected to methionine cycle metabolism disorder interference. PNI + MI group was subjected to peripheral nerve injury in methionine cycle metabolism interference model. Statistical significance is indicated as $*p<0.05$, $**p<0.01$ and $***p<0.001$ compared with the control group

In this study, it was revealed that FA partially mitigated Hcy levels. In the methionine cycle disorder model group, the blockage of the methionine cycle leads to a decrease in SAM and the accumulation of Hcy, as the remethylation or transsulfuration pathways of Hcy may be disrupted (Avila et al., 2005). The active form of FA in the body is 5-methyltetrahydrofolate (5-MTHF), which acts as a methyl donor in the remethylation process of Hcy (Scaglione & Panzavolta, 2014). After FA treatment, the levels of 5-MTHF increase, potentially promoting this process, thereby reducing Hcy levels. Additionally, SAM is an allosteric activator of cystathionine beta-synthase (CBS). Low SAM levels may inhibit CBS, leading to Hcy accumulation. FA supplementation may help restore SAM levels, activate CBS, promote the transsulfuration pathway of Hcy, and thus reduce Hcy levels.

The methionine cycle plays a vital role in synthesizing methyl donors like S-adenosylmethionine (SAM), crucial for DNA methylation (Rolland et al., 2015; Tassinari et al., 2024; Zubiete-Franco et al., 2016). Disruptions in this cycle can alter DNA methylation, affecting gene expression, nerve cell survival, proliferation, and differentiation, which challenges nerve regeneration after injury (Zhang, 2018). FA, as a key regulator of the methionine cycle, supports SAM synthesis, thereby influencing DNA and histone methylation and gene expression (Cao et al., 2022; Clare et al., 2019; Menezo et al., 2022; Mentch & Locasale, 2016). Elevated homocysteine in PNI patients suggests methionine cycle dysregulation contributes to nerve damage (Shandal & Luo, 2016). Abnormal DNA methylation is also implicated in various nerve injuries (Portela & Esteller, 2010).

In this study, FA supplementation restored methionine cycle metabolism, reduced DNM3 promoter methylation, and relieved methylation-associated inhibition, thereby enhancing DNM3 expression. These findings suggest FA regulates DNM3 by participating in SAM and homocysteine metabolism, modulating DNA methylation to promote nerve

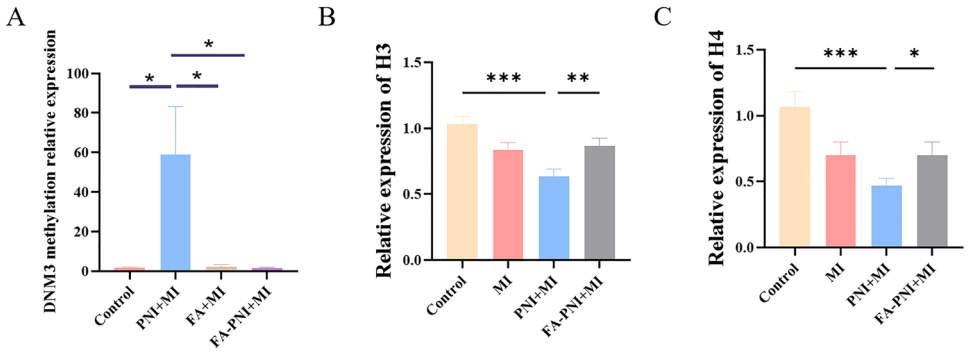

Fig. 3 Folic acid (FA) affects the epigenetic modification of DNM3. **A** Methylation analysis of the DNM3 promoter region in sciatic nerve tissue from rats with peripheral nerve injury (PNI). **B** and **C** Chromatin immunoprecipitation (ChIP) to analyze histone modifications (H3 and H4) in the DNM3 promoter region. MI group was subjected to methionine cycle metabolism disorder interference. PNI + MI group was subjected to peripheral nerve injury in methionine cycle metabolism interference model. Statistical significance is expressed as $*p < 0.05$ and $**p < 0.01$ compared with the control group

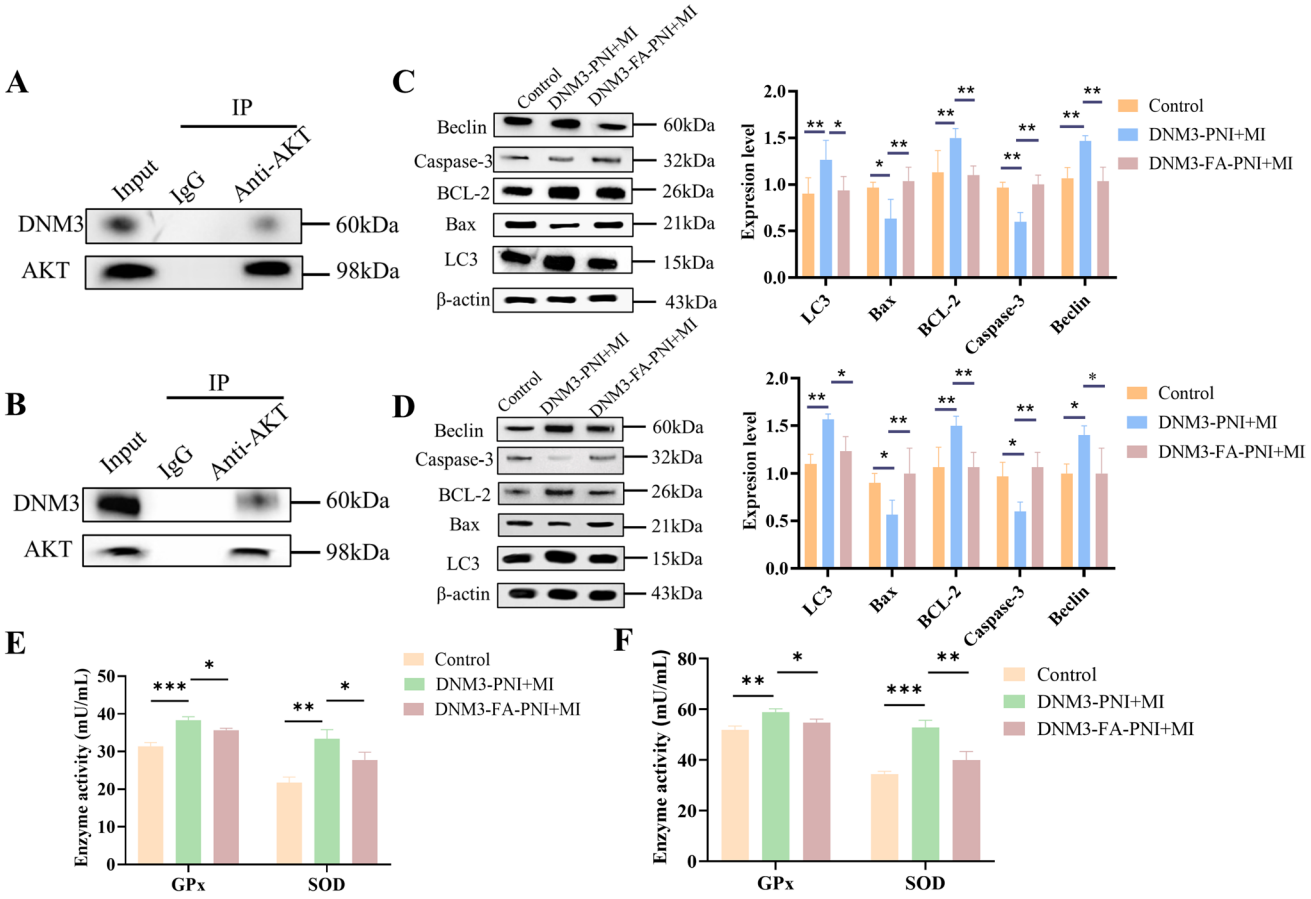

Fig. 4 Folic acid (FA) promotes the repair of peripheral nerve injury (PNI) via the DNM3-AKT pathway. **A** Immunoprecipitation to investigate the interaction between DNM3 and AKT in Schwann cells and neurons. **B** Immunoprecipitation to investigate the interaction between DNM3 and AKT in rats with PNI. **C** Western blot analysis of the expression levels of apoptosis-related proteins BCL-2, Bax, Caspase-3, and autophagy-related proteins LC3 and Beclin-1 in Schwann cells and neurons. **D** Western blot analysis of apoptosis-related proteins BCL-2, Bax, Caspase-3, and autophagy-related proteins LC3 and Beclin-1 downstream of AKT in Schwann cells and neurons. **E** ELISA to measure the activity of oxidative stress-related factors such as glutathione peroxidase (GPx) and superoxide dismutase (SOD) in Schwann cells and neurons. **F** ELISA to measure the activities of oxidative stress-related factors, GPx and SOD, in rats with PNI. Statistical significance is expressed as $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ compared with the control group

repair. DNM3 influences cell proliferation, apoptosis, and metabolism via interaction with AKT, an essential neuroprotective pathway for axon regeneration (Fayard et al., 2010; Huang et al., 2021; Ma et al., 2019; Zheng et al., 2024). Overexpression of DNM3 has been linked to inhibiting AKT phosphorylation, inducing tumor cell autophagy and apoptosis (Fa, 2021). This study confirms DNM3 positively regulates the AKT pathway, and FA supplementation amplifies this effect, potentially through dual regulation of DNM3 and AKT. Additionally, FA appears to influence cell metabolism and survival by modulating AKT activity, with both mechanisms synergistically promoting nerve repair.

AKT plays a pivotal role in various cellular processes, including metabolism, growth, and survival. Upon activation, AKT translocates to different cellular compartments, including the mitochondria, where it can phosphorylate various substrates to modulate mitochondrial dynamics and function (Sugiyama et al., 2019). The interplay between DNM3 and AKT is significant in the context of mitochondrial dynamics. Studies have shown that activation of the PI3K/AKT pathway can lead to the induction of heme oxygenase-1 (HO-1), an enzyme that plays a role in protecting cells from oxidative stress. The induction of HO-1 has been associated with the regulation of mitochondrial dynamics, including processes like fusion and fission, which are essential for maintaining mitochondrial function and integrity (Shi et al., 2019). Furthermore, research indicates that dynamin-related proteins, such as DNM3, are involved in the regulation of mitochondrial fission and fusion processes (Liu et al., 2021). These processes are critical for maintaining mitochondrial function and are influenced by various signaling pathways, including the AKT pathway. Together, DNM3 and AKT are both integral to the regulation of mitochondrial dynamics. DNM3 facilitates mitochondrial fission, while AKT influences mitochondrial function through various signaling pathways, including the PI3K/AKT pathway.

Despite the promising findings, limitations remain. The methionine cycle interference models, although valuable, may not fully replicate pathological disruptions. While the rat model offers clinical insights, the complexity of human physiology requires caution when extrapolating results to clinical applications. Moreover, the specific molecular interactions of DNM3 with the AKT pathway, including binding sites and interacting proteins, need further investigation.

Conclusions

In conclusion, FA promotes peripheral nerve repair by modulating the DNM3-AKT pathway, presenting significant research and therapeutic potential. Future studies should focus on elucidating the precise mechanisms of FA's regulation of this pathway to develop targeted therapies and personalized treatment strategies for peripheral nerve injuries.

Author Contributions Study design/planning: Weibo Kang, Yanli Zhang. Data collection/entry: Weibo Kang, Yanli Zhang, Wei Cui, Hua Meng, Duo Zhang. Data analysis/statistics: Weibo Kang, Yanli Zhang. Data interpretation: Weibo Kang, Yanli Zhang. Preparation of manuscript: Weibo Kang, Yanli Zhang, Wei Cui, Hua Meng, Duo Zhang. Literature analysis/search: Weibo Kang, Yanli Zhang. Funds collection: Weibo Kang.

Funding The study was supported by Natural Science Foundation of Capital Medical University of China (#PYZ2017082).

Data Availability Data are available upon reasonable request by contacting the corresponding author.Consent for Publication Not applicable.

Declarations

Conflict of interest Authors declare that they have no conflict of interest to disclose.

Ethical Approval Animal studies were approved by Animal Welfare Ethics Committee of Beijing Neurosurgery Institute (#2124210). This study was performed in strict accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication No. 85-23 Rev. 1985).

References

An, S., Shi, J., Huang, J., Li, Z., Feng, M., & Cao, G. (2024). HIF-1alpha induced by hypoxia promotes peripheral nerve injury recovery through regulating ferroptosis in DRG neuron. *Molecular Neurobiology, 61*, 6300–6311. https://doi.org/10.1007/s12035-024-03964-5

Avila, M. A., Berasain, C., Prieto, J., Mato, J. M., Garcia-Trevijano, E. R., & Corrales, F. J. (2005). Influence of impaired liver

methionine metabolism on the development of vascular disease and inflammation. *Current Medicinal Chemistry Cardiovascular and Hematological Agents, 3*, 267–281. https://doi.org/10.2174/1568016054368197

Bolandghamat, S., & Behnam-Rassouli, M. (2020). Recent findings on the effects of pharmacological agents on the nerve regeneration after peripheral nerve injury. *Current Neuropharmacology, 18*, 1154–1163. https://doi.org/10.2174/1570159X18666200507084024

Brown, E. E., Blauwendraat, C., Trinh, J., Rizig, M., Nalls, M. A., Leveille, E., Ruskey, J. A., Jonvik, H., Tan, M. M. X., Bandres-Ciga, S., Hassin-Baer, S., Brockmann, K., Infante, J., Tolosa, E., Ezquerra, M., Ben Romdhan, S., Benmahdjoub, M., Arezki, M., Mhiri, C., … International Parkinson Disease Genomics Consortium. (2021). Analysis of DNM3 and VAMP4 as genetic modifiers of LRRK2 Parkinson's disease. *Neurobiology of Aging, 97*(148), e117-e148.e124. https://doi.org/10.1016/j.neurobiolaging.2020.07.002

Cao, R., Xie, J., & Zhang, L. (2022). Abnormal methylation caused by folic acid deficiency in neural tube defects. *Open Life Sciences, 17*, 1679–1688. https://doi.org/10.1515/biol-2022-0504

Chen, Y., Wang, Z., Xie, Y., Guo, X., Tang, X., Wang, S., Yang, S., Chen, K., Niu, Y., & Ji, W. (2012). Folic acid deficiency inhibits neural rosette formation and neuronal differentiation from rhesus monkey embryonic stem cells. *Journal of Neuroscience Research, 90*, 1382–1391. https://doi.org/10.1002/jnr.23030

Clare, C. E., Brassington, A. H., Kwong, W. Y., & Sinclair, K. D. (2019). One-carbon metabolism: Linking nutritional biochemistry to epigenetic programming of long-term development. *Annual Review of Animal Biosciences, 7*, 263–287. https://doi.org/10.1146/annurev-animal-020518-115206

El Soury, M., Fornasari, B. E., Carta, G., Zen, F., Haastert-Talini, K., & Ronchi, G. (2021). The role of dietary nutrients in peripheral nerve regeneration. *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms22147417

Fa, J. (2021). Dynamin 3 overexpression suppresses the proliferation, migration and invasion of cervical cancer cells. *Oncology Letters, 22*, 524. https://doi.org/10.3892/ol.2021.12785

Fayard, E., Xue, G., Parcellier, A., Bozulic, L., & Hemmings, B. A. (2010). Protein kinase B (PKB/Akt), a key mediator of the PI3K signaling pathway. *Current Topics in Microbiology and Immunology, 346*, 31–56. https://doi.org/10.1007/82_2010_58

Finnell, R. H., Spiegelstein, O., Wlodarczyk, B., Triplett, A., Pogribny, I. P., Melnyk, S., & James, J. S. (2002). DNA methylation in Folbp1 knockout mice supplemented with folic acid during gestation. *Journal of Nutrition, 132*, 2457S-2461S. https://doi.org/10.1093/jn/132.8.2457S

Huang, N., Li, S., Xie, Y., Han, Q., Xu, X. M., & Sheng, Z. H. (2021). Reprogramming an energetic AKT-PAK5 axis boosts axon energy supply and facilitates neuron survival and regeneration after injury and ischemia. *Current Biology, 31*, 3098-3114.e3097. https://doi.org/10.1016/j.cub.2021.04.079

Huang, Y., Ye, K., He, A., Wan, S., Wu, M., Hu, D., Xu, K., Wei, P., & Yin, J. (2024). Dual-layer conduit containing VEGF-A—Transfected Schwann cells promotes peripheral nerve regeneration via angiogenesis. *Acta Biomaterialia, 180*, 323–336. https://doi.org/10.1016/j.actbio.2024.03.029

Hussain, G., Wang, J., Rasul, A., Anwar, H., Qasim, M., Zafar, S., Aziz, N., Razzaq, A., Hussain, R., de Aguilar, J. G., & Sun, T. (2020). Current status of therapeutic approaches against peripheral nerve injuries: A detailed story from injury to recovery. *International Journal of Biological Sciences, 16*, 116–134. https://doi.org/10.7150/ijbs.35653

Iskandar, B. J., Nelson, A., Resnick, D., Skene, J. H., Gao, P., Johnson, C., Cook, T. D., & Hariharan, N. (2004). Folic acid supplementation enhances repair of the adult central nervous system. *Annals of Neurology, 56*, 221–227. https://doi.org/10.1002/ana.20174

Kang, W. B., Chen, Y. J., Lu, D. Y., & Yan, J. Z. (2019). Folic acid contributes to peripheral nerve injury repair by promoting Schwann cell proliferation, migration, and secretion of nerve growth factor. *Neural Regeneration Research, 14*, 132–139. https://doi.org/10.4103/1673-5374.243718

Liu, J., Song, X., Yan, Y., & Liu, B. (2021). Role of GTPase-dependent mitochondrial dynamins in heart diseases. *Frontiers in Cardiovascular Medicine, 8*, 720085. https://doi.org/10.3389/fcvm.2021.720085

Lopes, B., Sousa, P., Alvites, R., Branquinho, M., Sousa, A. C., Mendonca, C., Atayde, L. M., Luis, A. L., Varejao, A. S. P., & Mauricio, A. C. (2022). Peripheral nerve injury treatments and advances: One health perspective. *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms23020918

Ma, Y., Guan, L., Han, Y., Zhou, Y., Li, X., Liu, Y., Zhang, X., Zhang, W., Li, X., Wang, S., & Lu, W. (2019). siPRDX2-elevated DNM3 inhibits the proliferation and metastasis of colon cancer cells via AKT signaling pathway. *Cancer Management and Research, 11*, 5799–5811. https://doi.org/10.2147/CMAR.S193805

Menezo, Y., Elder, K., Clement, A., & Clement, P. (2022). Folic acid, folinic acid, 5 methyl tetrahydrofolate supplementation for mutations that affect epigenesis through the folate and one-carbon cycles. *Biomolecules*. https://doi.org/10.3390/biom12020197

Mentch, S. J., & Locasale, J. W. (2016). One-carbon metabolism and epigenetics: Understanding the specificity. *Annals of the New York Academy of Sciences, 1363*, 91–98. https://doi.org/10.1111/nyas.12956

Piedrahita, J. A., Oetama, B., Bennett, G. D., van Waes, J., Kamen, B. A., Richardson, J., Lacey, S. W., Anderson, R. G., & Finnell, R. H. (1999). Mice lacking the folic acid-binding protein Folbp1 are defective in early embryonic development. *Nature Genetics, 23*, 228–232. https://doi.org/10.1038/13861

Portela, A., & Esteller, M. (2010). Epigenetic modifications and human disease. *Nature Biotechnology, 28*, 1057–1068. https://doi.org/10.1038/nbt.1685

Poulose, S. M., Miller, M. G., Scott, T., & Shukitt-Hale, B. (2017). Nutritional factors affecting adult neurogenesis and cognitive function. *Advances in Nutrition, 8*, 804–811. https://doi.org/10.3945/an.117.016261

Reynolds, E. H. (2002). Benefits and risks of folic acid to the nervous system. *Journal of Neurology, Neurosurgery and Psychiatry, 72*, 567–571. https://doi.org/10.1136/jnnp.72.5.567

Reynolds, E. H. (2006). Vitamin B_{12}, folic acid, and the nervous system. *Lancet Neurology, 5*, 949–960. https://doi.org/10.1016/S1474-4422(06)70598-1

Rolland, M., Dalsgaard, J., Holm, J., Gomez-Requeni, P., & Skov, P. V. (2015). Dietary methionine level affects growth performance and hepatic gene expression of GH-IGF system and protein turnover regulators in rainbow trout (*Oncorhynchus mykiss*) fed plant protein-based diets. *Comparative Biochemistry and Physiology Part b: Biochemistry and Molecular Biology, 181*, 33–41. https://doi.org/10.1016/j.cbpb.2014.11.009

Scaglione, F., & Panzavolta, G. (2014). Folate, folic acid and 5-methyltetrahydrofolate are not the same thing. *Xenobiotica, 44*, 480–488. https://doi.org/10.3109/00498254.2013.845705

Shandal, V., & Luo, J. J. (2016). Clinical manifestations of isolated elevated homocysteine-induced peripheral neuropathy in adults. *Journal of Clinical Neuromuscular Disease, 17*, 106–109. https://doi.org/10.1097/CND.0000000000000108

Shi, G., Zhou, X., Wang, X., Zhang, X., Zhang, P., & Feng, S. (2020). Signatures of altered DNA methylation gene expression after central and peripheral nerve injury. *Journal of Cellular Physiology, 235*, 5171–5181. https://doi.org/10.1002/jcp.29393

Shi, J., Yu, J., Zhang, Y., Wu, L., Dong, S., Wu, L., Wu, L., Du, S., Zhang, Y., & Ma, D. (2019). PI3K/Akt pathway-mediated HO-1 induction regulates mitochondrial quality control and attenuates endotoxin-induced acute lung injury. *Laboratory Investigation, 99*, 1795–1809. https://doi.org/10.1038/s41374-019-0286-x

Sugiyama, M. G., Fairn, G. D., & Antonescu, C. N. (2019). Akt-ing up just about everywhere: Compartment-specific Akt activation and function in receptor tyrosine kinase signaling. *Frontiers in Cell and Developmental Biology, 7*, 70. https://doi.org/10.3389/fcell.2019.00070

Tassinari, V., Jia, W., Chen, W. L., Candi, E., & Melino, G. (2024). The methionine cycle and its cancer implications. *Oncogene, 43*, 3483–3488. https://doi.org/10.1038/s41388-024-03122-0

Vijayavenkataraman, S. (2020). Nerve guide conduits for peripheral nerve injury repair: A review on design, materials and fabrication methods. *Acta Biomaterialia, 106*, 54–69. https://doi.org/10.1016/j.actbio.2020.02.003

Wallingford, J. B., Niswander, L. A., Shaw, G. M., & Finnell, R. H. (2013). The continuing challenge of understanding, preventing, and treating neural tube defects. *Science, 339*, 1222002. https://doi.org/10.1126/science.1222002

Wang, M. L., Rivlin, M., Graham, J. G., & Beredjiklian, P. K. (2019). Peripheral nerve injury, scarring, and recovery. *Connective Tissue Research, 60*, 3–9. https://doi.org/10.1080/03008207.2018.1489381

Yang, J. K., Song, J., Huo, H. R., Zhao, Y. L., Zhang, G. Y., Zhao, Z. M., Sun, G. Z., & Jiao, B. H. (2017). DNM3, p65 and p53 from exosomes represent potential clinical diagnosis markers for glioblastoma multiforme. *Therapeutic Advances in Medical Oncology, 9*, 741–754. https://doi.org/10.1177/1758834017737471

Zhang, N. (2018). Role of methionine on epigenetic modification of DNA methylation and gene expression in animals. *Animal Nutrition, 4*, 11–16. https://doi.org/10.1016/j.aninu.2017.08.009

Zheng, T., Jiang, T., Ma, H., Zhu, Y., & Wang, M. (2024). Targeting PI3K/Akt in cerebral ischemia reperfusion injury alleviation: From signaling networks to targeted therapy. *Molecular Neurobiology, 61*, 7930–7949. https://doi.org/10.1007/s12035-024-04039-1

Zubiete-Franco, I., Garcia-Rodriguez, J. L., Martinez-Una, M., Martinez-Lopez, N., Woodhoo, A., Juan, V. G., Beraza, N., Lage-Medina, S., Andrade, F., Fernandez, M. L., Aldamiz-Echevarria, L., Fernandez-Ramos, D., Falcon-Perez, J. M., Lopitz-Otsoa, F., Fernandez-Tussy, P., Barbier-Torres, L., Luka, Z., Wagner, C., Garcia-Monzon, C., … Varela-Rey, M. (2016). Methionine and S-adenosylmethionine levels are critical regulators of PP2A activity modulating lipophagy during steatosis. *Journal of Hepatology, 64*, 409–418. https://doi.org/10.1016/j.jhep.2015.08.037