Schwann cells-derived exosomal miR-21 participates in high glucose regulation of neurite outgrowth

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Highlights
The miR-21 was decreased in serum exosomes and sciatic nerve of DPN rats
High glucose inhibited SC viability and downregulated the expression of miR-21
Exosomes derived from SC cultured in high glucose inhibited the neurite outgrowth
SC-derived exosomes rich in miR-21 accelerated the neurite outgrowth of neuron
Schwann cells-derived exosomal miR-21 participates in high glucose regulation of neurite outgrowth

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SUMMARY
As a common complication of diabetes, the pathogenesis of diabetic peripheral neuropathy (DPN) is closely related to high glucose but has not been clarified. Exosomes can mediate crosstalk between Schwann cells (SC) and neurons in the peripheral nerve. Herein, we found that miR-21 in serum exosomes from DPN rats was decreased. SC proliferation was inhibited, cell apoptosis was increased, and the expression of miR-21 in cells and exosomes was downregulated when cultured in high glucose. Increasing miR-21 expression reversed these changes, while knockdown of miR-21 led to the opposite results. When cocultured with exosomes derived from SC exposed to high glucose, neurite outgrowth was inhibited. On the contrary, neurite outgrowth was accelerated when incubated with exosomes rich in miR-21. We further demonstrated that the SC-derived exosomal miR-21 participates in neurite outgrowth probably through the AKT signaling pathway. Thus, SC-derived exosomal miR-21 contributes to high glucose regulation of neurite outgrowth.

INTRODUCTION
Approximately 20% of type I diabetic (T1D) and 50% of type II diabetic (T2D) patients are affected by diabetic peripheral neuropathy (DPN) (Hicks and Selvin, 2019). DPN usually manifests as a feeling of pain and numbness. DPN not only has a tremendous impact on patient’s quality of life but also affects the mortality rate (approximately 25%–50% within 5–10 years). The pathogenesis of DPN has not yet been fully elucidated; however, hyperglycemia is viewed as a major factor that plays an important role in the development of DPN (Levitt et al., 1996).

MicroRNAs (miRNAs) are small RNA with a length of 18–28 nucleotides (Jagadeeswaran et al., 2012), which can mediate gene silencing. They are involved in almost all biological processes (O’Brien et al., 2018), and their importance in the field of peripheral nerve injury (PNI) and regeneration has been widely recognized (Sullivan et al., 2018). MiR-21 was upregulated after PNI (Karl et al., 2017; Sakai and Suzuki, 2013, Yu et al., 2011). Upregulation of miR-21 plays an important role in promoting Schwann cells (SC) proliferation, cell apoptosis of adult dorsal root ganglion neurons, and axon regeneration (Luo et al., 2017; Zhou et al., 2015; Ning et al., 2020). Interestingly, reduced miR-21 levels were found in the peripheral nerve and circulation of T2D model mice (Wang et al., 2020a; Wu et al., 2020).

SC form a myelin sheath to protect axons (Salzer, 2015) and there is a close metabolic interaction between SC and axons (Boucanova and Chrast, 2020). Exosomes are extracellular vesicles secreted by cells and are in a size range of 40–160 nm (Kalluri and LeBleu, 2020). As miRNA carriers (Yu et al., 2016), SC-derived exosomes can be specifically internalized by axons and enhance regeneration after PNI (Lopez Verrilli et al., 2013). Additionally, exosomes can also reverse the reduction in miR-21 levels induced by diabetes, promote neurite outgrowth, and improve sciatic nerve conduction velocity (NCV) in DPN mice (Wang et al., 2020a).

Here, we explored whether miR-21 levels were reduced in the peripheral nerve and circulation of T1D DPN model rats, then we focused on whether SC-derived exosomal miR-21 participates in high glucose regulation in NG108-15 cells (simulation of peripheral neurons (Ching et al., 2018)) neurite outgrowth, to provide a
reasonable basis for the application of SC-derived exosomal miR-21 for investigating the mechanisms and treatment of DPN.

RESULTS

The expression of miR-21 was decreased in serum exosomes and sciatic nerve of DPN rats

To investigate the expression changes of miR-21 in serum exosomes and peripheral nerves of DPN rats, we first established the T1D DPN model and found that the NCV of DPN rats was significantly lower than that of the control group (Figure 1A). In addition, the number of axons and myelin was significantly reduced in DPN rats compared with the control group, and the shape of myelin was changed (Figure 1B). These results demonstrated that the DPN model was successfully established. The expression of miR-21 in serum exosomes and sciatic nerve was detected by qPCR (n = 10 rats). (C) The level of miR-21 was decreased in serum exosomes and sciatic nerve of DPN rats (n = 10 rats). For the above, data are represented as mean ± SD (t-test: **p < 0.01, ***p < 0.001 versus the control group).

High glucose inhibited SC proliferation, increased cell apoptosis, and downregulated the expression of miR-21 in cells and exosomes

We then explored the impact of a high-glucose environment on SC and the changes of miR-21 in vitro. Cell viability varies regularly with glucose concentration (Figures S1A and S1B), and therefore 3 typical concentrations were chosen. Effects of high glucose on SC were detected after treatment with low glucose (1 g/L), normal glucose (4.5 g/L), or high glucose (18 g/L) by performing a CCK-8 cell viability assay. The results showed that cell proliferation of SC has been inhibited in the high glucose group compared with the low and normal glucose group at 48 h and 72 h; the low glucose group had the best cell viability. In future
experiments, the normal glucose group was excluded, and 72 h was used as the time-point since the differences between the groups were obvious at 72 h (Figure 2A).

Figure 2. High glucose conditions inhibited SC proliferation, increased cell apoptosis, and downregulated the expression of miR-21 in cells and exosomes

(A) The cell viability of SC was examined by CCK-8 assay, high glucose causes cell viability to decrease (n = 10-well, one-way ANOVA: ***p < 0.001 versus the low glucose group. ###p < 0.001 versus the normal glucose group).

(B) The expression of miR-21 in SC and exosomes was detected by qPCR, and miR-21 was downregulated in the high glucose group compared with that of the low glucose group (n = 10 samples; t-test: ***p < 0.001 versus the low glucose group).

(C) Exosome markers (CD9 and CD63) were detected by Western blot after exosomes were isolated by ultracentrifugation.

(D and E) Protein expression of p-AKT, t-AKT, and GAPDH was detected by Western blot (D), and the expression of the AKT signaling pathway was decreased in the high glucose group compared with that of the low glucose group. (E) Gray value statistics of Western blot (n = 6 samples, nonparametric tests (Mann-Whitney Test, same below): **p < 0.01 versus the low glucose group).

(F and G) TUNEL assay (F) was used to detect cell apoptosis (red), apoptosis increased in the high glucose group compared with the low glucose group. Scale bar: 50 μm.

(G) Cell apoptosis statistics (n = 20 fields of view per group, nonparametric tests: ***p < 0.001 versus the low glucose group). For the above, data are represented as mean ± SD.

Exosomes were isolated by ultracentrifugation and were characterized by exosome markers (CD9 and CD63) detected by Western blot (Figure 2C). After RNA purification from SC and exosomes, miR-21 expression was detected by qPCR. Expression of miR-21 in cells and exosomes was downregulated in the high glucose group compared with the low glucose group (Figure 2B). Detection of protein expression of p-AKT, t-AKT, and GAPDH by Western blot indicated that the expression of proteins associated with the AKT signaling pathway was significantly decreased under high glucose conditions in SC (Figures 2D and 2E). We used the TUNEL assay to identify and quantify apoptotic cells and observed increased cell apoptosis of SC in the high glucose group (Figures 2F and 2G). The above results indicate that miR-21 may play an important role in the pathogenesis of DPN.
Increasing levels of miR-21 enabled SC viability and reduced cell apoptosis in high glucose conditions

To clarify the regulatory role of miR-21 in DPN in vitro, we next explored the effects of miR-21 on SC in high glucose. We performed a qRT-PCR assay to verify that miR-21 expression was upregulated after transfection of miR-21 mimic compared with the MC group (n = 10 samples, t-test: ***p < 0.001 versus the MC group). The cell viability of SC was examined by CCK-8 assay and upregulation of miR-21 resulted in a significant increase in SC viability compared with the MC group (n = 10-well; t-test: p < 0.001 versus the MC group).

(C and D) Protein expression of p-AKT, t-AKT, Bcl-2, Bax, C-cas3, and GAPDH in SC was detected by Western blot (C), results showed that compared with the MC group, the upregulation of miR-21 resulted in increased expression of Bcl-2/Bax, while decreased expression of C-cas3/GAPDH. (D) Gray value statistics of Western blot (n = 4 samples, nonparametric tests: *p < 0.05 of p-AKT/AKT, Bcl2/Bax and C-cas3/GAPDH versus the MC group).

(E and F) TUNEL assay (E) to detect SC cell apoptosis (red) showed that increasing miR-21 levels reduced cell apoptosis in SC under high glucose conditions compared with the MC group. Scale bar: 50 µm. (F) Cell apoptosis statistics (n = 20 fields of view per group, t-test: ***p < 0.001 versus the MC group). For the above, data are represented as mean ± SD.

Increasing levels of miR-21 enabled SC viability and reduced cell apoptosis in high glucose conditions

To clarify the regulatory role of miR-21 in DPN in vitro, we next explored the effects of miR-21 on SC in high glucose. We performed a qRT-PCR assay to verify that miR-21 expression was upregulated after transfection of the miR-21 mimic (Figure 3A). The upregulation of miR-21 led to a significant increase in SC viability compared with the mimic control (MC) group (Figure 3B). The AKT signaling pathway was activated, as shown by Western blot (Figures 3C and 3D). We also determined Bcl-2/Bax and Cleaved Caspase-3 (C-cas3/GAPDH) protein expression because these proteins are related to the regulation of cell apoptosis;
a TUNEL assay was also performed. The results showed that upregulation of miR-21 causes an increase in the expression of Bcl-2/Bax and a concomitant decrease in the expression of C-cas3/GAPDH (Figures 3C and 3D), suggesting that under high glucose conditions, increasing levels of miR-21 reduced cell apoptosis of SC. The TUNEL assay results also confirmed these findings (Figures 3E and 3F).

SC-derived exosomes induced by high glucose levels inhibited the neurite outgrowth of NG108-15 cells

Degeneration of distal axons of peripheral neurons progresses in DPN, and exosomes can exchange materials and information between SC and peripheral neurons and their axons. To detect the effect of exosomes secreted by SC on neuronal cell lines under high glucose, exosomes (L-EXO and H-EXO) were extracted from the cell culture supernatants of SC cultured in low glucose or high glucose for 72 h. NG108-15 cells exposed to high glucose conditions were added with L-EXO or H-EXO daily for 3 days. Then, total RNA and protein were isolated from the cells. PCR and Western blot were used to detect the AKT signaling pathway and neurite growth-related factors (DNMT3A, and GAP43), respectively. The results demonstrated that the AKT signaling pathway and neurite growth-related factors were suppressed in NG108-15 cells treated with H-EXO compared to L-EXO (n = 10 samples; t-test: ***p < 0.001 versus the L-EXO group).

Immunocytochemistry staining of βIII Tubulin (red) to observe neurite outgrowth, H-EXO significantly reduced neurite outgrowth in NG108-15 cells compared to L-EXO. Scale bar: 25 μm. (E) Neurite length quantification (n = 10 fields of view per group, t-test: ***p < 0.001 versus the L-EXO group). For the above, data are represented as mean ± SD.

Figure 4. High glucose-induced SC-derived exosomes inhibited the neurite outgrowth of NG108-15 cells in high glucose

(A) Expression of AKT1, DNMT3A, and GAP43 in NG108-15 was detected by PCR, and AKT signaling pathway and neurite outgrowth-related factors (DNMT3A and GAP43) were inhibited in NG108-15 cells treated with H-EXO compared to L-EXO (n = 10 samples; t-test: ***p < 0.001 versus the L-EXO group).

(B and C) Western blot (B) was used to detect the protein expression of p-AKT, t-AKT, DNMT3A, and GAP43 in NG108-15 cells, the results had the same trend as the PCR results. (C) Gray value statistics of Western blot (n = 4 samples, nonparametric tests: *p < 0.05 of p-AKT/AKT, Bcl2/Bax and C-cas3/GAPDH versus the L-EXO group).

(D and E) Immunocytochemistry staining (D) of βIII Tubulin (red) to observe neurite outgrowth, H-EXO significantly reduced neurite outgrowth in NG108-15 cells compared to L-EXO. Scale bar: 25 μm. (E) Neurite length quantification (n = 10 fields of view per group, t-test: ***p < 0.001 versus the L-EXO group). For the above, data are represented as mean ± SD.
Finally, we explored the effect of altering the expression of miR-21 in exosomes secreted by SC on neuronal cell lines. Two stable SC cell lines transfected using lentiviral vectors expressing empty or the precursor of miR-21-5p, respectively, were constructed. These 2 SC cell lines were cultured in DMEM with 10% exosome-free FBS for 72 h, and exosomes (MC-EXO and miR-21-EXO) were extracted from the cell culture supernatant. NG108-15 cells exposed to high glucose conditions were added with MC-EXO or miR-21-EXO for 3 days, and then total RNA and protein were isolated from the cells. Interestingly, the AKT signaling pathway and neurite growth-related factors were elevated in NG108-15 cells treated with miR-21-EXO compared to MC-EXO (n = 10 samples, t-test: ***p < 0.001 versus the MC-EXO group). Western blot (B) was used to detect the protein expression of p-AKT, t-AKT, DNMT3A, and GAP43 in NG108-15 cells, the results had the same trend as the PCR results. (C) Gray value statistics of Western blot (n = 6 samples, nonparametric tests: *p < 0.05 of p-AKT/AKT/GAP43/GAPDH, **p < 0.01 of DNMT3A/GAPDH versus the MC-EXO group). Immunocytochemistry staining (D) of beta III Tubulin (red) to observe neurite outgrowth. Scale bar: 25 μm. (E) Neurite length quantification (n = 10 fields of view per group, t-test: ***p < 0.001 versus the MC-EXO group). For the above, data are represented as mean ± SD.

**Figure 5. SC-derived exosomes rich in miR-21 accelerated the neurite outgrowth of NG108-15 cells under high glucose conditions**

(A) Expression of AKT1, DNMT3A, and GAP43 in NG108-15 was detected by PCR, and AKT signaling pathway and neurite outgrowth-related factors (DNMT3A, GAP43) were enhanced in NG108-15 cells treated with miR-21-EXO compared to MC-EXO (n = 10 samples, t-test: ***p < 0.001 versus the MC-EXO group).

**MiR-21 knockdown in SC inhibited cell proliferation and increased cell apoptosis**

Furthermore, we also explored the effect of miR-21 knockdown in SC to further clarify the role of miR-21 in DPN. SC were transfected with miR-21 NC control or its inhibitor for 48 h. We performed an RT-qPCR assay to verify the expression of miR-21 was downregulated after transfection of the miR-21 inhibitor (Figure 6A). SC proliferation was significantly inhibited after the downregulating of miR-21 compared with the NC control group (Figure 6B). A TUNEL assay showed that downregulation of miR-21 significantly promoted SC apoptosis (Figures 6C and 6D).
DISCUSSION

Some diabetic patients have small lesions on nerve fibers, though DPN diagnosis has not been confirmed (Stino and Smith, 2017; Divisova et al., 2012) and there are currently no proven treatments for DPN (Azmi et al., 2021). Despite the complex pathogenesis of DPN, it has been documented that hyperglycemia plays a major role in the pathogenesis of DPN (Grisold et al., 2017; Pai et al., 2021). Thus, we explored the relationship between glucose concentration and SC viability. DMEM with 1 g/L or 4.5 g/L glucose is commonly used in media, and we found that SC viability in the low glucose group (1 g/L) was better than that in the normal glucose group (4.5 g/L) (Figure S1). The reason may be related to the fact that the glucose concentration of 4.5 g/L is higher than the normal blood glucose concentration in vivo when the carbon source is not depleted (Li et al., 2022). Besides, 1 g/L was also used in many SC studies as the control group (Yuan et al., 2022; Liu et al., 2021). Therefore, we think it is more reasonable to use 1 g/L as the control in the subsequent experiment. A high glucose concentration of more than 5.4 g/L is used in the literature. According to the reference (Wang et al., 2020b) and our experiment results (Figure S1), high glucose concentration and cell viability are negatively correlated and 18 g/L was used as the high glucose concentration. Furthermore, SC apoptosis was increased at high glucose concentrations and was positively correlated with the concentration (Liu et al., 2016). In conclusion, high glucose affects SC status and may mediate DPN.

Multiple miRNAs were down- and upregulated in diabetes, and have been shown not only to play an important role in the pathogenesis of T2D (Moura et al., 2014; Ozdemir and Feinberg, 2019) but also to be potential biomarkers for the development and progression of T2D as estimated by their circulating levels (Dehwah et al., 2012). Recent studies have reported that miR-21 is involved in regulating T2D glucose metabolism by promoting insulin secretion, further revealing the potential mechanism of miR-21 in regulating the pathogenesis of DPN (Liu et al., 2022a, 2022b). As a widely studied miRNA molecule, miR-21 was found to be closely related to peripheral neuropathy (Karl et al., 2022). Previous studies have focused on experiments in vivo, which have confirmed reduced levels of miR-21 in peripheral nerve and circulation in T2D model mice (Wang et al., 2020a; Wu et al., 2020), suggesting that miR-21 is involved in DPN. Here, we...
demonstrated that miR-21 levels were also reduced in the peripheral nerve and circulation exosomes of T1D model rats, further suggesting exosomal miR-21 has a close relationship with DPN.

The AKT signaling pathway can be positively regulated by miR-21 (Deng et al., 2016), and is related to the proliferation and apoptosis of SC (Liu et al., 2020; Ma et al., 2021). It is also reported that miR-21 can protect neurons from cell apoptosis by targeting the AKT signaling pathway (Lv et al., 2020; Feng et al., 2018a). This suggests that the AKT signaling pathway may be involved in miR-21-mediated DPN. When SC were exposed to a high-glucose environment, the proliferation of SC was inhibited and cell apoptosis increased, and the expression of miR-21 and p-AKT in cells and exosomes was markedly decreased. By knocking down miR-21 for an in-depth study, SC viability was reduced and cell apoptosis was increased, thus further confirming the involvement of miR-21 in the effect of glucose on SC. Protective effects on SC exposed to high glucose were found when the expression of miR-21 was upregulated, and p-AKT expression increased. It has also been reported that upregulating the level of miR-21 inhibits neuronal apoptosis and improves proliferation activity (Zhan et al., 2022). In conclusion, changes in the expression of miR-21 in vivo and exosomes in SC under a high glucose environment play a profound role in regulating the occurrence and development of DPN.

Exosomes have therapeutic potential for the treatment of diabetes and diabetic complications (Hu et al., 2020). Exosomes, as extracellular vesicles, are rich in a variety of proteins and genetic material. Many studies have supported the roles of exosomes in intercellular communication through paracrine signaling in DPN (Singh et al., 2021; Fan et al., 2020). As essential components of exosomes, miRNAs have attracted much attention in exosomal function studies (Liu et al., 2022b; Fan et al., 2021). Additionally, in vitro data showed that SC-derived exosomes promoted neurite outgrowth of DPN. Above all, SC-derived exosomes modulation of miRNAs contributes to this therapy (Wang et al., 2020a, 2020b). It has been discovered that exosomes from SC that were exposed to high glucose conditions had high expression of miR-28, miR-31a, and miR-130a, and played a role in promoting the development of DPN (Jia et al., 2018). Healthy SC-derived exosomes were enriched with miR-21 and were effective for the treatment of DPN in T2D mice. In addition, exosomal miR-21 from adipose mesenchymal stem cells (MSCs) can also promote diabetic wound healing (Lv et al., 2020). Pathological processes of DPN include oxidative stress (Zhang et al., 2020b), inflammatory reaction (Feng et al., 2018b), and autophagy (Chung et al., 2018), which can be regulated by miR-21 (Yuan et al., 2020; Loboda et al., 2016; Zhang et al., 2020a), underscoring the importance of further exploring the role of miR-21 in DPN.

NG108-15 cells have been extensively used as neuronal cell lines in the literature (Ching et al., 2018; Kingham et al., 2007; Fu et al., 1997). When treated with SC-derived exosomes exposed to high glucose, NG108-15 cells' neurite outgrowth was inhibited and p-AKT expression was decreased compared with SC-derived exosomes exposed to low glucose. Finally, to further confirm the effect of exosomal miR-21 on neurite growth of neurons, we obtained exosomes with high expression of miR-21 by stably transfecting SC cell lines by using lentivirus, and observed that neurite outgrowth in NG108-15 cells was accelerated when treated with SC-derived exosomes rich in miR-21; moreover, p-AKT expression was also increased. Thus, SC-derived exosomal miR-21 participates in the high glucose regulation of NG108-15 cells neurite outgrowth. It has also been reported that the upregulation of exosomal miR-21 inhibits neuronal apoptosis by activating the AKT pathway (Sun et al., 2021; Cong et al., 2021), and increased the protein synthesis of axons, which has been shown to enhance the regeneration of injured axons. Moreover, the inhibition of exosomal miR-21 eliminated the protective effects of neurons via the AKT pathway (Gao et al., 2020). Our previous results also showed that SC-derived exosomal miR-21 promoted neurite outgrowth in vitro (Liu et al., 2022a, 2022b), while upregulation of miR-21 in MSCs-derived exosomes can also inhibit neuronal apoptosis (Ku et al., 2019). Together with results from in vivo studies (Wang et al., 2020a), we believe that SC-derived exosomal miR-21 is involved in the pathogenesis of DPN and may be a promising target for DPN treatment. The number of samples was determined by commonly used animal and cell experiments (Kwon et al., 2021). Ideally, the sample size should be calculated based on the t-test and F-test. In conclusion, a high-glucose environment mediated neuronal axon growth through exosomal miR-21 secreted by SC and may be an important mechanism for the development of DPN.

Neuronal damage occurs in the prediabetic stage (Celikbilek et al., 2014), and differentially expressed RNAs play critical roles in regulating the functions of SC involved in the pathogenesis of DPN (Wang et al., 2020b). AKT signaling pathway has been proven to be candidate genes for DPN (Guo et al., 2020). Our results demonstrated that the expression of miR-21 was decreased in serum exosomes and sciatic nerve of T1D DPN rats, and the
content of miR-21 in SC and SC-derived exosomes was decreased in a high-glucose environment, which was deleterious to SC and neurite growth. Conversely, increasing the expression of SC-derived exosomal miR-21 promoted neurite growth, with the AKT signaling pathway likely being the downstream pathway. SC-derived exosomal miR-21 participates in the high glucose regulation of NG108-15 cells’ neurite outgrowth. Overall, our research provides valuable new insights into DPN treatment by targeting SC-derived exosomal miR-21.

Limitations of the study
1) AKT signaling pathway needs further study, 2) the use of primary cells to generate more convincing data, and 3) in vivo experiments of SC-derived exosomal miR-21 based on existing research results.

STAR METHODS
Detailed methods are provided in the online version of this paper and include the following:
- Key Resources Table
- Resource Availability
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- Experimental Model and Subject Details
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  - Culture of NG108-15 cells and injection of SC exosomes
  - Quantitative Real-time PCR (qPCR)
  - Western blot
  - Immunocytochemistry and neurite outgrowth
- Quantification and Statistical Analysis

Supplemental Information
Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.105141.

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Author Contributions
Hai-dong Guo and Shui-jin Shao designed the study, and Yu-pu Liu, Ming-yue Tian, and Yi-duo Yang performed most of the biological experiments; Han Li, Tian-tian Zhao, and Jing Zhu fed the animals and collected the samples; Yu-pu Liu and Guo-hong Cui wrote the manuscript; Ming-yue Tian, Fang-fang Mou, and Guo-hong Cui carried out the data analysis and revised the manuscript. All authors reviewed and approved the final manuscript.

Declaration of Interests
No potential conflicts of interest were disclosed by the authors.

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# STAR★METHODS

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Rabbit anti-NF200   | Sigma-Aldrich | Cat# N4142; RRID:AB_477272 |
| Rabbit anti-MBP     | Abcam  | Cat# ab40390; RRID:AB_1141521 |
| Rabbit anti-CD9     | Abcam  | Cat# ab92726; RRID:AB_10561589 |
| Rabbit anti-CD63    | Abcam  | Cat# ab108950; RRID:AB_10863101 |
| Rabbit anti-p-AKT   | CST    | Cat# 4060; RRID:AB_2315049 |
| Rabbit anti-t-AKT   | CST    | Cat# 4691; RRID:AB_915783 |
| Rabbit anti-Bcl-2   | Abcam  | Cat# ab59348; RRID:AB_2064155 |
| Rabbit anti-Bax     | CST    | Cat# 2772; RRID:AB_10695870 |
| Rabbit anti-Cleaved Caspase-3 | CST | Cat# 9664; RRID:AB_2070042 |
| Rabbit anti-DNMT3A  | Abcam  | Cat# ab2850; RRID:AB_303355 |
| Rabbit anti-GAP43   | Abcam  | Cat# ab12274; RRID:AB_2247459 |
| Mouse anti-GAPDH    | Proteintech | Cat# CL594-60004; RRID:AB_2919886 |
| Rabbit anti-beta III Tubulin | Abcam | Cat# ab18207; RRID:AB_444319 |
| Goat anti-rabbit Alexa Fluor 555 | Invitrogen | Cat# A27039; RRID:AB_2536100 |
| Anti-rabbit HRP     | CST    | Cat# 7074; RRID:AB_2099233 |
| Anti-mouse HRP      | CST    | Cat# 7076; RRID:AB_330924 |
| Anti-rabbit Alexa Fluor 555 | Invitrogen | Cat# A-21428; RRID:AB_2535849 |
| **Chemicals, peptides, and recombinant proteins** | | |
| miR-21-5p mimic     | Ribobio | miR10000790-1-5 |
| miR-21-5p inhibitor | Ribobio | miR20004711-1-5 |
| TRizol             | Life technologies | A33251 |
| miRcute Plus miRNA First-Strand cDNA Synthesis Kit | Tiangen | KR211-02 |
| FastKing gDNA Dispelling RT SuperMix Kit | Tiangen | KR118-02 |
| Lipofectamine 3000  | Invitrogen | L3000015 |
| Lipofectamine 2000  | Invitrogen | 11668-019 |
| CCK-8              | Kumamoto | CK04-11 |
| FBS                | Invitrogen | C0235 |
| DMEM               | Coming Cellgro™ | 10-013-CV |
| TUNEL reaction buffer | Invitrogen | C10246 |
| Hoechst 33342      | Invitrogen | 62249 |
| ECL                | Millipore | WBKLS0100 |
| Bradford protein assay | Sigma-Aldrich | 20130 |
| Streptozotocin     | Sigma-Aldrich | 158127 |

## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Shui-jin Shao (shaoshujin@163.com).
**Materials availability**

This study did not generate new unique reagents and all materials mentioned in the manuscript are available from the lead contact on request.

**Data and code availability**

Data reported in this paper will be shared by the lead contact upon request. This paper does not report novel gene/RNA sequences and original code. Any additional information from the data reported in this paper is available from the lead contact on request.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Animals**

Ethics approval: All experimental procedures involved in this study were authorized by the animal ethics committee of Shanghai University of TCM and the Animal Research Committee of Shanghai (ethics number: PZSHUTCM200628003). The animal experiments were complied with the ARRIVE guidelines as well as the National Institute of Health’s Guide for the Care and Use of Laboratory Animals. And efforts were made to reduce the number of rats and improve the utilization rate of rats as far as possible during the whole procedure.

Establishment of the T1D DPN model: 20 male SD rats weighing between 180 and 190g (7-week-old) were purchased from the Animal Experimental Center of Shanghai University of Traditional Chinese Medicine (license number: SYXX2020-0009). After one week of adaptive feeding, rats were randomly divided into 2 groups. Streptozotocin (STZ; 20,130 Sigma-Aldrich) was administrated to induce T1D DPN by an intraperitoneal injection of 50 mg/kg (DPN group), and the control group was treated with 0.1 M citrate buffer with PH-4.5. Blood glucose levels were estimated after 48 h, and rats with glucose levels of more than 16.7 mM was supposed to have diabetics. Blood glucose levels were measured at 0, 1, 4, and 8 weeks to ensure that blood glucose levels were higher than 16.7 mM.

At the end of the eighth week, nerve conduction velocity (NCV) of the sciatic nerve was measured by an operator blinded to the treatment. The RM6240 Biological Signal Collecting System (Chengdu Instrument Factory, Chengdu, China) was used to record compound motor action potentials (CMAPs). After the rat was anesthetized with pentobarbital sodium, the stimulating electrodes were inserted proximal and distal to the injury site, respectively. The recording electrode was inserted into the gastrocnemius muscle with 5–10 V intensity of electrical stimulation and 0.2 ms of wavelength. NCV was calculated by dividing the distance between two stimulating points by the latency difference between two points.

After the measurement, the rats were sacrificed, and the sciatic nerve was extracted for immunofluorescence staining on transverse frozen sections. Neurofilament heavy chain (NF200; rabbit polyclonal 1:80; Sigma-Aldrich N4142) and myelin basic protein (MBP; rabbit polyclonal 1:100; Abcam ab40390) were used to stain axons and myelin, and goat anti-rabbit Alexa Fluor 555 (1:1,000; A-21428 Invitrogen) was used as the secondary antibody.

**METHOD DETAILS**

**Isolation of exosomes from serum**

The serum of rats was extracted by blood collection from the abdominal aorta and was subjected to centrifugation (10,000 g for 30 min at 4°C) at once, followed by ultracentrifugation at 100,000 g for 70 min (CS120FNX Hitachi; Japan). 0.1M cold PBS (PBS; PH 7.4) was used to wash the precipitation containing the exosomes, and the solution was centrifuged again at 100,000 g for 70 min; the exosomes were then resuspended in PBS.

**Culture of SC and transfection**

RSC96 (rat SC line; CRL-2765 ATCC) cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM; 10-013-CV Corning Cellgro) containing low glucose (1 g/L), normal glucose (4.5 g/L) or high glucose (18 g/L) with 10% fetal bovine serum (FBS; C0235 Invitrogen). The incubator environment was 37°C, with 5% CO₂.

SC cultured in high glucose within 24 h were transfected with miR-21 control (MC) or miR-21-5p mimic (miR-21) (miR10000790-1-5 Ribobio; Guangzhou; China) combined with Lipofectamine 3000 reagent (L3000015
Invitrogen) according to the manufacturer’s instructions. SC cultured in low glucose within 24 h were transfected with miR-21 control (NC) or miR-21-5p inhibitor (miR-21) (miR20004711-1-5 Ribobio; Guangzhou; China) combined with Lipofectamine 2000 reagent (11,668-019 Invitrogen) according to the manufacturer’s instructions.

**Cell counting Kit-8 (CCK-8)**

RSC96 cells were plated on poly-L-lysine-coated 96-well plates (353072 Corning Falcon) at a density of $1 \times 10^4$ cells/ml. 72 h after different glucose concentration treatments or transfection under high glucose, 10 μL of CCK-8 (CK04-11 Dojido; Kumamoto; Japan) was added to each well, and the cells were incubated for 1 h at 37°C. The absorbance was read at 450 nm using a microplate reader (Synergy 2; BioTek; USA).

**Terminal dUTP nick-end labeling assay (TUNEL)**

At 72 h after treatment with different concentrations of glucose or transfection under high glucose, SC were fixed for 0.5 h on ice and then washed in PBS. For the TUNEL assay, the cells were incubated with 50 μL TUNEL reaction buffer (C10246 Invitrogen) for 1 h at 37°C in the dark. Nuclei were counterstained with Hoechst 33,342 (62,249 Invitrogen). The percentage of apoptotic SC were determined by counting the number of TUNEL-positive cells/total number of cells.

**Isolation and characterization of exosomes derived from SC**

Two methods were used to cultivate SC separately: 1) SC were cultured in DMEM with 10% exosome-free FBS (obtained by ultracentrifugation at 100,000 g for 20 h) containing low glucose (1 g/L) or high glucose (18 g/L). 2) Construction of 2 stably transfected SC cell lines, transfected using lentiviral vectors expressing an empty vector or a precursor of miR-21-5p respectively; SC were then cultured in DMEM with 10% exosome-free FBS.

After 72 h, the supernatant was subjected to a series of centrifugations (300 x g for 10 min, 2000 g for 10 min, 10,000 g for 30 min at 4°C), followed by ultracentrifugation at 100,000 g for 70 min (Hitachi; Japan). Cold 0.1M PBS (PH 7.4) was used to wash the precipitate containing the exosomes, and the solution was centrifuged again at 100,000 g for 70 min; the exosomes were then resuspended in PBS. The Bradford protein assay (5,000,204 BioRad) was used to measure the concentration of total protein in the exosomes (method 1: L-EXO and H-EXO; method 2: MC-EXO and miR-21-EXO).

Exosome markers (CD9, CD63; Abcam; UK) were detected by Western blot. The exosomal lysate was mixed with loading buffer, subjected to 10% SDS-PAGE electrophoresis, and then transferred to polyvinylidene fluoride (PVDF) membranes (FP28 Millipore, USA). The following primary antibodies were used: anti-CD9 (rabbit monoclonal 1:2000; Abcam ab92726), anti-CD63 (mouse monoclonal 1:1000; Abcam ab108950). Secondary antibodies used were anti-rabbit HRP (1:8000; CST 7074) and anti-mouse HRP (1:8000; CST 7076). Protein expression levels were visualized by enhanced chemiluminescence (ECL; WBKLS0100 Millipore).

**Culture of NG108-15 cells and injection of SC exosomes**

NG108-15 cells (HB-12317 ATCC) were cultured in DMEM with 10% FBS and supplemented with 0.1 mM hypoxanthines, 400 nM aminopterin, and 16 μM thymidine (H9377, A1784, and T1895 Sigma-Aldrich). NG108-15 cells were plated on a 60 mm cell culture dish or a 35 mm confocal dish in DMEM and cultured in 10% exosome-free FBS. After plating, 4 types of SC exosomes (L-EXO and H-EXO; MC-EXO and miR-21-EXO; 15/5 μg resuspended in PBS) were added daily for 3 days.

**Quantitative Real-time PCR (qPCR)**

SC and NG108-15 cells were trypsinized and collected by centrifugation, and the total RNA of cells and sciatic nerve was extracted from the cells by TRizol (A33251 Life technologies; Carlsbad; CA). The miRNeasy Serum/Plasma Advanced Kit (Qiagen 217,184) was used to isolate the miRNAs from SC exosomes and serum exosomes. miRcute Plus miRNA First-Strand cDNA Synthesis Kit and FastKing qDNA Dispelling RT Super-Mix Kit (KR211-02 and KR118-02 Tiangen, Beijing, China) was used to reverse-transcribe total RNA and miRNAs and for amplification.
Western blot
RIPA and protease inhibitors (ThermoFisher Scientific) were used to extract protein lysates from SC and NG108-15 cells, and a BCA protein assay kit (23,225 Pierce; Rockford; IL) was used to measure the protein concentration. The steps were as described above. The membranes were incubated with primary antibodies at 4°C overnight after blocked with 5% skim milk at room temperature for 1 h. The following primary antibodies used were: anti-p-AKT (rabbit polyclonal 1:2000; CST 4060), anti-t-AKT (rabbit polyclonal 1:1000; CST 4691), anti-Bcl-2 (rabbit monoclonal 1:1000; Abcam ab59348), anti-Bax (rabbit polyclonal 1:1000; CST 2772), anti-Cleaved Caspase-3 (C-cas3)(rabbit polyclonal 1:1000; CST 9664), anti-DNMT3A (rabbit monoclonal 1:500; Abcam ab2850), anti-GAP43 (rabbit monoclonal 1:1000; Abcam ab12274), anti-GAPDH (mouse monoclonal 1:20,000; Proteintech 60,004). After addition of the anti-rabbit or antimouse secondary antibody at room temperature for 2 h, the protein bands on the membranes were detected using an enhanced chemiluminescence system and a Bio-Spectrum Gel Imaging System, respectively. The gray value statistics was analyzed by ImageJ.

Immunocytochemistry and neurite outgrowth
After treatment with SC exosomes for 3 days, NG108-15 cells cultured in a 35 mm confocal dish were fixed with ice-cold 4% paraformaldehyde (PFA; 158,127 Sigma-Aldrich) for 20 min, permeabilized with 0.5% Triton X-100 (X100 Sigma-Aldrich) for 15 min and blocked with 10% goat serum (G9023 Sigma-Aldrich) for 1 h. Anti-beta III Tubulin (bIII) (rabbit monoclonal 1:2000; Abcam ab18207) was used as the primary antibody over-night at 4°C, and goat anti-rabbit Alexa Fluor 555 (1:1,000; Invitrogen A27039) was used as the secondary antibody 2 h at room temperature. Hoechst 33,342 was used to stain the nuclei. After washing with PBS, fluorescently labeled NG108-15 cell bodies and neurites were imaged using an inverted fluorescence microscope (IXS3, Olympus, Japan). Neurite length in the images was measured using the ImageJ program.

QUANTIFICATION AND STATISTICAL ANALYSIS
Parametric test data presented as bar graphs (blue) were mean values ±SD. SPSS 22.0 was used to detect the normality and uniform variance of the sample (satisfy normality and uniform variance when p > 0.05). Statistical analysis was performed using one-way ANOVA followed by Scheffe’s post hoc multiple-comparison for 3-group or a Student’s t test was used for 2-groups when the sample satisfy normality and uniform variance (Lg-transformation was conducted to normalize the distribution of the sample which didn’t satisfy normality and uniform variance). Nonparametric tests were used when the samples were few, had high variability, or did not satisfy uniform variance. Nonparametric test data were presented as bar graphs (red) are the median and interquartile range. Differences with p < 0.05 were considered statistically significant.