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ARTICLE

Apoptosis and autophagy inhibited by autophagy-related circular RNA in Schwann cells via miR-144-3p/PI3K/AKT/ mTOR pathway

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Diabetic peripheral neuropathy is a common complications of Type 2 Diabetes and its main pathological feature is myelin sheath damage of peripheral nerve that was induced by Schwann cells (SCs) apoptosis. Increasing evidence suggested that noncoding RNAs might play a role in improving DPN because of its ability to prevent SCs apoptosis. In this study, we explore the effect of autophagy-related circular RNA on preventing SCs apoptosis and its underlying mechanism. Our study found that ACR could relieve HG-aroused RSC96 cell apoptosis, autophagy, and oxidative stress via reducing miR-144-3p and then promoting PI3K/AKT/ mTOR pathway activation, which provides some clues that ACR might be effective and feasible candidate for the treatment of DPN.

Keywords: autophagy-related circular RNA; diabetic peripheral neuropathy; Schwann cells; miR-144-3p; apoptosis

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Diabetes mellitus (DM), a chronic metabolic disorder, gets more and more public attention [1]. World Health Organization estimated that about 422 million people suffered from DM in 2018 worldwide [2]. Type 2 DM encompasses the most prevalent form of the disease, which is associated with a wide range of complications. One common of complications is diabetic peripheral neuropathy (DPN) that has been defined as peripheral nerve dysfunction in diabetes [1, 3]. The disorder affects both somatic and autonomic peripheral nerves with significant morbidities, such as pain, sensory loss, foot ulcers, and potentially, lower extremity amputations. These morbidities have been reported affecting about fifty percent of diabetic patients with a high morbidity, mortality and quality of life. It was well known that one of the mainly characteristic pathogenesis of DPN is myelin sheath damage [4]. Myelin sheath played a critical role in nerve development by ensheathing the axons and providing structural and functional support to the nerves in the peripheral nervous system. Studies have confirmed that myelin sheath damage leaded to neuropathy of peripheral nerve in diabetic patients and experimental diabetic models [4, 5]. Diabetic patients showed that a loss of myelinated fibers observed in transverse nerve sections [4]. Moreover, the similar structural abnormalities have been found in experimental diabetic rats, and showed increased morphological alterations in myelinated fibers of the sciatic nerve, including demyelination and degenerating myelinated fibers as well as myelinated fiber loss [5]. In the peripheral nervous system, myelin sheath was formed by

Schwann cells (SCs).

SC is important regulator of nerve function, including regulating and maintaining myelin thickness and providing neurotrophic support [6-8]. It has found that SCs loss or damage was noted in patients suffering from diabetic neuropathy [9]. In addition, previous studies have also demonstrated that apoptosis of SCs was occurred in the progress of DPN in experimental animals. De Gregorio et al. reported that SCs apoptosis was increased as diabetic progress in diabetic (db/db) compared with non-diabetic (db/+) mice [10]. Delaney and his colleague found apoptotic SCs with classic features of chromatin clumping and prominent vacuolation, as well as the disruption of myelin surrounding the axons in a diabetic animal's model of streptozotocin (STZ)-treated rats [9]. Moreover, other researchers also reported that SCs apoptosis played a role in DPN procession, while, preventing apoptosis of SCs could ameliorate injury of the myelin sheath in DPN [11]. These studies demonstrated that SCs apoptosis is one of the mechanisms that cause DPN via inducing myelin sheath damage. Therefore, controlling apoptosis of SCs was contributed to protect against myelin sheath damage and then slow the peripheral nerve degeneration in DPN.

Circular RNAs (circRNAs), a type of novel discovered single‐stranded and closed noncoding RNAs in cells, are formed by reverse cleavage of mature messenger RNA (mRNA) exon [12]. In recent years, circRNAs have attracted widespread attention in the field of genomics [13]. Compared with linear RNA, circRNA are more conservative and stable due to covalently closed loop and the deficiency of polyadenylation tail. Recent literatures discovered that circRNAs acted as sponge of microRNAs (miRNAs), another type of noncoding RNAs in cells, and bond to miRNAs sites to release the inhibitory effects of miRNAs on target mRNAs [14,15]. CircRNAs exhibit gene expression regulatory functions and then engage in the development of human diseases generally via negative modulating miRNAs expression [16]. In terms of DPN, Wang et al found that circHIPK3 expression was positively related to the neuropathic pain of patients with type 2 diabetes, and silencing circHIPK3 could relieve neuropathic pain in diabetic rats through reducing miRNA-124 expression [17]. Autophagy-related circular RNA (ACR), a newly discovered circRNA, was proved to take part in the regulation of cardiomyocytes autophagy, which could weaken myocardial ischemia/reperfusion damage via repressing cardiomyocytes autophagy and death [18]. However, until now, there is no any literature can be searched regarding the possible impact of ACR on neuroglial cell apoptosis and autophagy in DPN. In recent years, study has found that miRNAs were involved in many important processes of diabetes through the regulation of cytokine expression [15]. Recently, miR-144-3p has been discovered to be associated with the modulation of apoptosis and autophagy of cisplatin-resistance of human thyroid carcinoma cells [19].

This study tried to investigate the underlying effect and mechanism of ACR protecting SCs against apoptosis in the diabetic rats, which provides some clues that ACR might be effective and feasible candidate for the treatment of DPN.

Materials and methods

Cell culture and HG irritation

RSC96 cells, a kind of myelin cells in rat peripheral system, were utilized to establish the DPN cell model. RSC96 cells were supplied by American Type Culture Collection (CRL-2765; VA) and grown in Dulbecco modified Eagle medium (DMEM; PM150210; Procell Inc) containing 10% fetal bovine serum (164210; Procell Inc) with 5% CO2 and 95% air at 37°C. D-glucose (purity >98.0%) was purchased from MedChem Express (HY-B0389; NJ). D-glucose powder was dissolved in DMEM to a final concentration of 150 mM. Next, RSC96 were cultured in DMEM containing 150 mM D-glucose for 48 hours to simulate HG condition.

qRT-PCR

Total RNAs were separated using RiboPure RNA Purification Kit (AM1924; Invitrogen). Complementary DNA (cDNA) was composited using High-Capacity RNA-to-cDNA Kit (4387406; Applied Biosystems). Then, TaqMan noncoding RNA Assay (4426961; Applied Biosystems) was carried out for measuring ACR expression and compared to β-actin. mirVana qRT-PCR miRNA Detection kit (AM1558; Invitrogen) was used for measuring miR-144-3p expression and compared to U6 small nuclear RNA expression.

Lentivirus transfection

Full-length sequence of ACR was sub-cloned into PLCDH-circ vector (GS0104; Geneseed Biotech Co) to form PLCDH-ACR. Unloaded PLCDH-circ vector was utilized as negative control (NC). Lentifectin transfection reagent (G074; Applied Biological Materials Inc) was used for PLCDH-ACR and unloaded PLCDH-circ vector transfection. miR-144-3p mimic and its NC mimic were supplied by Life Technologies.

Cell viability and apoptosis

Cell Counting Kit-8 assay (40203; Yeasen Biotechnology Co) was carried out for testing RSC96 cell viability. The absorbance of every group at 450 nm was detected using Microplate reader. Annexin V-PE/7-AAD Apoptosis Detection Kit (40310; Yeasen Biotechnology Co) was used for testing RSC96 cell apoptosis. Results were measured using Attune NxT Acoustic Focusing Cytometer (Invitrogen).

ROS generation

ROS expression in RSC96 cells was detected by 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA; C3890; APExBIO) staining. RSC96 cells were cultivated into six-well plate (1×10^5 cells/well) and subjected to different irritation. Then, cells were gathered in line with the experimental group, rinsed with phosphate buffer saline and stained using 10 μM DCFH-DA for twenty minutes at 37°C protected from light. Subsequently, the ROS level in every group was detected using Attune NxT Acoustic Focusing Cytometer (488 nm excitation and 521 nm emission) and shown as the percentage of control.

Western blotting

Total proteins were separated by Radioimmunoprecipitation assay lysis buffer (20101ES60; Yeasen Biotechnology Co containing Halt Protease Inhibitor Cocktail (78438; Thermo Fisher Scientific). Bradford Protein Quantification Kit (20202ES76; Yeasen Biotechnology Co) was used for measuring protein concentration. Some of the primary antibodies, including Bax (#2772), caspase 3 (#9662), caspase 9 (#9508), p62 (#5114), Beclin-1 (#3738), LC3 (#4108), and β-actin (#4970) were purchased from Cell Signaling Technology. Other primary antibodies, including p-PI3K (ab182651), t-PI3K (ab191606), p-AKT (ab38449), t-AKT (ab18785), p-mTOR (ab137133), and t-mTOR (ab2732) were purchased from Abcam Biotechnology. Secondary antibodies, including anti-mouse immunoglobulin G (IgG; H+L; DyLight 680 Conjugate; #5470) and anti-rabbit IgG (H+L; DyLight 680 Conjugate; #5366), were purchased from Cell Signaling Technology. The relative expression of proteins was normalized against β-actin. The gray values of proteins were quantified by utilizing the ImageJ software (NIH, Bethesda, MD).

Statistical analysis

Data was expressed as mean ± Standard error (SEM) and analyzed by Graphpad Prism 5 using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test.

Results

HG lowered ACR expression in RSC96 cells

In order to know the ACR expression after HG irritation in RSC96 cells, HG irritation model was established and the circular RNA ACR expression was measured. HG irritation reduces the ACR expression in RSC96 cells ($P < 0.05$), which indicates that ACR might play an important role in the pathophysiology of HG irritation (Figure 1).

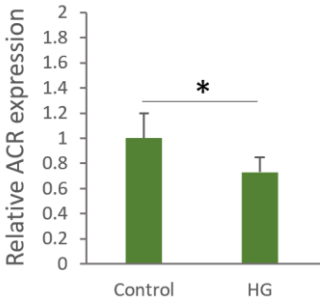

Figure 1. ACR expression in RSC96 cells. In RSC96 cells which were subjected to high glucose (HG) irritation, the circular RNA ACR expression was measured. The ACR expression in RSC96 cells was decreased significantly. *$P < 0.05$.

Exogenous PLCDH-ACR transfection inhibited RSC96 cell apoptosis and autophagy

In order to know the ACR role in RSC96 cellS, exogenous PLCDH-ACR transfection was applied to overexpressing ACR in RSC96 cells (Figure 2A; $P < 0.05$). Figure 2B showed that HG irritation decreased the RSC96 cell viability ($P < 0.05$), while exogenous PLCDH-ACR transfection alleviated the HG-aroused decrease of RSC96 cell viability ($P < 0.05$). Figure 2C presented that HG irritation elevated the RSC96 cell apoptosis ($P < 0.05$), while exogenous PLCDH-ACR transfection decreased the HG-aroused RSC96 cell apoptosis ($P < 0.05$). In addition, HG irritation raised the Bax, cleaved/procaspase 3 (Cleaved/Procaspase 3), and cleaved/procaspase 9 expression in RSC96 cells (Figure 2D; $P < 0.05$). In contrast with the HG+PLCDH group, the Bax, cleaved/procaspase 3, and cleaved/ procaspase 9 expression were reduced in HG+PLCDH-ACR group ($P < 0.05$). Furthermore, Figure 2E pointed out that HG irritation decreased the p62 expression, but raised the Beclin-1 and LC3-II/LC3-I expression in RSC96 cells ($P < 0.05$), which suggested that HG irritation could cause RSC96 cell autophagy. However, exogenous PLCDH-ACR transfection notably inhibited the impacts of HG irritation on p62, Beclin-1, and LC3-II/ LC3-I expression in RSC96 cells ($P < 0.05$).

Exogenous PLCDH-ACR transfection reduced HG-aroused ROS generation

The ROS levels were measured in RSC96 cells after HG irritation and/or exogenous PLCDH-ACR transfection. As presented in Figure 3, HG irritation enhanced the ROS level in RSC96 cells ($P < 0.05$), while exogenous PLCDH-ACR transfection reduced the ROS level in HG-irritated RSC96 cells ($P < 0.05$).

Reduced miR-144-3p expression in HG-irritated RSC96 cells

Subsequently, the miR-144-3p expression in RSC96 cells

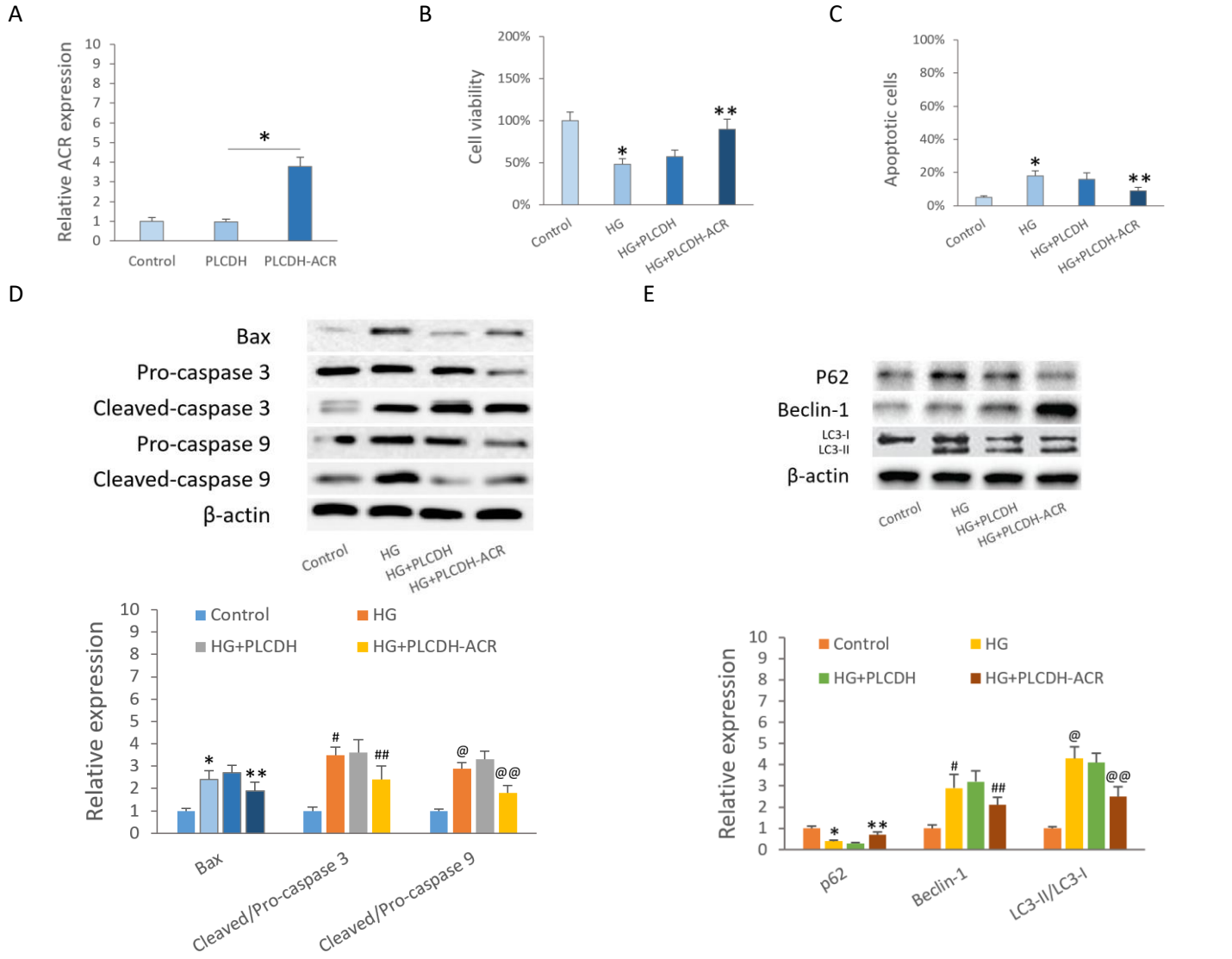

Figure 2. Exogenous PLCDH-ACR transfection relieved HG-aroused RSC96 cell apoptosis and autophagy. Accompanying with exogenous PLCDH or PLCDH-ACR transfection, the ACR expression in RSC96 cells were measured (A). RSC96 cells were subjected to HG irritation and/or exogenous PLCDH-ACR transfection. Then cell viability (B), cell apoptosis (C), Bax, caspase 3, caspase 9, p62, Beclin-1, and LC3 expression were tested, respectively (D,E). $^{*,\#,@}P < 0.05$, $^{**,\#\#,@@}P < 0.05$.

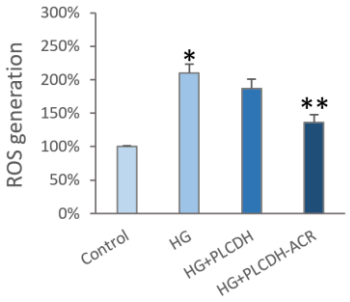

Figure 3. HG-aroused ROS generation. Exogenous PLCDH-ACR transfection relieved HG-aroused ROS generation after HG irritation and/or exogenous PLCDH-ACR transfection. *P < 0.05, **P < 0.05.

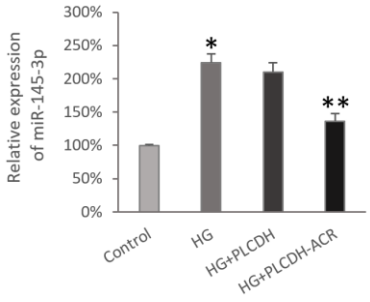

Figure 4. Exogenous PLCDH-ACR transfection decreased miR-144-3p expression in HG-irritated RSC96 cells after HG irritation and/or exogenous PLCDH-ACR transfection. *P < 0.05, **P < 0.05.

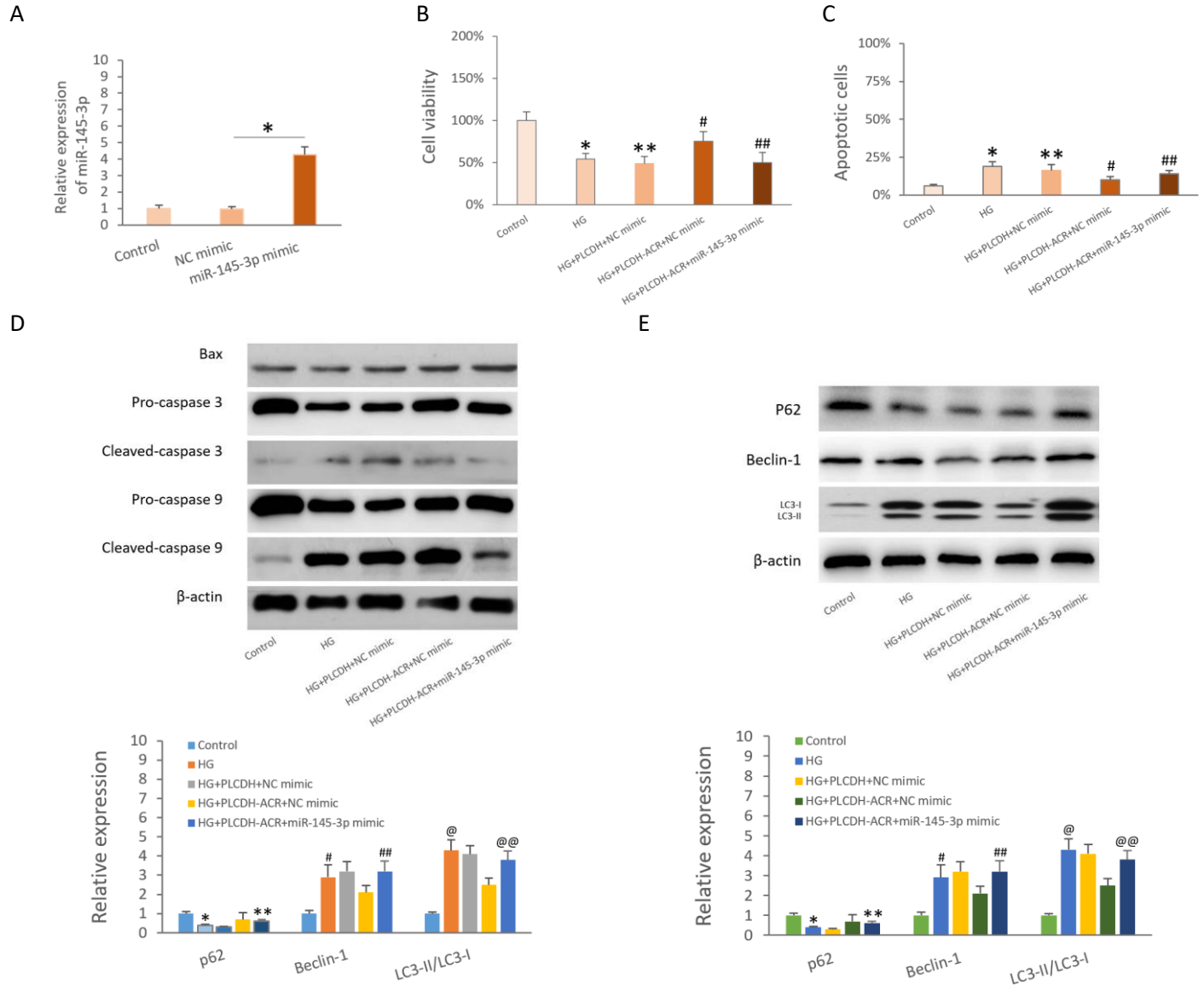

Figure 5. Role of miR-144-3p in ACR on HG-aroused RSC96 cell apoptosis and autophagy. Overexpression of miR-144-3p in RSC96 cells is observed (A). HG-aroused RSC96 cell viability and apoptosis after exogenous PLCDH-ACR transfection were both inhibited by miR-144-3p mimic transfection (B and C). In contrast with HG+PLCDH-ACR+NC mimic group, the Bax, cleaved/procaspase 3, and cleaved/procaspase 9 expression were all enhanced in HG+PLCDH-ACR+miR-144-3p mimic group (D). Furthermore, Beclin-1 and LC3-II/LC3-I expression in HG-irritated RSC96 cells were reduced by miR-144-3p mimic transfection (E). $^{*,\#,@}P < 0.05$, $^{**,\#\#,@@}P < 0.05$.

was tested after HG irritation and/or exogenous PLCDH-ACR transfection. Data in Figure 4 showed that HG irritation raised the miR-144-3p expression in RSC96 cells ($P < 0.05$). In contrast with the HG+PLCDH group, the miR-144-3p expression was decreased in the HG+PLCDH-ACR group ($P < 0.05$).

Apoptosis and autophagy regulated by miR-144-3p

miR-144-3p mimic was applied to overexpress miR-144-3p in RSC96 cells (Figure 5A; $P < 0.05$). HG-aroused RSC96 cell viability and apoptosis after exogenous PLCDH-ACR transfection were both inhibited by miR-144-3p mimic transfection (Figure 5B and C; $P < 0.05$). In contrast with HG+PLCDH-ACR+NC mimic group, the Bax, cleaved/procaspase 3, and cleaved/procaspase 9 expression were all enhanced in HG+PLCDH-ACR+miR-144-3p mimic group (Figure 5D; $P < 0.05$). Furthermore, Beclin-1 and LC3-II/LC3-I expression in HG-irritated RSC96 cells were reduced by miR-144-3p mimic transfection (Figure 5E; $P < 0.05$).

ROS generation regulated by miR-144-3p

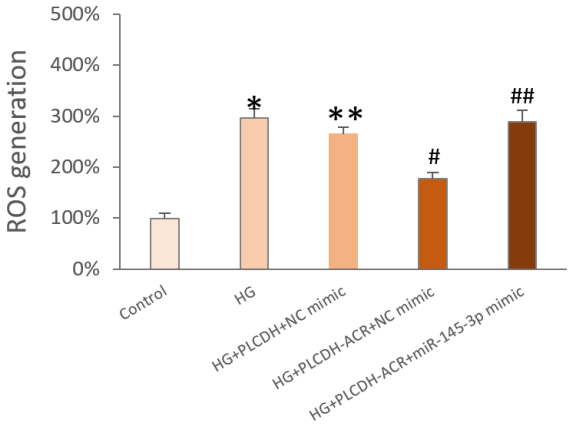

Figure 6. miR-144-3p engaged in the impact of ACR on HG-aroused ROS generation. Compared to HG+PLCDH-ACR+NC mimic group, the ROS level in RSC96 cells was raised in HG+PLCDH-ACR+miR-144-3p mimic group. $^{*,\#}P < 0.05$, $^{**,\#\#}P < 0.05$.

Whether miR-144-3p engaged in the impact of ACR on HG-aroused ROS generation in RSC96 cells was also probed. Data in Figure 6 showed that relative to HG+PLCDH-ACR+NC mimic group, the ROS level in RSC96 cells was raised in HG+PLCDH-ACR+miR-144-3p mimic group ($P < 0.05$), which illustrated that exogenous PLCDH-ACR transfection relieved HG-aroused ROS generation in RSC96 cells also could be achieved through reducing miR-144-3p expression.

miR-144-3p/PI3K/AKT/ mTOR pathway regulated by ACR

In order to know the miR-144-3p/PI3K/AKT/mTOR pathway regulated by ACR, the PI3K/AKT/mTOR pathway activity in RSC96 cells was assessed. HG irritation repressed the PI3K/AKT/mTOR pathway in RSC96 cells through reducing p/t-PI3K, p/t-AKT, and p/t-mTOR expression (Figure 7; $P < 0.05$). However, exogenous PLCDH-ACR transfection notably reversed the HG irritation-aroused inactivation of PI3K/AKT/mTOR pathway ($P < 0.05$). Besides, miR-144-3p mimic transfection inhibited the impact of exogenous PLCDH-ACR transfection on HG-aroused inactivation of PI3K/AKT/mTOR pathway ($P < 0.05$).

Discussion

DPN is the most common complication of diabetes, affecting up to 50% of diabetic patients, and it makes an important contribution to pain, loss of sensation, numbness, injury and lower extremity amputation [1, 3]. This disorder is characterized by myelination defects, vascular insufficiency and axonal atrophy. Because complex pathophysiology are implicated in the DPN, available treatments to date consist of improved metabolic control and a focus on symptoms effective medications [3, 4], while, there are no effective

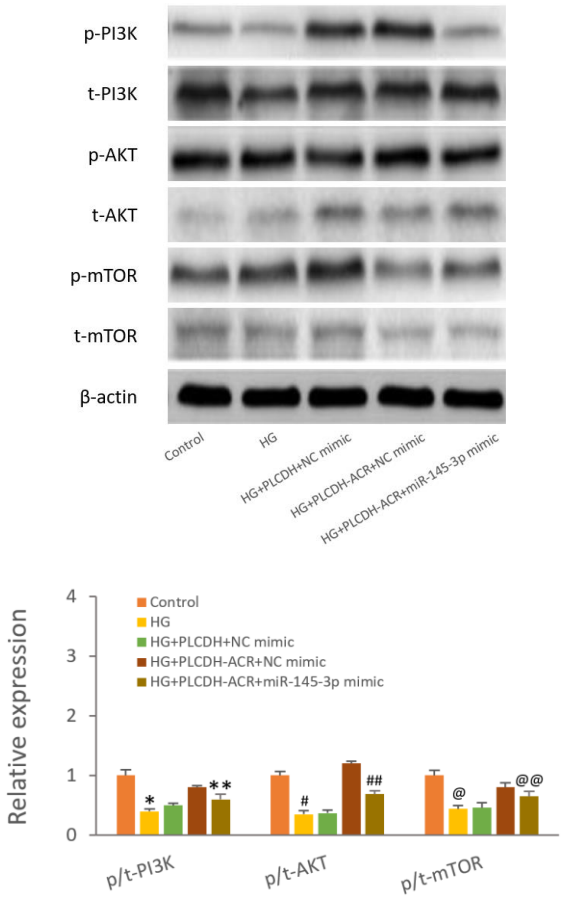

Figure 7. Exogenous PLCDH-ACR transfection promoted PI3K/AKT/mTOR pathway through miR-144-3p. HG irritation repressed the PI3K/AKT/ mTOR pathway in RSC96 cells through reducing p/t-PI3K, p/t-AKT, and p/t-mTOR expression. Meanwhile, miR-144-3p mimic transfection inhibited the impact of exogenous PLCDH-ACR transfection on HG-aroused inactivation of PI3K/AKT/mTOR pathway. $^{*,\#,@}P < 0.05$, $^{**,\#\#,@@}P < 0.05$.

treatments doing concentrate on fundamental mechanisms in the pathogenesis of neuropathy.

SCs are special gliocytes and peripheral myelin–forming cells in the peripheral nervous system. It is an essential element in the pathogenesis and development of peripheral nerves as well as maintaining normal morphology and function. Preview study suggested the important role of the SCs as a support cell for neuronal function, SCs may be a natural target for treating DPN. Recently, it was demonstrated that SCs apoptosis induced myelin sheath damage, which is considered one of the mechanisms leading to DPN [9-11]. Moreover, preventing apoptosis of SCs ameliorated the injury of the myelin sheath in DPN [11].

In the present study, HG irritation decreased Schwann RSC96 cell viability, but elevated cell apoptosis, which was accompanied by the raised Bax, cleaved/procaspase 3 and

cleaved/procaspase 9 expression. As key proteins participating in cell autophagosome formation, Beclin-1 and LC3-II expression were both enhanced in RSC96 cells after HG irritation. On the contrary, as a negative marker of cell autophagy, p62 expression was decreased after HG irritation [20, 21]. Apoptosis and autophagy are two kinds of programmed cell deaths associated with disease suppression. Autophagy is a cell degradation process that provides cells with alternative energy by recovering damaged organelles, which helps cells maintain intracellular environment and viability under metabolic stress [22]. Apoptosis refers to the process by which cells can end its life under certain physiological or pathological conditions, following its own procedures [23]. At present, the relationship between autophagy and apoptosis in diabetes is mainly divided into two types: first, autophagy antagonizes apoptosis by damaging organelles and changing endoplasmic reticulum folding protein, thereby inhibiting apoptosis and protecting cells [24, 25]. Second, autophagy is the premise of apoptosis, autophagy is activated after deprivation of nutrition and eventually leads to apoptosis [26]. In our paper, the data revealed that exogenous ACR relieved HG-aroused RSC96 cell apoptosis and autophagy, indicating that autophagy and apoptosis might co-operate in DPN. Besides, earlier literatures proved that persistent HG environment-caused oxidative stress in neuroglial cells contributed to the neuroglial cell apoptosis and autophagy [27, 28]. Herein, followed by HG irritation, the ROS level in RSC96 cells was boosted, which suggested that the oxidative stress was increased in RSC96 cells after HG irritation. Taken together, these outcomes illustrated that HG-irritated RSC96 cells could be applied to probe the impact of ACR on DPN. CircRNAs are a new type of noncoding RNAs produced by nonsequential back-splicing of pre-mRNA transcripts [29]. Being different from traditional linear noncoding RNAs (containing 5′ and 3′ ends), circRNAs present a covalent closed circular structure [30]. So, it is not influenced by RNA exonuclease and can maintain a more stable expression in cells. Some circRNAs have been discovered to be dysregulated in diabetes and diabetes-related complications, including DPN,which suggested that circRNAs might be as effective diagnostic biomarkers and therapeutic targets for diabetes and diabetes-related complications [31, 32]. ACR is a recently discovered circRNA, which has been reported to engage in the modulation of cardiomyocytes autophagy. In the current research, we discovered that HG irritation lowered the ACR expression in RSC96 cells. What's more, exogenous PLCDH-ACR transfection notably relieved the RSC96 cell apoptosis, autophagy, and ROS generation aroused by HG, which illustrated that ACR engaged in the modulation of Schwann cell apoptosis, autophagy, and oxidative stress under HG environment, and implied that ACR might be as the potential diagnostic biomarker and therapeutic target for DPN. CircRNAs, including ACR, generally exert regulatory functions on cellular biological processes via acting as miRNAs sponge. miRNAs can bind to the 3′-untranslated region of the target mRNAs and then cause posttranscriptional gene expression silencing, while circRNAs can weaken the posttranscriptional gene expression silencing function of miRNAs on target mRNAs [33]. Meanwhile, miR-144-3p has been discovered to be associated with the modulation of apoptosis and autophagy of cisplatin-resistance of human thyroid carcinoma cells [19].

Herein, we discovered that HG irritation raised the miR-144-3p expression in RSC96 cells, while exogenous PLCDH-ACR transfection decreased the miR-144-3p expression in HG- irritated RSC96 cells. What's more, overexpression of miR-144-3p mitigated the impacts of exogenous PLCDH-ACR transfection on HG-aroused RSC96 cell apoptosis, autophagy and ROS generation. These outcomes illustrated that ACR engaged in the modulation of Schwann cell apoptosis, autophagy, and oxidative stress under HG environment at least be implemented through reducing miR-144-3p. PI3K/AKT/mTOR signaling pathway is proved to be essential for cell survival [34]. Previous literatures reported that HG irritation could repress PI3K/AKT/mTOR pathway in Schwann cells [35, 36]. Moreover, miR-144-3p has been demonstrated to attend to the regulation of PI3K/AKT/mTOR pathway [37]. In consistent with the previous literatures, PI3K/AKT/mTOR pathway was also repressed by HG irritation in our experiment. We discovered that exogenous PLCDH-ACR transfection reversed the HG-aroused PI3K/AKT/mTOR pathway repression, while miR-144-3p overexpression inhibited the influence of exogenous PLCDH-ACR transfection, which illustrated that ACR relieved HG-aroused RSC96 cell apoptosis, autophagy, and oxidative stress could be via reducing miR-144-3p and then promoting PI3K/AKT/mTOR pathway activation. To sum up, this study confirmed the impacts of ACR on Schwann cell apoptosis, autophagy, and ROS generation under HG environment. ACR could relieve HG-aroused RSC96 cell apoptosis, autophagy, and oxidative stress via reducing miR-144-3p and then promoting PI3K/AKT/mTOR pathway activation.

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Conflicting interests

The authors have declared that no conflict of interests exist.

References

1. Sima AA, Sugimoto K. Experimental diabetic neuropathy: an update. Diabetologia 1999;42(7):773-788.
2. Global Report on Diabetes, World Health Organization, 2018.

3. Vinik AI, Park TS, Stansberry KB, et al. Diabetic neuropathies. Diabetologia 2000;43(8):957-973.

4. Sinnreich M, Taylor BV, Dyck PJ. Diabetic neuropathies. Classification, clinical features, and pathophysiological basis. Neurologist 2005;11(2):63-79.

5. Sugimoto K, Murakawa Y, Sima AA. Diabetic neuropathy--a continuing enigma. Diabetes Metab Res Rev 2000;16(6):408-433.

6. Eckersley L. Role of the Schwann cell in diabetic neuropathy. Int Rev Neurobiol 2002;50:293-321.

7. Mizisin AP. Mechanisms of diabetic neuropathy: Schwann cells. Handb Clin Neurol 2014;126:401-428.

8. Dey I, Midha N, Singh G, et al. Diabetic Schwann cells suffer from nerve growth factor and neurotrophin-3 underproduction and poor associability with axons. Glia 2013;61(12):1990-1999.

9. Delaney CL, Russell JW, Cheng HL, et al. Insulin-like growth factor-I and over-expression of Bcl-xL prevent glucose-mediated apoptosis in Schwann cells. J Neuropathol Exp Neurol 2001;60(2):147-160.

10. De Gregorio C, Contador D, Campero M, et al. Characterization of diabetic neuropathy progression in a mouse model of type 2 diabetes mellitus. Biol Open 2018;7(9). pii: bio036830.

11. Pan H, Ding Y1, Yan N, et al. Trehalose prevents sciatic nerve damage to and apoptosis of Schwann cells of streptozotocin-induced diabetic C57BL/6J mice. Biomed Pharmacother 2018;105:907-914.

12. Hombach S, Kretz M. Non-coding RNAs: Classification, Biology and Functioning. Adv Exp Med Biol 2016;937:3-17.

13. López-Jiménez E, Rojas AM, Andrés-León E. RNA sequencing and Prediction Tools for Circular RNAs Analysis. Adv Exp Med Biol 2018;1087:17-33.

14. Militello G, Weirick T, John D, et al. Screening and validation of lncRNAs and circRNAs as miRNA sponges. Brief Bioinform 2017;18(5):780-788.

15. Thomson DW, Dinger ME. Endogenous microRNA sponges: evidence and controversy. Nat Rev Genet 2016;17(5):272-283.

16. Rong D, Sun H, Li Z, et al. An emerging function of circRNA-miRNAs-mRNA axis in human diseases. Oncotarget 2017;8(42):73271-73281.

17. Wang L, Luo T, Bao Z, et al. Intrathecal circHIPK3 shRNA alleviates neuropathic pain in diabetic rats. Biochem Biophys Res Commun 2018;505(3):644-650.

18. Zhou LY, Zhai M, Huang Y, et al. The circular RNA ACR attenuates myocardial ischemia/reperfusion injury by suppressing autophagy via modulation of the Pink1/ FAM65B pathway. Cell Death Differ 2019;26(7):1299-1315.

19. Feng Liu,Jin Zhang,Long Qin, et al. Circular RNA EIF6 (Hsa_circ_0060060) sponges miR-144-3p to promote the cisplatin-resistance of human thyroid carcinoma cells by autophagy regulation. Aging (Albany NY) 2018; 10(12): 3806-3820.

20. Cui D, Sun D, Wang X, et al. Impaired autophagosome clearance contributes to neuronal death in a piglet model of neonatal hypoxic-ischemic encephalopathy. Cell Death Dis 2017;8(7):e2919.

21. Jiang P, Mizushima N. LC3- and p62-based biochemical methods for the analysis of autophagy progression in mammalian cells. Methods 2015;75:13-18.

22. Parzych KR, Klionsky DJ. An overview of autophagy: morphology, mechanism, and regulation. Antioxid Redox Signal 2014;20(3):460-473.

23. Kaczanowski S. Apoptosis: its origin, history, maintenance and the medical implications for cancer and aging. Phys Biol 2016;13(3):031001.

24. Lim YM, Lim H, Hur KY, et al. Systemic autophagy insufficiency compromises adaptation to metabolic stress and facilitates progression from obesity to diabetes. Nat Commun 2014;5:4934.

25. Ding WX1, Ni HM, Gao W, et al. Differential effects of endoplasmic reticulum stress-induced autophagy on cell survival. J Biol Chem 2007;282(7):4702-4710.

26. Wei Y, Sinha S, Levine B. Dual role of JNK1-mediated phosphorylation of Bcl-2 in autophagy and apoptosis regulation. Autophagy 2008;4(7):949-951.

27. Sifuentes-Franco S, Pacheco-Moisés FP, Rodríguez-Carrizalez AD, et al. The Role of Oxidative Stress, Mitochondrial Function, and Autophagy in Diabetic Polyneuropathy. J Diabetes Res 2017;2017:1673081.

28. Wang BB, Wang JL, Yuan J, et al. Sugar composition analysis of fuzi polysaccharides by HPLC-MS(n) and their protective effects on schwann cells exposed to high glucose. Molecules 2016; 21(11). pii: E1496.

29. Conn SJ, Pillman KA, Toubia J, et al. The RNA binding protein quaking regulates formation of circRNAs. Cell 2015; 160(6):1125-1134.

30. Ebbesen KK, Hansen TB, Kjems J. Insights into circular RNA biology. RNA Biol 2017; 14(8):1035-1045.

31. Ghasemi H, Sabati Z, Ghaedi H, et al. Circular RNAs in β-cell function and type 2 diabetes-related complications: a potential diagnostic and therapeutic approach. Mol Biol Rep 2019;46(5):5631-5643.

32. Zhao Z, Li X, Jian D, et al. Hsa_circ_0054633 in peripheral blood can be used as a diagnostic biomarker of pre-diabetes and type 2 diabetes mellitus. Acta Diabetol. 2017; 54(3):237-245.

33. Mohr AM, Mott JL. Overview of microRNA biology. Semin Liver Dis 2015; 35(1):3-11.

34. Yu JSL, Cui W. Proliferation, survival and metabolism: the role of PI3K/AKT/mTOR signalling in pluripotency and cell fate determination. Development 2016; 143(17):3050-3060.

35. Anitha M, Gondha C, Sutliff R, et al. GDNF rescues hyperglycemia-induced diabetic enteric neuropathy through activation of the PI3K/Akt pathway. J Clin Invest 2006; 116(2):344-356.

36. Li R, Wu Y, Zou S, et al. NGF attenuates high glucose-induced ER stress, preventing schwann cell apoptosis by activating the PI3K/Akt/GSK3β and ERK1/2 pathways. Neurochem Res 2017; 42(11):3005-3018.

37. Liu S, Gao G, Yan D, et al. Effects of miR-145-5p through NRAS on the cell proliferation, apoptosis, migration, and invasion in melanoma by inhibiting MAPK and PI3K/AKT pathways. Cancer Med. 2017;6(4):819-833.