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Original Article

The effect of neuregulin-1 on the survival of Schwann-like cells via the PI3K/Akt/Bad pathway

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Abstract: Here, we investigated the molecular mechanism underlying the regulatory effects of neuregulin-1 (NRG) on the differentiation of Schwann cells (SCs) and evaluated the role of NRG in the phosphoinositide-3-kinase (PI3K)/protein kinase B (Akt)/Bad pathway. NRG1 was used to induce the differentiation of mouse bone marrow mesenchymal cells (BMSCs) into SCs. The phosphorylation levels of Akt, extracellular signal-regulated kinase (ERK), and Bad were monitored. The phosphorylation of Akt, ERK, and Bad was blocked after the suppression of PI3K ($P < 0.05$), resulting in the apoptosis of SCs ($P < 0.05$). The blockade of mitogen-activated protein kinase (MEK) resulted in the inhibition of the phosphorylation of only ERK and Bad but failed to affect the survival of SCs. The blockade of the mammalian target of rapamycin (mTOR) also had no effect on the survival of SCs ($P < 0.05$). The PI3K/Akt/Bad and MEK/ERK1/2/Bad pathways were activated during the NRG1-induced differentiation of BMSCs into SCs, but NRG1 could only activate the PI3K/Akt/Bad pathway to promote the survival of SCs. In summary, this study demonstrates that NRG1 can activate PI3K and its downstream signal Akt, ultimately leading to the phosphorylation of Bad and the survival of SCs.

Keywords: Neuregulin-1, ErbB, PI3K/Akt/Bad, bone marrow mesenchymal cells, schwann cells, differentiation induction, survival, mice

Introduction

Schwann cells (SCs) secrete several neurotrophin factors, which nourish and support the proliferation and metabolism of nerve cells and regulate axonal regeneration and myelin thickening. SC transplantation in nerve defects may promote the regeneration of neurons and achieve partial functional recovery [1, 2]. However, there are limitations, such as insufficient source and the rejection of allogeneic SCs, easy apoptosis, and survival difficulty.

Bone marrow mesenchymal stem cells (BMSCs) are non-hematopoietic stem cells present in the bone marrow. These are characterized by abundant sources, simple sampling, easy isolation/purification/amplification, and transplantability. Under certain *in vitro* conditions, BMSCs may differentiate into SCs, indicative of their potential applications to replace SCs for transplantation [3-5].

In the peripheral nervous system, apoptosis is one of the mechanisms responsible for matching the number of glial cells with axons. During development or after nerve injury, there is an increase in the number of SCs that proliferate along the growing axons; however, the limited level of neuregulin (NRG) produced by the axons may affect the survival of SCs. Hence, the SCs growing away from the axon contact point may undergo apoptosis, thereby demanding extensive transplantation and significantly limiting their clinical applications [6, 7].

Factors such as fibronectin-2 (FGF-2), insulin-like growth factor (IGF), and NRG are known to

effectively prevent apoptosis [8-10]. NRG is a primary survival factor secreted during the maturation of Schwann-type precursor cells [11]. Mice knocked out for *ErbB3* expression completely lack SCs or precursors, demonstrating the importance of NRG in the survival of SCs [12]. Aside from its role in promoting survival, NRG can mediate the differentiation, proliferation, and migration of SCs by binding and activating ErbB2/ErbB3 heterodimers [13]. ErbB2 itself does not bind to NRG but may bind to ErbB3, which then forms a high-affinity receptor complex with NRG [14]. However, the mechanism underlying the NRG-mediated downstream signaling pathways of ErbB2/ErbB3 that regulate the survival of SCs remain unclear, restricting the clinical applications of NRG. The phosphoinositide-3-kinase (PI3K)/protein kinase B (Akt) pathway is one of the key signaling pathways that regulate cellular apoptosis. Studies have shown that NRG can mediate the survival of rat SCs through the activation of the PI3K/Akt pathway [15].

Here, we prepared an *in vitro* rat BMSC model, blocked the PI3K/Akt/Bad pathway using the PI3K inhibitor wortmannin and the MEK inhibitor U0126, and determined the expressions of p-Akt, Akt, extracellular signal-regulated kinase 1/2 (ERK1/2), p-ERK1/2, and Bad to reveal the role of the PI3K/Akt/Bad pathway in the survival of mouse BMSC-derived SCs. Our results indicate that NRG1 could inhibit apoptosis and related signaling molecules through the activation of the PI3K/Akt/Bad pathway. Thus, NRG1 can be used to regulate apoptosis and obtain a large pool of SC-like cells. Our results provide an experimental basis for nerve injury clinical cell transplantation therapy.

Materials and methods

Isolation, culture, and purification of BMSCs

Mice femurs and tibias (SPF grade, provided by the Experimental Animal Center of Qingdao University) were excised under aseptic conditions; the osteophytes were cut at both ends, and the marrow cavity was exposed. The marrow cavity was thoroughly rinsed with α-MEM (Gibco, USA). The rinsing solution was mixed with 10% fetal bovine serum (FBS) (Gibco, USA) and 1% a penicillin-streptomycin solution (Solarbio, Beijing, China) and centrifuged. The supernatant was discarded and the pellets were suspended in a culture medium. The suspended cells were inoculated into a 25 cm^2 culture flask and cultured at 37°C under 5% CO_2. Wall-adherent growing BMSCs were considered to be relatively pure cells. After 24 h, the medium was changed for the first time by gently shaking the flask and aspirating the culture solution. The flask was rinsed twice with phosphate-buffered saline (PBS) to remove any unattached and miscellaneous cells; the medium was then changed every 72 h for 2-3 weeks. After reaching 85%-90% confluency, the cells were treated with 0.25% trypsin/ethylenediaminetetraacetic acid (EDTA; Gibco, USA) and replated into new culture flasks at a density of 10^5 cells/cm^2.

Differentiation induction of BMSCs

To induce the differentiation of MSCs into SC-like cells, the cells were digested with 0.25% trypsin (containing EDTA) and plated in a 6-cm culture flask at a density of 10^5 cells/cm^2. The medium comprised α-MEM and 1 mM β-mercaptoethanol (Sigma, Japan). After 24 h, the medium was removed and the flask was washed thrice with PBS, followed by the addition of α-MEM supplemented with 10% FBS (Gibco, USA), and all-trans retinoic acid (RA; 35 ng/mL) (Sigma, Japan). After 72 h, the flask was washed thrice with PBS and the cells were incubated in α-MEM supplemented with 10% FBS, 20 ng/mL basic fibroblast growth factor (bFGF; PeproTech, USA), 5 mM forskolin (Sigma, Japan), 5 ng/mL platelet-derived growth factor (PDGF)-BB (PeproTech, USA), and 200 ng/mL HRG (PeproTech, USA). The medium was changed once every 3 days for a total of 10 days. The cells were divided into the following groups: the total inducer group (TI), the HRG-free group (HRG-free), and the inhibitor group (I). Group I was treated with either the PI3K inhibitor (50 mM wortmannin), the MEK inhibitor (50 mM U0126), or the mTOR inhibitor (100 nM rapamycin). The SC-like cells from Group I were cultured in a serum-free medium for 20 h and treated with different pathway inhibitors.

Immunocytochemical analysis

After reaching 95%-100% confluency, two sets of cells from the induction and non-induction groups were removed and rinsed thrice with pre-warmed 1× PBS (Solarbio, Beijing, China) for 10 min. The cells were incubated with 4%

Table 1. Primer sets used for RT-PCR

Gene	Primer (5'-3')	Product size (bp)	Cycle No.	Reference (Gene bank No.)
LNGF-R	Forward: TGGGAAGTGGGACATTCTTT	90	40	NM-012610
	Reverse: CAAACACATGCTAAACCGTACC			
S100β	Forward: ACTGGTCAACGAGGACAACC	169	28	NM-013191
	Reverse: GTATGGCTGGGACTCTCGAA			
NGF	Forward: CTGCCTGGAGTTCATCAG	82	35	XM-227525
	Reverse: CAGGCAAGTCAGCCTCTTCT			
NT-3	Forward: CGACGTCCCTGGAAATAGTC	77	35	NM-031073
	Reverse: TGGACATCACCTTGTTCACCT			
BDNF	Forward: TATGGCCTCCATCCCATTT	89	35	NM-012513
	Reverse: GCATCACTGCTATGCCATATTT			
GDNF	Forward: CTAAGATGAAGTTATGGGATGTCG	96	35	NM-019139
	Reverse: CTTCGAGAAGCCTCTTACCG			

formaldehyde at room temperature for 20-30 min and 0.2% Triton X-100 (permeabilization) for 2-5 min. The cells were rinsed thrice with 1× PBS (10 min each time) and blocked with 5% bovine serum albumin at room temperature for 30 min. The cells were probed with rabbit anti-rat s-100 (1:10000) (Dako, Denmark), rabbit anti-glial fibrillary acidic protein (GFAP; 1:200, Santa Cruz, USA), and rabbit anti-p75 (1:200, Santa Cruz, USA). The negative control was treated with 0.01 mol/L PBS. All groups were cultured overnight in a wet box at 4°C. Following overnight incubation, the cells were washed thrice with 1× PBS (10 min each time) and treated with Alexa Fluor 546 goat anti-rabbit IgG (1:1000) (Invitrogen, USA) for 30 min in the dark. The cells were washed thrice with 1× PBS (10 min each time), and incubated for 30 min with 4',6-diamidino-2-phenylindole (DAPI) solution (Sigma, Japan) at room temperature. Images were obtained under a fluorescence microscope.

Reverse-transcriptase polymerase chain reaction (RT-PCR)

The cells induced for 7 days were trypsinized and collected; total RNA was extracted using the Trizol method (Invitrogen, USA). The extracted RNA was diluted for 10 times with diethyl pyrocarbonate (DEPC) water and its concentration determined (three-time determination for the mean, with the ratio of A260 to A280 being 1.8-2.0). The primers for s-100, p75, and GFAP were designed by Primer 3.0, compared with the NCBI gene pool, and synthesized by Sangon Biotech (Shanghai) Co., Ltd. The primer sequences are shown in **Table 1**. The RT-PCR reaction was carried out according to the kit's instructions (Takara, Japan) for the synthesis of cDNA. The reverse transcription product was subjected to PCR in a total reaction volume of 20 μL. The PCR conditions were as follows: predenaturation at 95°C for 5 min, denaturation at 95°C for 10 s, annealing at 57°C for 20 s, and extension at 72°C for 15 s (40 cycles). Each sample was repeated in three wells, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as the internal control. The PCR products were subjected to agarose gel electrophoresis and imaged with an UV-gel electrophoresis imager (UVP, UK).

Western blot analysis

The cells from each group were collected, counted, and lysed, and the protein levels were quantified using the Bradford assay. After the addition of a loading buffer, 10 mg of protein samples were denatured at 95°C for 5 min and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis using GAPDH (Cell Signaling Technology, USA) as the loading control. The proteins separated on the gel were transferred onto a polyvinylidene fluoride (PVDF) membrane and blocked with 5% skim milk after 1.5 h. Following 1 h incubation at room temperature, the blots were treated with primary rabbit anti-Akt (Cell Signaling Technology, USA), p-Akt (Cell Signaling Technology, USA), ERK1/2 (Cell Signaling Technology, USA), p-ERK1/2 (Cell Signaling Technology, USA), and Bad (Cell Signaling Technology, USA) for overnight at 4°C. The membranes were washed

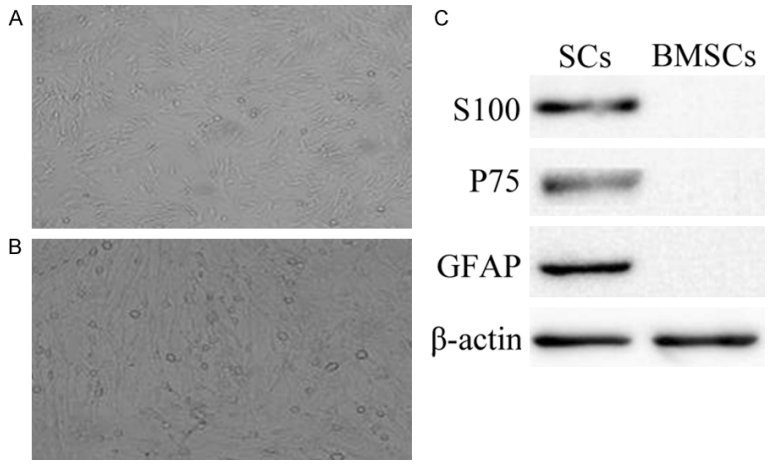

Figure 1. NRGs induce BMSCs into SC-like cells. A. A large amount of BMSCs after primary culture. B. After adding NRG, there are several axons and dendrite-like processes observed to get out of the body. C. The expression of S-100, GFAP, p75 is significantly increased compared to the untreated cultures.

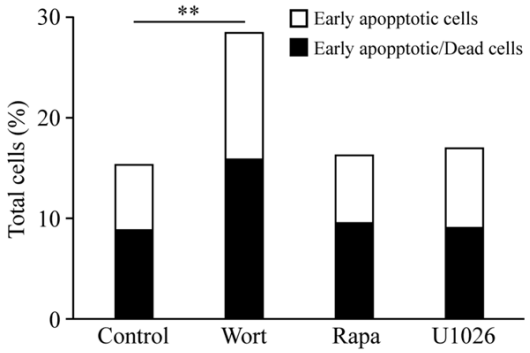

Figure 2. SCs were serum-deprived for 24 h and then treated with pathway inhibitors. A PI3K inhibitor (50 mM LY24902) exacerbated apoptosis in response to serum withdrawal and blocked NRG-mediated rescue, but the aMEK inhibitor (50 mM PD98059) and the m-TOR inhibitor (100 nM rapamycin) did not. The bars represent the means of 6 standard deviations from three independent experiments.

with TBST (Solarbio, Beijing, China), incubated with a secondary antibody for 1 h, and developed using an enhanced chemiluminescence (ECL) kit (Thermo Fisher Scientific, USA). The results were observed on a UVP imager.

Statistical analysis

All the data were statistically analyzed using GraphPad Prism 5, and the independent sample test was used for the intergroup comparisons. A value of $P < 0.05$ was considered statistically significant.

Results

NRG induced BMSC differentiation into SC-like cells

Most primary cultured cells adhered to the wall within 24 h and were round or elliptical in shape with a uniform morphology. The cells gradually showed cytoplasmic processes; the culture medium was changed every 72 h. After 6 days, the cells were mainly spindle-shaped and had a uniform morphology, abundant cytoplasms, large nuclei, and obvious nucleoli. After about 3 weeks of cultivation, the cells reached 90% confluency. The cells were then digested with 0.25% trypsin/EDTA; after passage, the cells were completely adherent within 20 h and fully integrated at day 10. The morphology of the cells changed 24 h after induction, as evident from the stretched processes that intertwined to form a network (**Figure 1A**).

Upon growth and reaching 95%-100% confluency, the expression levels of the SC-specific marker genes encoding s-100, GFAP, and p75 were examined to determine the effect of NRG on SC differentiation. The results of western blotting showed that all the three markers were expressed after induction (**Figure 1B**). These data indicate that BMSCs had differentiated into SC-like cells.

Blockade of PI3K significantly increased the apoptosis of SC-like cells

The addition of the PI3K inhibitor wortmannin after 24 h of serum-free cultivation significantly increased the number of apoptotic SC-like cells. The addition of the MEK inhibitor U0126 and the m-TOR inhibitor rapamycin could slightly increase the number of apoptotic cells, but the difference was not significant as compared with the number observed in group TI. Thus, these inhibitors failed to significantly block the NRG-mediated survival of SC-like cells (**Figure 2**). These results indicate that NRG promoted the survival of SC-like cells through the activation of the PI3K/Akt/Bad pathway.

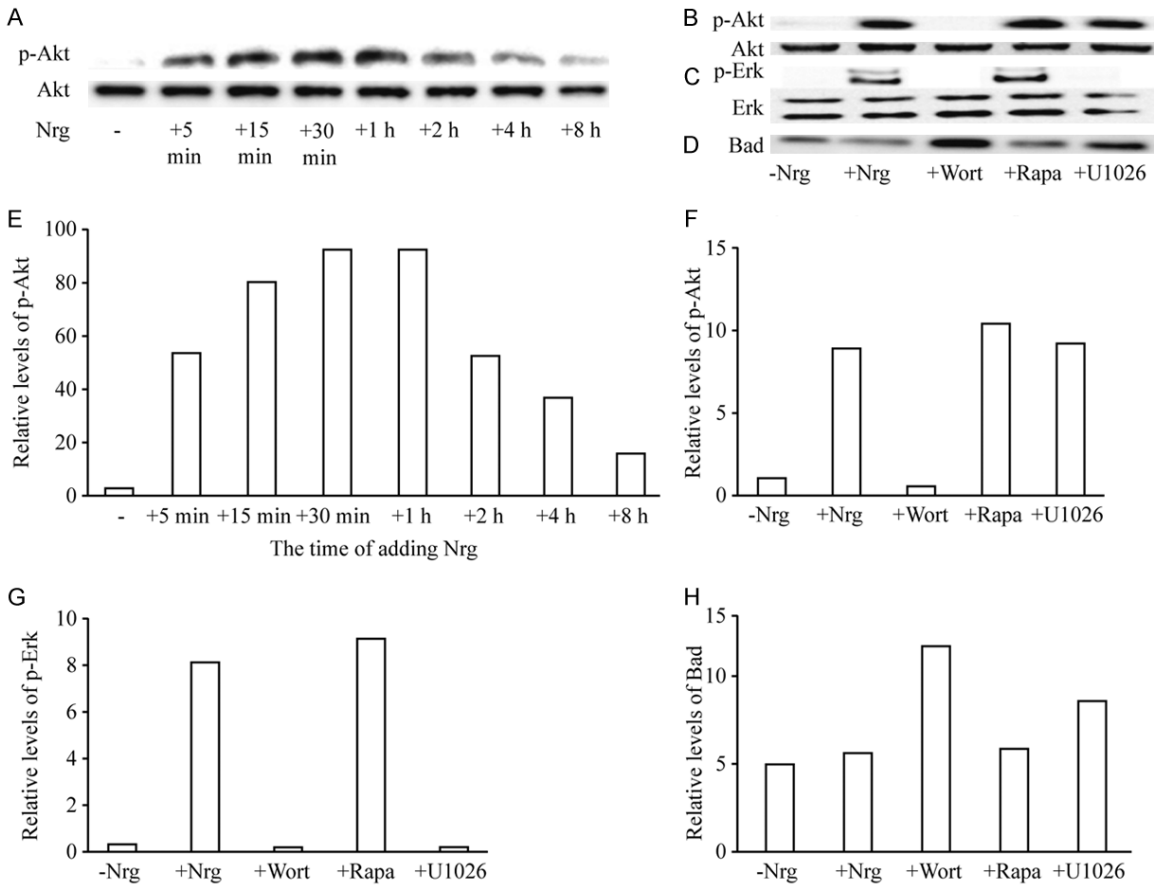

Figure 3. NRG induces the phosphorylation of Akt and Erk and dual phosphorylation of Bad. Notes: A. The level of p-Akt is at its highest at 30 min and decreases with time, but the level of Akt is stable. B. After adding Wortmannin, the phosphorylation of Akt is blocked, but U0126 and rapamycin have no influence on it. C. Wortmannin and U0126 can block the phosphorylation of Erk, but rapamycin can't. D. NRG can induce the phosphorylation of Bad in at least two sites. It can be observed by the retarded migration of Bad. E. The expression of the p-Akt protein of each period after adding NRG. D. The expression of the ERK1/2 protein in the three groups of cells. F. The expression of the p-Akt protein in the all groups. G. The expression of the p-Erk protein in the all groups. H. The expression of the dual phosphorylation of Bad in the all groups.

NRG activated the PI3K downstream signal Akt and consequently phosphorylated Bad

The NRG treatment rapidly increased the level of p-Akt within 5 min which gradually decreased after 1 h. No p-Akt was observed after 8 h. This observation indicates that NRG exerted a significant time-dependent effect on the expression of p-Akt (**Figure 3A**). NRG-mediated PI3K phosphorylation was blocked by the PI3K blocker wortmannin but was unaffected by rapamycin or U0126. Thus, the activation of Akt is dependent on the phosphorylation of PI3K but independent of MEK and m-TOR. NRG also promoted the phosphorylation of ERK1/2, and this effect was completely abrogated by wortmannin and U0126 but not rapamycin (**Figure 3C**). Thus, the phosphorylation of ERK1/2 is dependent on the activation of PI3K and MEK but independent of m-TOR. NRG-treated SC-like cells showed the phosphorylation of ERK1/2 (**Figure 3C**) and Akt (**Figure 3A**, **3B**) along with the delayed migration of Bad in the gel (**Figure 3D**). The phosphorylation of MEK and ERK1/2 has been known to induce phosphorylation of the serine 112 of Bad [11], while Akt activation is known to induce the phosphorylation of the serine 136 site of Bad [12]. The delayed migration of Bad in the gel is suggestive of its phosphorylation at two sites. The PI3K inhibitor wortmannin and the MEK inhibitor U0126 blocked the migration and double phosphorylation of Bad; we did not observe similar inhibitory effects with rapamycin.

Discussion

SCs play an important role in peripheral nerve regeneration and promote the sheathing of peripheral nerve axons (the satellite cells of the peripheral nervous system) [16]. These cells also secrete a variety of neurotrophic factors, which nourish and support the proliferation and metabolism of nerve cells and regulate the regeneration of axons and the formation of myelin. The transplanted SCs in nerve defects are thought to promote nerve regeneration by crossing nerve defects and reaching the corresponding target cells, thereby achieving partial functional recovery. However, the lack of a suitable SC source has led to difficulties, such as the rejection of allogeneic SCs, apoptosis, and survival difficulty, thereby restricting clinical applications [17]. BMSCs are adult pluripotent stem cells derived from the original germ layer and may be induced to differentiate into ectodermal-derived nerve cells under specific conditions. BMSCs can be self-derived and have an ideal *in vitro* expansion rate; these are expected to serve as replacements for the transplantation therapy of SCs and may provide a potential solution for SC transplantation to repair nerve damage. Studies have shown that BMSCs may be directionally induced to differentiate into SC-like cells, which have shown the feasibility of repairing peripheral nerve defects and restoring the function of their dominant regions after forming artificial nerves [18]. Based on the method of Woodbury [19], we established a new method for the induction of the differentiation of mouse BMSCs into Schwann-like cells. Immunofluorescence results showed that the cells not only underwent morphological changes after induction by this method but also exhibited the characteristics of SCs at the molecular level [20].

Apoptosis is one of the mechanisms through which the peripheral nervous system maintains a balance in the number of glial cells and axons. During development and after nerve injury, SCs proliferate along the growing axons. If the number of cells is too high, the cells compete with the NRG produced by the axons and affect the survival of SCs. SCs away from axons lack NRG and subsequently undergo apoptosis [9, 10]. Therefore, research studies have been focused on regulating the apoptotic level of SCs.

Many biological properties of SCs are affected by NRG-1, which is known to promote the differentiation of neural crest stem cells into glial cells. NRG-1 promotes the proliferation and migration of SC precursors and provides important signals for myelination. After denervation injury, NRG-1 affects the activity of SCs. NRG-1 is expressed in early developing neurons; SC precursors are capable of expressing the corresponding receptors such as ErbB2. NRG may bind and activate ErbB2/ErbB3 heterodimers. Mice with ErbB3 knockout completely lack SCs and their precursors, indicative of the importance of NRG in SC survival. Studies have shown that NRG plays an important role in the maturation of SCs. The survival of SC precursors is independent of NRG but that of mature SCs relies on NRG to a large extent [21]. However, the ErbB2/ErbB3 downstream signaling pathways that maintain the NRG-mediated survival of SC precursors are still unclear. The PI3K/Akt signaling pathway is closely related to cellular apoptosis. Studies have shown that PI3K/Akt may attenuate acrylamide-induced apoptosis [22] and induce the apoptosis of human breast cancer cells [23] and cardiac muscle cells [24]. Studies have also shown that MEK can regulate the expression of the cell death protein B cell lymphoma 2 family [25]; MEK phosphorylates ERKs, which in turn activate RSKs and stimulate the phosphorylation of Bad at threonine 112 [26].

Our study found that NRG receptor signaling can rapidly activate PI3K in SC-like cells after cultivation for 24 h in a serum-free medium and that the addition of a PI3K inhibitor can significantly aggravate apoptosis and block the phosphorylation of Akt, MEK, and Bad (**Figure 3**). Thus, PI3K is a common upstream signal of Akt, MEK, and Bad. The NRG-mediated phosphorylation of PI3K can be blocked by the PI3K blocker wortmannin but not by rapamycin or U0126 (**Figure 3**). Thus, Akt and MEK are the common parallel downstream signals of PI3K. Activated PI3K can rapidly induce the phosphorylation of MEK and Akt, ultimately leading to the phosphorylation of different serine residues in Bad. The addition of the MEK inhibitor U0126 and the m-TOR inhibitor rapamycin only slightly increased the number of apoptotic cells, but the difference was not statistically significant as compared with the number reported for group TI. U0126 and rapamycin also

failed to significantly block the stimulatory role of NRG in the survival of SC-like cells. Thus, NRG1 promotes the survival of SC-like cells through the activation of the PI3K/Akt/Bad pathway.

Although a rapid phosphorylation of ERK1/2-Bad at serine 112 in SCs by NRG1 can be observed, the MEK inhibitor U0126 fails to block the NRG-mediated increase in SC survival [27]. Although the activation of MEK can terminate the phosphorylation of Bad and exert anti-apoptotic effects through the release of Bcl-2 and Bcl-xl [28], this signaling pathway may not be involved in the NRG1-mediated survival of SCs. The roles of MEK/Bad and mTOR signaling pathways need to be confirmed in further studies. The evaluation of other downstream signals of the NRG/ERBB pathway that mediate anti-apoptosis effects may be the focus of our next experimental studies.

In conclusion, this study constructs an *in vitro* induction model of mouse BMSCs, improves the method of inducing differentiation of mouse BMSCs into SC-like cells, and demonstrates the NRG1-mediated phosphorylation of Bad through the activation of PI3K and its downstream signal Akt to promote SC survival. Our results provide an experimental basis for the *in vitro* acquisition of large quantity of SCs and cell therapy for clinical neurological defect.

Disclosure of conflict of interest

None.

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References

[1] Castelnovo LF, Bonalume V, Melfi S, Ballabio M, Colleoni D and Magnaghi V. Schwann cell development, maturation and regeneration: a focus on classic and emerging intracellular signaling pathways. Neural Regen Res 2017; 12: 1013-1023.

[2] Jessen KR and Mirsky R. The origin and development of glial cells in peripheral nerves. Nat Rev Neurosci 2005; 6: 671-682.

[3] Gnecchi M, Danieli P and Cervio E. Mesenchymal stem cell therapy for heart disease. Vascul Pharmacol 2012; 57: 48-55.

[4] Dezawa M, Takahashi I, Esaki M, Takano M and Sawada H. Sciatic nerve regeneration in rats induced by transplantation of in vitro differentiated bone-marrow stromal cells. Eur J Neurosci 2001; 14: 1771-1776.

[5] Lin L, Lin H, Bai S, Zheng L and Zhang X. Bone marrow mesenchymal stem cells (BMSCs) improved functional recovery of spinal cord injury partly by promoting axonal regeneration. Neurochem Int 2018; 115: 80-84.

[6] Ramli K, Aminath Gasim I, Ahmad AA, Hassan S, Law ZK, Tan GC, Baharuddin A, Naicker AS, Htwe O, Mohammed Haflah NH, B H Idrus R, Abdullah S and Ng MH. Human bone marrow-derived MSCs spontaneously express specific schwann cell markers. Cell Biol Int 2019; 43: 233-252.

[7] O'Neill P, Lindsay SL, Pantiru A, Guimond SE, Fagoe N, Verhaagen J, Turnbull JE, Riddell JS and Barnett SC. Sulfatase-mediated manipulation of the astrocyte-Schwann cell interface. Glia 2017; 65: 19-33.

[8] Jessen KR, Brennan A, Morgan L, Mirsky R, Kent A, Hashimoto Y and Gavrilovic J. The schwann cell precursor and its fate: a study of cell death and differentiation during gliogenesis in rat embryonic nerves. Neuron 1994; 12: 509-527.

[9] Gambhir HS, Raharjo E, Forden J, Kumar R, Mishra C, Guo GF, Grochmal J, Shapira Y and Midha R. Improved method to track and precisely count Schwann cells post-transplantation in a peripheral nerve injury model. J Neurosci Methods 2016; 273: 86-95.

[10] Chen MS, Kim H, Jagot-Lacoussiere L and Maurel P. Cadm3 (Necl-1) interferes with the activation of the PI3 kinase/Akt signaling cascade and inhibits Schwann cell myelination in vitro. Glia 2016; 64: 2247-2262.

[11] Dong Z, Brennan A, Liu N, Yarden Y, Lefkowitz G, Mirsky R and Jessen KR. Neu differentiation factor is a neuron-glia signal and regulates survival, proliferation, and maturation of rat Schwann cell precursors. Neuron 1995; 15: 585-596.

[12] Riethmacher D, Sonnenberg-Riethmacher E, Brinkmann V, Yamaai T, Lewin GR and Birchmeier C. Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. Nature 1997; 389: 725-730.

[13] Grinspan JB, Marchionni MA, Reeves M, Coulaloglou M and Scherer SS. Axonal interactions regulate Schwann cell apoptosis in developing peripheral nerve: neuregulin receptors and the role of neuregulins. J Neurosci 1996; 16: 6107-6118.

[14] Sliwkowski MX, Schaefer G, Akita RW, Lofgren JA, Fitzpatrick VD, Nuijens A, Fendly BM, Cerione RA, Vandlen RL and Carraway KL 3rd.

Coexpression of erbB2 and erbB3 proteins reconstitutes a high affinity receptor for heregulin. J Biol Chem 1994; 269: 14661-14665.
[15] Li Y, Tennekoon GI, Birnbaum M, Marchionni MA and Rutkowski JL. Neuregulin signaling through a PI3K/Akt/Bad pathway in Schwann cell survival. Mol Cell Neurosci 2001; 17: 761-767.
[16] Eckersley L. Role of the Schwann cell in diabetic neuropathy. Int Rev Neurobiol 2002; 50: 293-321.
[17] Carey DJ and Bunge RP. Factors influencing the release of proteins by cultured Schwann cells. J Cell Biol 1981; 91: 666-672.
[18] Burden S and Yarden Y. Neuregulins and their receptors: a versatile signaling module in organogenesis and oncogenesis. Neuron 1997; 18: 847-855.
[19] Black IB and Woodbury D. Adult rat and human bone marrow stromal cells differentiate into neurons. Blood Cells Mol Dis 2001; 27: 632-636.
[20] Jessen KR and Mirsky R. Developmental regulation in the schwann cell lineage. Adv Exp Med Biol 1999; 468: 3-12.
[21] Gavrilovic J, Brennan A, Mirsky R and Jessen KR. Fibroblast growth factors and insulin growth factors combine to promote survival of rat Schwann cell precursors without induction of DNA synthesis. Eur J Neurosci 1995; 7: 77-85.
[22] Sun G, Wang X, Li T, Qu S and Sun J. Taurine attenuates acrylamide-induced apoptosis via a PI3K/AKT-dependent manner. Hum Exp Toxicol 2018; 960327118765335. [Epub ahead of print].
[23] Zhang H, Xu HL, Wang YC, Lu ZY, Yu XF and Sui DY. 20(S)-protopanaxadiol-induced apoptosis in MCF-7 breast cancer cell line through the inhibition of PI3K/AKT/mTOR signaling pathway. Int J Mol Sci 2018; 19: 1053.
[24] Lv X, Xu T, Wu Q, Zhou Y, Huang G, Xu Y and Zhong G. 6-gingerol activates PI3K/akt and inhibits apoptosis to attenuate myocardial ischemia/reperfusion injury. Evid Based Complement Alternat Med 2018; 2018: 9024034.
[25] Chao DT and Korsmeyer SJ. BCL-2 family: regulators of cell death. Annu Rev Immunol 1998; 16: 395-419.
[26] Bonni A, Brunet A, West AE, Datta SR, Takasu MA and Greenberg ME. Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. Science 1999; 286: 1358-1362.
[27] Rahmatullah M, Schroering A, Rothblum K, Stahl RC, Urban B and Carey DJ. Synergistic regulation of Schwann cell proliferation by heregulin and forskolin. Mol Cell Biol 1998; 18: 6245-6252.
[28] Scheid MP, Schubert KM and Duronio V. Regulation of bad phosphorylation and association with Bcl-x(L) by the MAPK/Erk kinase. J Biol Chem 1999; 274: 31108-31113.