RETRACTED ARTICLE: Stem-like cells of various origins showed therapeutic effect to improve the recovery of spinal cord injury

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ABSTRACT
We aimed to evaluate the therapeutic effects of exosomes, which were collected from human neuro-epithelial stem cells (HNESCs) treated by miR-29b mimics, on the treatment of spinal cord injury (SCI). Computational analysis, real-time PCR, Western blot analysis and TUNEL assay, a BBB score system, the Nissl staining and IHC assay were conducted to explore the molecular signalling pathway underlying the function of exosomes in SCI. Exosomes isolated from cells treated with HNESC exhibited the strongest inhibitory effect on cell apoptosis while exhibiting the highest level of miR-29b expression and the lowest levels of PTEN and caspase-3 expression. Moreover, PTEN and caspase-3 were identified as the direct target genes of miR-29b. The exosomes isolated from the groups of HNESC and HNESC + miR-29b mimics exhibited in vivo therapeutic effects by restoring the BBB score and apoptosis index of post-SCI neuron cells to those of normal neuron cells, with the exosomes collected from the group of HNESC + miR-29b mimics showing the strongest effect. We suggested that the exosomes derived from the group of HNESC + miR-29b mimics exerted therapeutic effects on SCI by down-regulating the expression of PTEN/caspase-3 and subsequently suppressing the apoptosis of neuron cells.

Introduction

Each year, about 10,000 American people suffer from spinal cord injuries (SCIs). SCI pathology can be identified by both initial mechanical damage and secondary processes, such as the formation of free-radicals, anoxia, ischaemia and post-injury excitotoxicity occurring over several hours and several days [1]. The axonal regeneration in the central nervous system (CNS) seems to be inhibited partially by inhibitors associated with myelin, the loss of the intrinsic ability for overcoming inhibitory cues in adult neurons, and the formation of a scar barrier following lesions [1–4].

There are also multipotent stem cells in many types of tissues of adults [5,6]. Stem cells are most often isolated from the marrow in bones, but brain and muscle, blood vessels and skin also contain multipotent stem cells [7–9]. Multipotent stem cells can increase post-SCI neural repair through renewing host cells or facilitating the regeneration of axons of the host. It has been reported that the implantation of the cells derived from the spinal cord of foetuses or foetal nervous systems into injured or intact spinal cords can be achieved, although the differentiation of such stem cells is restricted.

As vesicles with a diameter of 30–100 nm, exosomes are generated by a number of cell types, and can be used as a new pool for the discovery of disease biomarkers. They can transfec and traffic miRNAs, mRNAs and proteins into the target cell for the communication between different cells and can probably lead to pathology [10]. In nervous systems, most researches on exosomes focussed on in vitro research. For instance, primary cells from the culture of cortical neurons from rats, differentiated neurons from palliums and cells from neuroglia have been confirmed to secrete exosomes [11–13].

MicroRNAs (miRNAs) are short non-coding RNAs with a length of 20–22 nt. It suppresses the expression of genes by interacting with mRNAs and targeting the corresponding three untranslated regions (UTRs). It is predicted that miRNAs target more than half of all the genes encoding proteins in human, rendering them significant in the regulation of a great number of developmental and physiological processes, such as proliferation, apoptosis, differentiation and development, via imperfectly targeting mRNAs of genes encoding proteins and regulating the expression of these genes at the transcriptional level or the post-transcriptional level [14,15].
The down-regulation of Sp1 by the miRNA miR-29b has been shown to upregulate the expression of PTEN, and finally caused the decrease in the phosphorylation of AKT. As an antagonist of the PI3K-AKT signalling pathway in cells in cancers, PTEN plays a key role in the suppression of tumours and is modulated by many factors, including Snail1, C-Jun, AP-2, Egr-1 and p53 [16–21]. In the authors’ previous study, it was indicated that Sp1 inhibited the expression of PTEN through targeting the promoter region of PTEN (918/913 bp) [22]. MiR-29b can also target the gene Sp1; hence, it was found that miR-29b overexpression enhanced the activity of the promoter of PTEN.

It has been previously reported that stem-like cells of various origins, including bone marrow mesenchymal stem cells (BMSMCs), differentiated PC12 (DPC12) cells, dental pulp-derived stem cells (DPDSCs), human embryonic stem cells (hESCs), human neuroepithelial stem cells (HNESCs) and adipose derived stem cells (ADSCs), showed certain therapeutic effects to improve the recovery of SCI [23–28]. In this study, we harvested the conditional medium from the culture of above cells or the exosomes isolated from such mediums to treat cultured cells to study their effects on H2O2-induced apoptosis. Furthermore, we treated SCI rats with exosomes fortified with or without miR-29b to investigate its effect on post-SCI recovery and the expression of possible miR-29b targets such as PTEN and CASPASE3.

Materials and methods

Animals and treatments with drugs

A total of 24 male adult SD rats with an average body weight of 200 ± 20 g were housed under a cycle of 12 h lightness/12 h darkness at 22 ± 2 °C. For assessing the therapeutic efficacy of exosomes of post-SCI recovery in terms of their locomotion activities, these rats were divided in a random manner into four groups with eight rats in each group: a sham group, a group of SCI, an SCI group treated with exosomes collected from undifferentiated PC12 cells, and an SCI group treated with exosomes collected from PC12 cells transfected with miR-29b. The exosomes used in the experiment were collected and purified from related cell lines. The purified exosomes were then intravenously injected into the rats with SCI. The rats in the sham group only underwent laminectomy and an intraperitoneal injection with 1% C6H12OH in 1 mL saline. The rats in the SCI group underwent operations shown below for SCI model establishment.

SCI model establishment

For different groups, the rats were subjected to anaesthetization first through an intraperitoneal injection using the saline containing chloral hydrate at a concentration of 10% at a dose of 0.33 mL/kg. Later on, the rats were fixed and then incised via the midlines of their backs for exposing their spinal cords. Then these rats experienced a T9/10 laminectomy. Subsequently, the rats of the sham group were subjected to an intraperitoneal injection with 1% C6H12OH in 1 mL saline before the closing of the incisions. The rats in the treatment groups were further subjected to the SCI model establishment using Allen technique. Briefly, the rats experienced a contusing operation through bearing an impactor (with a diameter of 2 mm and weight of 10 g) rapidly fell from a higher place with a distance of 25 mm onto the back side of the spinal cord. Following the closing of the incision, each rat was returned to the corresponding cage for future observations and experiments.

Isolation of RNA and real-time PCR

Total RNA was extracted from each sample using a Qiagen kit in accordance with the instructions of the manufacturer (Hilden, Germany). Calculation was then performed for obtaining the concentrations of the RNA samples based on the ratio of the OD value measured under 260 nm to the one measured under 280 nm. Reverse transcription was performed for obtaining cDNA utilizing a Qiagen kit (Hilden, Germany). Furthermore, primer design was performed using the software Primer 5.0 based on corresponding gene sequences obtained from GenBank. The design and synthesis of all primers were performed by Takara Bio Inc. (Tokyo, Japan). RT-PCR reaction was done using a 20-μL system containing distilled water (6 μL), DNA templates (2 μL), ROX reference dye II (0.4 μL), reverse primer (0.8 μL), forward primer (0.8 μL) and SYBR PremixExTaq (10 μL). The RT-PCR system was subjected to the following procedure for 40 cycles: 95 °C (30 s) → 95 °C (5 s) → 60 °C (30 s). The verification of the results of the PCR was based on the construction of a dissolution curve in which the control reference value was from the dissolution of U6 and β-actin. The relative expression of miR-21, miR-29b, miR-19b, miR-125b, miR-335, miR-30b, miR-341, miR-370, miR-221, miR-188, miR-24-3p, miR-206, PTEN and caspase-3 was calculated using U6 and β-actin as the internal control.

Exosome extraction

An Exiqon miRCURY Exosome Isolation Kit (Vedbaek, Denmark) was used to isolate exosomes from the conditional media of cultured BMSMCs, DPC12 cells, DPSCs, hESCs, HNESCs and ADSCs. Exosome extraction was performed in accordance with the instructions of the manufacturer. The exosomes from unconditioned cells were utilized as a control.

Exosome analysis using scanning electron microscopy

Exosome distribution was determined using scanning electron microscopy. Briefly, the isolated exosomes were subjected to 1 h of the incubation with Con A lectin (50 μg/mL) and then were fixed for 15 min in a glutaraldehyde solution (2.5%). Later on, the resultant exosomes were dried using gradient ethanol solutions and analyzed using a scanning electron microscope.
**Cell culture and transfection**

BMSMCs, DPC12 cells, DPSCs, hESC, HNESC and ADSCs were cultured to collect their conditional media, which were then used to culture SH-SY5Y and U251 cells. In addition, exosomes isolated from these conditional media were used to treat SH-SY5Y and U251 cells before the effect of exosomes was studied. At the same time, parallel groups were prepared using the same way as above and were treated with H2O2. Finally, SH-SY5Y and U251 cells were transfected with miR-29b mimics, PTEN siRNA or caspase-3 siRNA using Lipofectamine 2000 to study the effect of these compounds on PTEN expression.

**Vector construction, site-directed mutagenesis and luciferase assay**

The identification of the miR-29b-binding sites on the mRNA of PTEN and caspase-3 was performed using a bioinformatic tool. Later on, the regions of the mRNA of PTEN and caspase-3 containing the miR-29b target sites were separately cloned and inserted into Promega pcDNA vectors (Madison, WI). Meanwhile, site-specific mutagenesis was done in the miR-29b target sites of the PTEN and caspase-3 fragments for producing mutations, and the mutant sequences were also later cloned and inserted into Promega pcDNA vectors as mutant plasmids. U251 and SH-SY5Y cells were then co-transfected with miR-29b mimics or a scramble control in conjunction with mutant/wild type PTEN or caspase-3 mRNA using Invitrogen Lipofectamine 2000 (Carlsbad, CA, USA). Later on, these cells were collected after the transfection for 48 h and the detection of the activity of luciferase in the cells were done through a Promega Dual Luciferase Reporter Assay (Madison, WI, USA).

**Western blot analysis**

Cell collection was performed, and total proteins of the collected cells were extracted through the lysis of these cells and centrifugation. The concentration of the total proteins was determined using a Thermo Fisher Scientific BCA kit (Waltham, MA, USA) and then the proteins were subjected to 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The resultant proteins were then subjected to protein transfer using a polyvinylidene fluoride membrane at 25°C-phosphate dehydrogenase (GAPDH, internal control), anti-caspase-3 and anti-PTEN Abcam monoclonal primary antibodies (Cambridge, MA, USA) before being incubated at 25°C for 1 h with Abcam secondary antibodies label with HRP (Cambridge, MA, USA). The membrane was washed thrice using PBS for removing the superfluous primary antibodies and developed using an enhanced chemiluminescence (ECL) reagent. The Western blot protein bands were checked using an X-ray system. The GAPDH band was used for reference for the calculation of the relative level of PTEN and caspase-3 expression using the software Image J.

**TUNEL assay**

The status of the apoptosis of cells was identified through a TUNEL assay using a Beyotime Biotechnology TUNEL kit (Shanghai, China) in accordance with the instructions of the manufacturer.

**Immunohistochemistry**

A streptavidin-peroxidase method with three steps was used to perform histochemical immunostaining. Later on, 4% formalin was used to fix all the pathological specimens and then paraffin was used to embed the specimens. Subsequently, 2-μm-thick specimen slices were obtained through continuous slicing operation. Antigens of the tissue slices were recovered through boiling using a microwave. The resultant slices were then immersed in an H2O2 solution (3%) for 10 min for blocking endogenous oxidase. Later on, sera were used to remove antigen impurities on the slices. Then, the slices were subjected to incubation for 30 min at 25°C and washed thrice using PBS. The Abcam anti-PTEN and anti-caspase-3 primary antibodies (Cambridge, MA, USA) were hence added to the slices, which were then subjected to overnight incubation at 4°C. After being washed using PBS, the slices were subjected to 10 min incubation with the secondary antibody and hence this reaction was stopped through adding a peroxidase solution with an antiantibiotin label. The slices were stained with DAB. Haematoxylin was used to counter stain the resultant slices. Then the slices were dehydrated using anhydrous ethanol before being mounted using neutral gum. Later on, the nuclei in the slices were checked using a 400 microscope. Based on the staining intensity of the slices and the cell quantities, further determination was performed. Non-stained cells were judged as negative. Using light blue as the background colour, cells in brown were assessed as positive. In total, five view fields that did not overlap each other were selected for each slice for counting the positive cells.

**Nissl’s staining**

Collected tissues of spinal cords were used to prepare 10-μm-thick sections, which were then subjected to the Nissl staining for one day. A Sigma-Aldrich cresyl violet solution (1%) (St. Louis, MO) was used as the stain and 10 view fields were selected in a random manner for the corresponding slide of each of the tissue types, i.e. ventral horn, motor neurons and grey matter, for counting neurons. The software Image-Pro plus 6.0 was used to analyze positive cells of the Nissl staining.

**The Basso–Beattie–Bresnahan (BBB) score evaluation**

The recovery of the locomotion of rats of all the groups was evaluated based on BBB scores. Briefly, the BBB scores were assessed through tests in open fields, and these tests were performed on day 0, 1, 3, 7, 14, 21 and 28, separately, after the SCI model establishment.
**Statistical analysis**

The software SPSS 21.0 from SPSS (Chicago, IL, USA) was used to process the statistical data. All data were finally represented as mean ± standard deviations. The one-way ANOVA was used for data verification. *p*<0.05 represented a statistically significant level.

**Ethics statement**

This study was approved by our institutional animal ethics committee and follows the tenants of the Guide for the Care and Use of Laboratory Animal by International Committees.

**Results**

**Conditional medium collected from human neuroepithelial stem cells inhibited cell apoptosis**

The conditional medium collected from the H2O2 treated group (group 1), ADSCs (group 2), DPC12 cells (group 3), DP-DSCs (group 4), hESCs (group 5), HNESC (group 6) and BMSMCs (group 7) was utilized to treat SH-SYSY and U251 cells before cell apoptosis was detected using the TUNNEL assay. As shown in Figure 1(A), exosomes were isolated from above seven types of conditional media and examined by an electron microscope. In addition, Western blot analysis was also used to detect the expression of CD69, CD9 and CD83 in the exosomes collected from the above seven types of conditional media. The results showed no obvious difference among groups 2–7 (Figure 1(B)). Meanwhile, the exosomes isolated from the conditional media of six types of stem cells were used to treat SH-SYSY and U251 cells before cell apoptosis was detected using the TUNNEL assay. As shown in Figure 1(A), exosomes were isolated from above seven types of conditional media and examined by an electron microscope. In addition, Western blot analysis was also used to detect the expression of CD69, CD9 and CD83 in the exosomes collected from the above seven types of conditional media. The results showed no obvious difference among groups 2–7 (Figure 1(B)). Meanwhile, the exosomes isolated from the conditional media of six types of stem cells were used to treat SH-SYSY and U251 cells. The results showed that the exosome isolated from groups 2 to 7 inhibited the apoptosis of SH-SYSY (Figure 1(C)) and U251 (Figure 1(D)) cells compared with those isolated from group 1, while the inhibitory effect of the exosome isolated from group 6 was the strongest.

**Expression of miRNAs in the exosomes isolated from the conditional medium of stem cells**

SH-SYSY (Figure 2) and U251 (Figure 3) cells were transfected with exosomes isolated from the conditional media in groups 1–7, respectively. As shown in Figures 2 and 3, the expression of miR-21 (Figures 2(A) and 3(A)) in group 2, 3, 6 and 7 was much higher than that in group 1, and the level of miR-21 expression was the highest in group 3. In addition, the expression of miR-29b (Figures 2(B) and 3(B)) was high in groups 3–6, and group 6 exhibited the highest level of miR-29b expression. Moreover, the levels of miR-19b (Figures 2(C) and 3(C)) in groups 2, 3 and 6 were much higher than that in group 1, and group 3 exhibited the highest level of miR-19b. Additionally, the levels of miR-125b (Figures 2(D) and 3(D)) and miR-30b (Figures 2(F) and 3(F)) in groups 2, 6 and 7 were much higher than those in group 1, respectively. However, other miRNAs, including miR-335 (Figures 2(E) and 3(E)), miR-341 (Figures 2(G) and 3(G)), miR-370 (Figures 2(H) and 3(H)), miR-221 (Figures 2(I) and 3(I)), miR-188 (Figures 2(J) and 3(J)), miR-24-3p (Figures 2(K) and 3(K)) and miR-206 (Figures 2(L) and 3(L)), showed no significant difference expression among groups 1–7. Furthermore, the protein levels of PTEN (Figures 2(M) and 3(M)) and caspase-3 (Figures 2(N) and 3(N)) in groups 2–7 were much lower than those in group 1, while group 6 exhibited the lowest levels of PTEN and caspase-3 expression.

**MiR-29b directly targeted PTEN and caspase-3**

Online miRNA target prediction tools were used to explore the target genes of miR-29b. Consequently, PTEN and caspase-3 were identified as the direct target genes of miR-29b with the “seed sequence” located in the 3’UTR of PTEN (Figures 4(A) and 5(A)) and caspase-3 (Figures 4(C) and 5(C)). In the subsequent luciferase assay, the luciferase activity of wild-type PTEN 3’UTR but not that of mutant PTEN 3’UTR showed a stepwise decline in SH-SYSY (Figure 4(B)) and U251
Figure 5(B) cells treated with an increasing concentration of miR-19b mimics. The transfection of SH-SY5Y (Figure 4(D)) and U251 (Figure 5(D)) cells with miR-19b reduced the luciferase activity of wild-type 3’UTR of caspase-3 but not that of mutant 3’UTR of caspase-3 in a dose-dependent manner. Moreover, both miR-29b mimics and PTEN siRNA
Figure 3. The targets of exosomal miRNAs isolated from the conditional medium were identified in U251 cells (\(*p < 0.05\) vs. group 1). (A) The miR-21 levels in groups 2, 3, 6 and 7 were much higher than that in group 1, and the miR-21 level in group 3 was the highest. (B) MiR-29b was highly expressed in groups 3–6 compared with that in group 1, and the miR-29b level in group 6 was the highest. (C) MiR-19b was highly expressed in groups 2, 3 and 6, and the miR-19b level in group 3 was the highest. (D) MiR-125b was highly expressed in groups 2, 6 and 7, and the miR-125b level in group 6 was the highest. (E) MiR-335 level was comparable among groups 1–7. (F) MiR-30b was highly expressed in groups 2, 6 and 7, and the miR-30b level in group 4 was the highest. (G) MiR-341 level was comparable among groups 1–7. (H) MiR-370 level was similar among groups 1–7. (I) MiR-221 level showed no obvious difference among groups 1–7. (J) MiR-206 level was comparable among groups 1–7. (K) MiR-24-3p level was comparable among groups 1–7. (L) MiR-206 level was comparable among groups 1–7. (M) PTEN protein level in groups 2–7 was much lower than that in group 1, while the PTEN protein level in group 6 was the strongest. (N) Caspase-3 protein level in groups 2–7 was much lower than that in group 1, while the caspase-3 protein level in group 6 was the strongest.
inhibited the mRNA and protein expression of PTEN in SH-SY5Y (Figure 4(E)) and U251 (Figure 5(E)) cells, while caspase-3 siRNA exerted no effect on the expression of PTEN. Additionally, miR-29b mimics and caspase-3 siRNA inhibited the mRNA and protein expression of caspase-3 in SH-SY5Y (Figure 4(F)) and U251 (Figure 5(F)) cells, while PTEN siRNA exerted no effect on the expression of caspase-3.

**Exosomes collected from neuroepithelial stem cells exhibited in vivo therapeutic effects**

An animal model of SCI was established to evaluate the therapeutic effects of above exosomes in the treatment of SCI. In this study, the experimental rats were randomly divided into a sham group, an SCI group, a group of SCI rats treated with exosomes collected from neuroepithelial stem cells (HNESCs), and a group of SCI rats treated with exosomes collected from HNESC transfected with miR-29b mimics. According to the BBB scores of up to 28 days after model establishment, the rats in the SCI group (Figure 6(A)) showed evidently lower scores compared with those in the sham group, while the injection of exosomes collected from HNESC or HNESC transfected with miR-29b mimics increased the BBB score of SCI rats. In particular, the therapeutic effect of exosomes collected from HNESC transfected with miR-29b mimics was the strongest. The Nissl staining
Figure 6(B) assay also indicated that the exosomes collected from HNECs transfected with miR-29b mimics could alleviate neuron cell apoptosis in SCI rats. Moreover, the IHC assay of PTEN (Figure 7) and caspase-3 (Figure 8) expression showed that the PTEN and caspase-3 expression reached their peak levels in the SCI group, while the injection of exosomes collected from HNECs transfected with miR-29b mimics completely restored the levels of PTEN and caspase-3 expression to those in the sham group.

Finally, real-time PCR and Western-blot analysis were used to compare the mRNA and protein levels of PTEN and caspase-3 among the above four groups. As shown in Figure 9, the expression of PTEN mRNA (Figure 9(A)), PTEN mRNA protein (Figure 9(B)), caspase-3 mRNA (Figure 9(C)) and caspase-3 protein (Figure 9(D)) reached the highest level in the SCI group, while the injection of exosomes collected from HNECs transfected with miR-29b mimics completely restored the levels of PTEN and caspase-3 expression to those in the sham group.

Discussion

According to their definition, stem cells are specific cells capable of self-renewal and the differentiation into all types of cells [29]. However, neuroepithelial stem cells can only differentiate into astrocytes, oligodendrocytes and neurons [30].
Several studies have confirmed that neuroepithelial stem cells show a close association with glioblastoma cells in terms of their origin [31]. According to previous studies, the level of nestin expression in gliomas correlated with their potential to become malignant [32]. Previous studies have emphasized that trophic and mechanical support is important to ensure neurite extension and the survival of grafted cells [33]. It was also observed by the author that SC-NES cells improved the deficiency in locomotor activity and that the SCI lesions severity affected locomotor activity improvement [33–37]. In this study, we isolated exosomes from the conditional media collected from groups 1 to 7 and then treat culture cells with these conditional media and exosomes. Among all treatment groups, the exosomes collected from HNESC exhibited the strongest inhibitory effect against the cell apoptosis induced by the administration of H$_2$O$_2$.

Many types of cells can secrete exosomes, such as tumour cells, epithelial cells, mast cells, T cells, B cells, dendritic cells and reticulocytes. Exosomes are membrane vesicles with diameters of only 40–100 nm [38]. After being secreted into the extracellular environment, exosomes are still near the mother cell or probably can enter biological fluids including tumour effusions, amniotic fluid, cerebrospinal fluid, milk, urine and plasma to realize the long-distance exchange of biological information [39]. These types of exosomes are among the extracellular vesicles transporting different compounds between cells [40].

MiRNAs are small RNAs that are able to modulate the translation levels of various RNAs and can regulate various cellular functions [41]. Many miRNAs have been found in neurons and are crucial for the nervous system to develop normally [42–44]. In vivo researches have confirmed that miR-29b affected neuronal apoptosis in vitro. In the spinal cord lesion, the injection with miR-29b inhibitors also rescued neurons from death and the co-injection with both miR-29b and miR-29b inhibitors fully abolished the apoptosis induced by SCI. In summary, changes in miR-29b expression probably cooperatively facilitate the death of neurons in SCI by decreasing the expression of myeloid cell leukaemia sequence-1 (Mcl-1) against apoptosis and increasing the expression of BH3 proteins beneficial to apoptosis. In this study, we compared the expression of 12 miRNAs, PTEN and caspase-3 among the conditional medium of stem cells to find the miRNAs targets of exosomes isolated from the conditional medium. The results showed that the group treated by the medium of HNESC exhibited the highest level of miR-29b expression but the lowest levels of PTEN and caspase-3.

Previous researches showed that neonatal mice genetically lacking PTEN had axons with an increased level of post-SCI renewal ability. Zukor et al. recently found that neonatal mice (P0/P1) whose PTEN was silenced by anti-PTEN shRNA (AAV-shPTEN-GFP) could also achieve post-SCI regeneration of CST axons [45]. Ohtake et al. also confirmed that inactivating PTEN using the treatment with PTEN antagonist peptide (PAP)
enhanced serotonergic-fibre densities of caudal spinal cords and increased the sprouting of axons in the lesion [46]. According to these results and for adult mice, the deletion of PTEN can increase the regeneration of axons in the CST and enhance the recovery of motor functions of forelimbs. The gene of PTEN is strongly modulated by many factors, such as methylation and miRNAs [47]. MiR29b was reported to mediate the control of the methylation of PTEN, and such control was related to the influence of the knockdown of HOTAIR. It was indicated that miR-29b targets HOTAIR and the lacking for the miR-29b target site of HOTAIR inhibited the effect of miR-29b, indicating that HOTAIR facilitates the downregulation of the expression of PTEN.

Figure 8. Caspase-3 protein level reached its highest in the SCI group, the exosomes isolated from HNESCs treated with miR-29b mimics completely restored caspase-3 protein expression to the level seen in the sham group.

Figure 9. Exosomes isolated from HNESCs and HNESCs treated with miR-29b mimics altered PTEN and caspase-3 expression (*p value<.05 vs. sham group; **p value<.05 vs. SCI group). (A) The mRNA level of PTEN reached the highest level in the SCI group, while the injection of exosomes collected from HNESCs transfected with miR-29b mimics completely restored the levels of PTEN and caspase-3 expression to those in the sham group. (B) Western blots of PTEN protein among the four rats groups. (C) The mRNA level of caspase-3 reached the highest level in the SCI group, while the injection of exosomes collected from HNESCs transfected with miR-29b mimics completely restored the levels of PTEN and caspase-3 expression to those in the sham group. (D) Western blots of PTEN protein among the four rats groups.
by sponging miR-29b. Moreover, loss of the target HOTAIR led to an enhancement in miR-29b expression, while the overexpression of HOTAIR resulted in the decrease in the expression of miR-29b. Furthermore, miR-29b overexpression enhanced the level of PTEN, which was then increased again by siHOTAIR. In contrast, miR-29b inhibitor repressed the expression of PTEN induced by the knockdown of HOTAIR. These results confirmed that there is a HOTAIR/miR-29b/PTEN signalling network.

The caspase family of cysteine proteases modulates the implementation of the death programme for apoptotic cells in mammals [48]. Caspase-3 results in the cleavage of a number of crucial downstream substrates related to apoptotic-phenotype expression in vitro [49]. A potential mechanism for miR-29b to affect the activity of caspase 3/7 is to regulate the expression of apoptotic regulators, such as Mcl-1 and Bcl-2, which are known targets of the miR-29 family. Suppression of the function of miR-29b using an inhibitor resulted in a significant decrease in the activity of caspase 3/7 in comparison to BMSC that was transfected using an NT-miR. In the present study, it was indicated that both caspase-3 and caspase-1 were activated in the established SCI mouse model, and the application of zVAD-fmk significantly inhibited the activation and lowered the degree of apoptosis induced by SCI. The present study first indicated that apoptosis mediated by caspase played a significant role in post-SCI neurological dysfunction and tissue damage.

In this study, we found that the results of BBB scoring and the Nissl staining in different groups of rats validated the therapeutic effects of exosomes collected from HNESCs and HNESCs treated by miR-29b mimics. In addition, the exosomes collected from HNESCs treated by miR-29b mimics completely restored the levels of PTEN and caspase-3 to those in the sham group.

Conclusions
In summary, the findings of this study demonstrated that stem-like cells of various origins, including BMSMCs, DPC12 cells, DPDSs, hESCs, HNESCs and ADSCs, exerted a therapeutic effect to improve the recovery of SCI. In this study, we harvested the conditional media and exosomes from the above cells to treat culture cells and to study their effects on H2O2 induced apoptosis. Furthermore, we treated SCI rats with exosomes fortified with or without miR-29b to investigate the effect of miR-29b on post-SCI recovery and the expression of PTEN and CASPASE3.

Disclosure statement
No potential conflict of interest has been reported by the author(s).

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Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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