Biochemical and Biophysical Research Communications xxx (2016) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Hypoxia pretreatment and EPO-modification enhance the protective effects of MSC on neuron-like PC12 cells in a similar way

Jinli Feng*, Wei Wang*

Department of Neurology, The 309th Hospital of PLA, NO17, Heishanhu Road, Haidian District, Beijing, China

A R T I C L E I N F O

Article history:
Received 2 November 2016
Accepted 8 November 2016
Available online xxx

Keywords:
Mesenchymal stem cells (MSC)
PC12
Erythropoietin (EPO)
Hypoxia injuries

A B S T R A C T

Mesenchymal stem cells (MSC) based cell transplantation therapy is proved to be an attractive strategy with great potential for improvement of hypoxia induced neural damage. In the present study, MSCs were co-culture with PC12 to investigate its protective effects against hypoxia pretreatment, and the Lactate dehydrogenase (LDH) release assay, MTT and Anexin V staining were performed to analysis the cellular damage or apoptotic. RT-PCR and Western blotting were further used to investigate the underlying mechanism. The results indicate that hypoxia treatment results in the decrease of PC12 cell viability, yet co-culture with MSC could protect the PC12 from hypoxia induced damage. Hypoxia pre-activated or EPO transduced MSC with up-regulated erythropoietin (EPO) expression could further enhance MSC's protective effect against hypoxia induced cell damage, which was associated with high level of anti-apoptotic p-Akt and ration Bcl-2/Bax, and decreased Caspase 3 in PC12. Taken together, these data suggests high levels of MSC-mediated cyto-protection is closely tied to high gene expression levels of EPO. The up-regulation of EPO for enhanced MSC-mediated cyto-protection may has great potential for the MSC cellular therapy of neural or neuronal injuries induced by hypoxia.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

It has been proved that hypoxia induced neural tissues or neuronal cells injuries are closely related to many neural diseases, and the effective treatment is an unmet need [1–3]. Many strategies were employed to protect the neural or neuronal injuries from hypoxia. Especially, the mesenchymal stem cells (MSC) based cell transplantation therapy method was suggested to be an attractive strategy with great potential [4–7], and thus draw more and more attentions. Studies indicated that transplantation of MSC could promote neuronal regeneration and improve injury neural function via both MSCs themselves and the MSCs secreted paracrine or tropic factors [8–10].

Recently, erythropoietin (EPO) has been proved to be a potential hypoxia associated survival cytokine and it may directly protect neuronal cells like PC12 cells from hypoxia induced injury [11–13]. The studies also found that the up-regulation of anti-apoptotic members, such as Bcl-2 and Bcl-XL, as well as down-regulation of pro-apoptotic members, such as Bax and Bak may contribute to the MSCs protective effect of EPO on hypoxia induced neuronal injuries [14,15]. However, the exact protect mechanisms underlying MSC transplantation are still confusion and needs further elucidation.

To explore the potential signaling mechanism involved in EPO-expression protective effects as well as MSCs-mediated cyto-protection, the present study investigated the protective effects of EPO up-regulated MSC on PC12 by using a trans-well co-culture system and explored the underlying mechanisms as well.

2. Materials and methods

2.1. In vitro isolation and culture of MSC

MSCs were isolated from the bone marrow of adolescent, male Sprague-Dawley rats. Sprague-Dawley rat (150–180 g) were purchased from Beijing Weitong Lihua Experimental Animal Technology, Beijing, China. Primary MSCs were isolated from the tibias and femurs bone marrow of SD as previously described [16]. The isolated cells were cultured in Dulbecco's Modified Eagle Medium with low glucose (L-DMEM), supplementing with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Gibco). Passages at 3–4 were used in this study.

* Corresponding authors.
E-mail addresses: fengjinli886@tom.com (J. Feng), 13910581521@163.com (W. Wang).

http://dx.doi.org/10.1016/j.bbrc.2016.11.046
0006-291X/© 2016 Elsevier Inc. All rights reserved.

Please cite this article in press as: J. Feng, W. Wang, Hypoxia pretreatment and EPO-modification enhance the protective effects of MSC on neuron-like PC12 cells in a similar way, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.11.046

ARTICLE IN PRESS

2.2. Hypoxia mimetic or hypoxia induction of co-culture cells

The well-known hypoxia mimetic agent Cobalt chloride ($CoCl_2$, 0.6 mM), which has been widely used for CNS injury models construction *in vitro*, is used to induce hypoxia condition in the present study. MSCs and PC12 were co-culture in 6-well tissue culture plates with trans-well. The cellular numbers is 4×10^4 cells/cm^2 for PC12 cells and 2×10^4 cells/cm^2 for MSCs.

2.3. Cellular damage and apoptotic analysis

The Lactate dehydrogenase (LDH) release assay was used to measure the cellular damage caused by hypoxia condition according to the manufacturer's protocol. Shortly, the supernatant of the treated cells from different groups were centrifuged at 10, 000 rpm for 5 min. 120 μL/well of the resulted supernatant were incubated with 60 μL LDH working solution for 30 min under dark condition. The absorption was scanned at a wavelength of 490 nm.

2.4. Anexin V staining for apoptosis

To further analysis the apoptosis, Anexin V staining (Invitrogen) was performed. In short, the treated cells were staining with the Annexin V FITC according to the methods provided by manufacturer. The nuclear were stained with DAPI for 5 min. The samples were observed with an inverted microscope, (OLYMPUS IX70-S8F, Olympus). For each group, 10 random fields were chosen to count the TUNEL positive cells. The apoptosis level were defined as (Annexin V+/Total cells) $\times$ 100%.

2.5. Cell viability assessment

The cell viability was assessed by a 3-(4, 5-dimethylthiazol-2-yl)- 2, 5-diphenyltetrazolium bromide reduction (MTT) assay. In short, the cells were incubated with 5 mg/mL of MTT working solution for 4 h. The resulted formazan crystals were solved by DMSO and the 490 nm OD were measure by a micro-plate reader (Bio-RAD 680, USA).

2.6. SiRNA inference

The MSCs were transfected with Silencer EPO siRNA (siEPO), control negative siRNA (siCTL) and Lipofectamine 2000 for 24 h. After that, the cells were washed with PBS for 3 times before coculturing with PC12 cells.

2.7. EPO transfected

The EPO-GFP gene expressed lentivirus was constructed and brought from Jima company, Shanghai. The puromycin selection was performed to kill the non-transduced cells. In short, the MSCs were culture in 6 well plates at 1×10^5/cells. 24 h later, the cells culture medium were replaced with fresh medium containing lentivirus (MOI = 20) and cultured for 24 h. After washing with PBS for 3 times, the cells were added with fresh culture medium for 2 days and the puromycin were loaded to kill the non-transduced cells.

2.8. Western blotting

Western analysis was performed as described everywhere. Shortly, the cells were treated with RIPA lysis buffer and 10 μg protein was separated by 15% sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Then proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, USA) and blocked with 2% milk. The membrane was then incubated with anti-pAkt (1:1000), anti-Cleaved caspase-3 (1:1000), anti-Bcl-2 (1:1000), anti-Bax (1:1000), and anti-GAPDH (1:8000) antibodies (Cell Signaling Technology) overnight at 4 °C, a horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology) were used. Finally, the membrane was washed and the signals were detected. The expression level of proteins as normalized by quantity one.

2.9. Statistical analysis

SigmaStat Statistical Software (SPSS Inc, Chicago, IL, USA) was used for all statistical analyses. All data are presented as the mean ± SD. one-way analysis of variance (ANOVA) were used to compare the difference between different groups. P-value < 0.05 was considered statistically significant.

3. Results

3.1. The time-dependent apoptosis of PC12 induced by hypoxia

In order to confirm the protective effect of MSC on neural cells, the MSC and PC12 were co-cultured by Tans-well method and the cell viability at different time points were measured. As shown in Fig. 1, the cell viability of PC12 was significantly decreased by hypoxia treatment after 12 h alone. However, after co-culturing with MSCs, the viability was remarkable increased as compared with the PC12 alone after 12 h, 24 h and 48 h of incubation. Further measurement of EPO protein levels MSC in indicated that the EPO increased at a time-dependent way and the level reach the peak value after 24 h (Fig. 1).

3.2. EPO gene silencing decrease the cyto-protection of MSC on PC12 under hypoxia

In order to further investigate the role of EPO in the protective of MSC on PC12, the SiRNA experiment was performed to interfere the EPO expression of MSC. The cellular viability results indicated that the silence of EPO could significantly decrease the protective effect of MSC on PC12. The results confirmed the important role of EPO on the protective effect of MSC on neural cells (Fig. 1C).

3.3. The delay protective effects of MSC co-culturing on PC12

The above results suggested that the up-regulation of EPO were delayed during the protection of MSC under hypoxia condition and resulted in the delay protection of PC12, which might lead to the irreversible damage or even death of PC12. To evaluate the effect of delayed MSC treatment on PC12, the different hypoxia-post time points were selected to co-culture with MSC. As shown in Fig. 2, the co-culturing of MSC before hypoxia treatment with PC12 could resulted in the highest level of protective effect, yet the co-culture of MSC with PC12 after hypoxia could resulted significantly reduced cell viability ($p < 0.01$). The results suggested the hypoxia could result in irreversible damage of PC12, and the earlier co-culture resulted in the better protective effect of MSC.

3.4. The protective effects of hypoxia pre-activated MSC on PC12

Based on the results of aforementioned, we hypothesized that hypoxia could activated the up-regulation of EPO gene expression of MSC, which resulted in the protective effects on PC12. As shown in Fig. 3A & B, as compared with normal MSC, hypoxia pre-activated MSC could significantly increase the cell viability of PC12 ($p < 0.01$) and resulted in remarkable decreased LDH release of PC12, which

Please cite this article in press as: J. Feng, W. Wang, Hypoxia pretreatment and EPO-modification enhance the protective effects of MSC on neuron-like PC12 cells in a similar way, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.11.046

Download English Version:

https://daneshyari.com/en/article/5505806

Download Persian Version:

https://daneshyari.com/article/5505806

Daneshyari.com