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Hypoxia promotes bone marrow-derived mesenchymal stem cell proliferation through apelin/APJ/autophagy pathway

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Abstract

Bone marrow-derived mesenchymal stem cells (BMSCs) are a population of multipotent progenitors that have the capacity of proliferation and differentiation into mesenchymal lineage cells. The regulatory peptide apelin is the endogenous ligand for the G protein-coupled receptor APJ. Apelin, which can enhance BMSC proliferation, has mitogenic effects on a wide variety of cell types. We hypothesized that the increased apelin/APJ might be involved in the occurrence and development of hypoxia-induced BMSC proliferation. BMSCs from the bone marrow of 8- to 10-week-old C57BL/6J mice were cultured under either normoxia (21% oxygen) or hypoxia (1% oxygen) condition. Cell proliferation was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and 5-bromo-2′-deoxyuridine assay. Expressions of hypoxia-inducible factor (HIF)-1α, apelin, APJ, Beclin-1, and LC3II/LC3I were detected by western blot analysis. Results suggested that hypoxia enhanced the proliferation of BMSC in a time-dependent manner. The expressions of HIF-1α, apelin, APJ, Beclin-1, and LC3II/LC3I were increased in BMSCs induced by hypoxia. Small interfering RNA (siRNA)-HIF-1α that inhibited the hypoxia-induced expressions of apelin, APJ, Beclin-1, and LC3II/LC3I prevented hypoxia-induced BMSC proliferation. siRNA-APJ that inhibited the hypoxia-induced expressions of Beclin-1 and LC3II/LC3I reversed hypoxia-induced BMSC proliferation. siRNA-Beclin-1 also abolished hypoxia-induced cell proliferation. These data suggested that the apelin/APJ/autophagy signaling pathway might be involved in hypoxia-induced BMSC proliferation.

Key words: hypoxia, apelin, mesenchymal stem cell, autophagy, proliferation

Introduction

Apelin was initially isolated from bovine stomach and is an endogenous neuropeptide. It is a native ligand of the seven transmembrane G protein-coupled receptor (APJ) [1]. Apelin is a biologically active peptide which has several isoforms that were agonists for receptor APJ. It has been revealed that the C-terminal of the 77 amino-acid residues apelin preproprotein is cleaved to form shorter bioactive isoforms, including apelin-36, apelin-17, apelin-13, apelin-12, and so on [2].

Apelin and APJ were found in the central nervous system and in different peripheral tissues such as heart, lung, brain, spinal cord, adipose tissue, and gastrointestinal tract. Apelin/APJ system is involved in a wide range of physiological and pathological functions, including

dilatation of arteries, the systolic effect, regulation of fluid homeostasis, the adipoinsular axis, cell proliferation, and angiogenesis [3–5].

Functionally, apelin can stimulate cell proliferation [6–8]. Apelin can promote thymidine incorporation into the DNA in Chinese hamster ovary (CHO) cells. Apelin was also reported to promote gastric cell proliferation and the growth of human inotropic osteoblasts [9]. Our previous study has demonstrated that apelin significantly enhances bone marrow-derived mesenchymal stem cell (BMSC) proliferation via AKT signaling pathways [10]. BMSCs are multipotential cells that can be used in tissue engineering and regenerative medicine. Apelin/APJ, whose expression is inducible by hypoxic, appears to play a critical role in hypoxia-related proliferation. Apelin expression is increased in endothelial cells and vascular smooth muscle cells (VSMCs) during hypoxia phase *in vivo* and *in vitro* [11]. In rat myocardium and cardiomyocytes, the levels of apelin dramatically were increased in hypoxia condition. However, the relationship between the physiological response of the apelin/APJ system and hypoxia-induced BMSC proliferation is largely unknown.

Therefore, in the present study, we aimed to observe the effects of apelin/APJ on hypoxia-induced BMSC proliferation. This study showed that hypoxia stimulates the proliferation of BMSCs and promotes the expressions of hypoxia-inducible factor (HIF)-1α, apelin, APJ, Beclin-1, and LC3II/LC3I. The small interfering RNA (siRNA)-HIF-1α, siRNA-APJ, and siRNA-Beclin-1 can prevent hypoxia-induced BMSC proliferation. Here, we provided evidence that hypoxia promotes BMSC proliferation partly through the apelin/APJ/autophagy signaling pathways.

Materials and Methods

Reagents

The synthetic apelin-13 peptide (pGlu–Arg–Pro–Arg–Leu–Ser–His–Lys–Gly–Pro–Met–Pro–Phe) was purchased from Phoenix Biotech (Phoenix, USA) and rehydrated as a stock solution in phosphate-buffered saline (PBS) before use. Bicinchoninic acid protein assay kit was purchased from Hyclone (Logan, USA). Polyvinylidene difluoride (PVDF) membranes were purchased from Millipore Biosciences (Billerica, USA). Antibodies against HIF-1α (sc-10790), apelin (sc-33469), APJ (sc-33823), Beclin-1 (sc-11427), LC3I/LC3II (L7543), and α-Tubulin (sc-5546) were all purchased from Santa Cruz Biotechnology (Santa Cruz, USA). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG was purchased from Boster Biological Technology (Wuhan, China).

BMSC isolation and culture

BMSCs were obtained from the bone marrow of C57BL/6J mice aged 8–10 weeks. Under sterile conditions, the femur and tibia of mice were excised. Special attention was paid to remove all connective tissue attached to bones. Bone marrow plugs were extracted from the bones by flushing the bone marrow cavity with complete culture medium. The marrow plug suspension was dispersed by passing through pipettes of decreasing sizes. After a homogeneous cell suspension was achieved, the cells were centrifuged, resuspended in complete culture medium, plated, and incubated at 37°C humidified atmosphere with 5% CO_2 for 4 days before the first medium change. The mesenchymal population was isolated on the basis of its ability to adhere to the culture plate. At 90% confluence, the cells were trypsinized (0.25% trypsin–EDTA; Sigma, St Louis, USA) and passaged at 1:3 ratio. First-passage BMSCs were used in all experiments. The cells were maintained at normoxic conditions (21% O_2) as the control, and cultured under hypoxic conditions (1% O_2, 5% CO_2, and 94% N_2) as hypoxic experiments.

siRNA and transfection

In the 6-well tissue culture plates (Corning, New York, USA), 2×10^5 cells were seeded per well. siRNA-HIF-1α (sc-35562), siRNA-APJ (sc-44733), siRNA-Beclin-1 (sc-29798), or non-targeting siRNA control (sc-37007) was transfected into cells using the Tran Messenger Transfection Reagent (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and 5-bromo-2′-deoxyuridine assays

The proliferation of BMSCs was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were plated in 96-well plates, synchronized by incubation in Dulbecco's modified Eagle's media containing 0.5% fetal bovine serum (FBS) for 24 h. Then, medium was replaced by growth medium (containing 10% FBS) for proliferation experiments. Following the designated incubation time interval, 20 μl of MTT was added to each well of the 96-well plates and incubated for 4 h. After removing the medium, 150 μl of dimethyl sulfoxide was added and incubated for 15 min at 37°C. Finally, optical density (OD) values were measured at 570 nm. The proliferation of VSMCs was also determined by using 5-bromo-2′-deoxyuridine (BrdU) cell proliferation assay kit (Cell Signaling, Beverly, USA). After the designated incubation time interval, 10 μM BrdU was added to the plate and cells were incubated for 4 h. Finally, OD values were read at 450 nm.

Western blot analysis

Immunoblotting was performed using mouse BMSCs. Cells were washed twice with ice-cold PBS and lysed in HEPES buffer for 10 min on ice. After clarification of the cell lysates by centrifugation at 13,000 *g* for 15 min, the supernatants were collected. Aliquots containing 25 μg of protein were electrophoresed in 10% sodium dodecyl sulfate-polyacrylamide gels and transferred onto PVDF membranes. The membranes were blocked with Tris-buffered saline containing 5% milk and 0.1% Tween-20 for 1.5 h at room temperature. The membranes were incubated with the primary antibody diluted in blocking solution overnight, and then incubated with HRP-conjugated anti-IgG. Finally, membranes were detected with ECL kit (Santa Cruz) and densitometric analysis was carried out using image acquisition and analysis software.

Statistical analysis

All data are presented as the mean ± SEM of the indicated number of measurements. The one-way ANOVA test was used to determine the significance. A P-value of <0.05 was considered statistically significant.

Results

Hypoxia enhanced BMSC proliferation in a time-dependent manner

To examine the effects of hypoxia on BMSC proliferation, cells were incubated in the presence of normoxic (21% O_2) or hypoxic (1% O_2) atmosphere for 6, 12, and 24 h, and cell proliferation was measured by MTT and BrdU assays. MTT results showed that hypoxia promoted BMSC proliferation in a time-dependent manner, when compared with normoxic cells (**Fig. 1A**). Results of the BrdU assay also showed

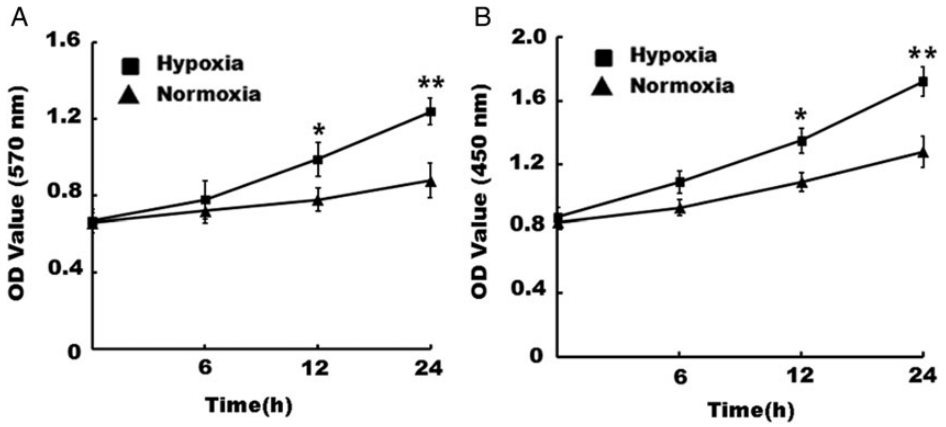

Figure 1. Hypoxia induced the proliferation of BMSCs in a time-dependent manner BMSC were cultured under normoxic (21% O_2) or hypoxic (1% O_2) atmosphere for 6, 12, and 24 h. MTT (A) and BrdU (B) results indicated that hypoxia promoted BMSC proliferation in a time-dependent manner compared with normoxic group. Data were presented as the mean ± SEM ($n = 6$). *$P < 0.05$ and **$P < 0.01$ vs. control.

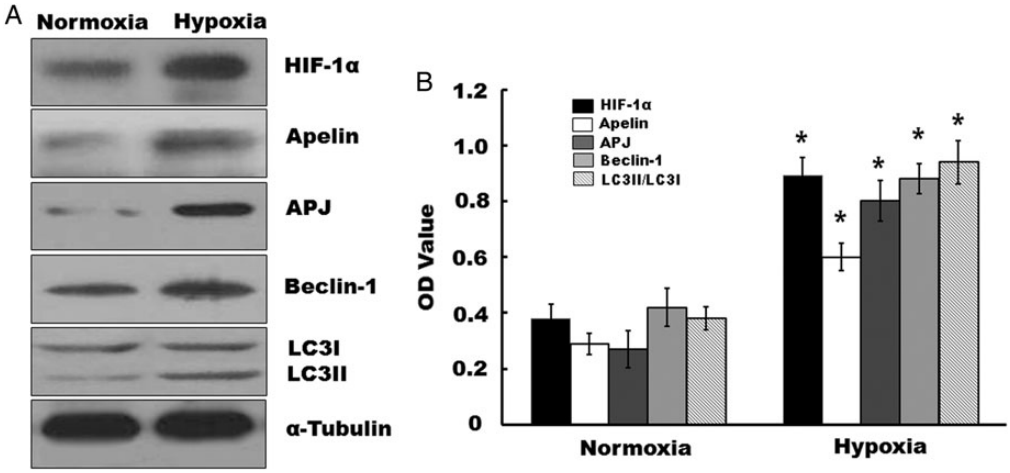

Figure 2. The expressions of HIF-1α, apelin, APJ, Beclin-1, and LC3II/LC3I were increased in BMSCs induced by hypoxia BMSCs were exposed to hypoxia for 24 h. Western blot results indicated that hypoxia stimulated the expression levels of HIF-1α, apelin, APJ, Beclin-1 (A), and LC3II/LC3I (B) compared with normoxic group. Data were presented as the mean ± SEM ($n = 4$). *$P < 0.05$ vs. control.

that hypoxia-induced BMSC proliferation in a time-dependent manner (**Fig. 1B**).

Hypoxia stimulated the expressions of HIF-1α, apelin, APJ, Beclin-1, and LC3II/LC3I in BMSCs

Western blot analysis of whole cell extracts was used to measure the expressions of HIF-1α, apelin, APJ, Beclin-1, and LC3II/LC3I in response to hypoxia. Results showed that the expression levels of HIF-1α, apelin, APJ, Beclin-1, and LC3II/LC3I were significantly higher in BMSCs exposed to hypoxic conditions for 24 h compared with those in BMSCs exposed to normoxia (**Fig. 2**).

siRNA-HIF-1α inhibited hypoxia-induced expressions of apelin, APJ, Beclin-1, and LC3II/LC3I

To determine the role of HIF-1α in hypoxia-induced apelin/APJ signaling in autophagy, BMSCs were pretreated with siRNA-HIF-1α for 24 h before incubation in the presence of normoxia or hypoxia for 24 h. Western blot results showed that hypoxia stimulated the expression of HIF-1α, and siRNA-HIF-1α inhibited the expression of HIF-1α under both normoxia and hypoxia conditions (**Fig. 3A,B**). Hypoxia also increased the expressions of spelin, APJ, Beclin-1, and LC3II/LC3I. siRNA-HIF-1α inhibited the expression of apelin, APJ, Beclin-1, and LC3II/LC3I induced by hypoxia (**Fig. 3A,C–F**).

Knock-down of APJ decreased the expressions of Beclin-1 and LC3II/LC3I induced by hypoxia in BMSCs

We further studied the effects of apelin/APJ on autophagy. BMSCs were pretreated with siRNA-APJ for 24 h, and then cultured under normoxia or hypoxia for 24 h. Western blot analysis showed that hypoxia induced the expressions of HIF-1α and apelin, but siRNA-APJ had no impact on the expressions of HIF-1α and apelin (**Fig. 4A–C**). siRNA-APJ specifically inhibited the expression of APJ under both normoxia and hypoxia conditions (**Fig. 4A,D**). Hypoxia also promoted the expressions of Beclin-1 and LC3II/LC3I, and siRNA-APJ decreased the expressions of Beclin-1 and LC3II/LC3I induced by hypoxia (**Fig. 4A,E,F**).

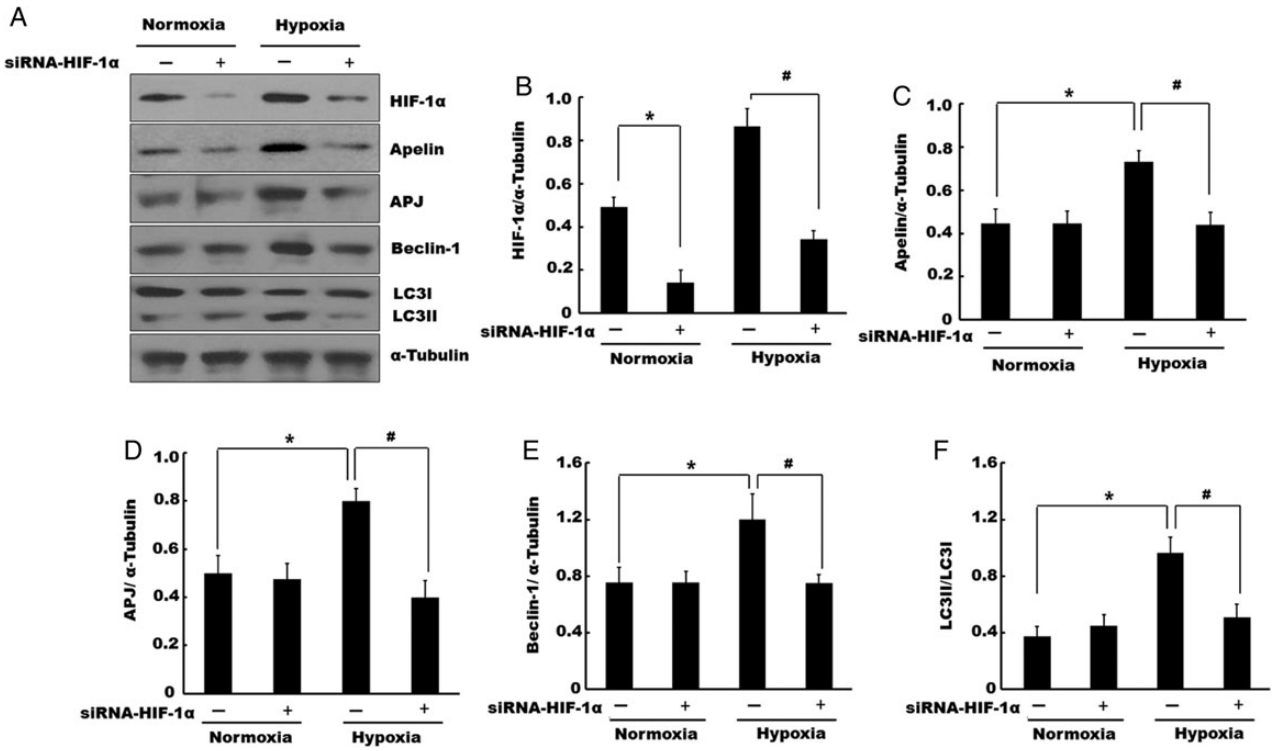

Figure 3. siRNA-HIF-1α reduced the hypoxia-induced expressions of apelin, APJ, Beclin-1, and LC3II/LC3I BMSCs were pretreated with siRNA-HIF-1α for 24 h then cultured in the presence of normoxia or hypoxia for 24 h. Western blot results indicated hypoxia stimulated the expressions of HIF-1α, apelin, APJ, Beclin-1, and LC3II/LC3I. SiRNA-HIF-1α inhibited the expression of HIF-1α under both normoxia and hypoxia conditions (A,B) and reduced the expressions of apelin, APJ, Beclin-1, and LC3II/LC3I induced by hypoxia (A,C–F). Data are presented as the mean ± SEM ($n = 4$). *$P < 0.05$ vs. control under normoxia condition and #$P < 0.05$ vs. control under hypoxia condition.

Knock-down of HIF-1α, APJ, and Beclin-1 inhibited hypoxia-induced cell proliferation

We silenced the expressions of HIF-1α, APJ, and Beclin-1 by transfecting small interfering RNA, and analyzed their effects on proliferation by using MTT and BrdU assays. siRNA-HIF-1α, siRNA-APJ, or siRNA-Beclin-1 was transfected into BMSCs for 24 h; then, the successfully transfected cells were incubated in the presence of normoxic or hypoxic atmosphere for 24 h. MTT results indicated that hypoxia promoted BMSC proliferation, but siRNA-HIF-1α, siRNA-APJ, and siRNA-Beclin-1 inhibited BMSC proliferation induced by hypoxia (**Fig. 5A**). BrdU results also showed that siRNA-HIF-1α, siRNA-APJ, and siRNA-Beclin-1 abolished hypoxia-induced BMSC proliferation (**Fig. 5B**).

Discussion

Self-renewal and multipotency are the key hallmarks of stem cells, permitting them to act as the fundamental units maintaining growth, homeostasis, and repair of many tissues. Among the different types of stem cells, MSCs are considered as a potential tool to treat degenerative diseases [12]. An increased proliferation rate is necessary for more efficient use of stem cells in regenerative therapies. Hypoxia is a strong stimulator of proliferation in pathological conditions such as malignant growth, atherosclerosis, and diabetic retinopathy by inducing expression of several angiogenic growth factors [13]. However, Fehrer *et al.* [14] demonstrated that bone marrow-derived MSCs cultured in 3% O_2 concentration showed significantly increased *in vitro* proliferative life span compared with cells cultured in the ambient O_2 environment. We also tested the effects of hypoxia on BMSC proliferation and got similar results. BMSCs were incubated in the presence of normoxic (21% O_2) or hypoxic (1% O_2) atmosphere for 6, 12, and 24 h. MTT and BrdU results showed that hypoxia promoted BMSC proliferation in a time-dependent manner compared with normoxic cells.

Under hypoxic conditions, these functions are usually regulated by several transcription factors such as HIFs, prolyl-hydroxylases, factor-inhibiting HIF-1, activator protein1, nuclear factor-κB, p53, and c-Myc [15]. Although interaction among all of the transcription factors is required for cellular response, HIFs (especially HIF-1) are the key regulators of cellular response to hypoxia. The expression of HIF-1α in response to hypoxia was also studied. Results showed that HIF-1α was significantly higher in BMSCs exposed to hypoxic conditions for 24 h than that in BMSCs exposed to normoxia.

Recently, it has been reported that HIF-1 regulates apelin expression in cardiomyocytes and in adipocytes [16]. HIF-1 was an oxygen-sensitive heterodimeric transcription factor that promoted the expression of genes containing hypoxia-responsive element (HRE). Putative HREs were found in the apelin promoter by *in silico* analysis [17]. In this study, it was found that hypoxia stimulated the expression of apelin and its receptor APJ. Knock-down of HIF-1α by siRNA inhibited hypoxia-induced expression of apelin and APJ.

Apelin has mitogenic effects on a wide variety of cell types and is capable of stimulating the growth of retinal endothelial cells, the growth of the human umbilical vein endothelial cells, the proliferation of gastric cells, and the growth of human osteoblasts [18,19].

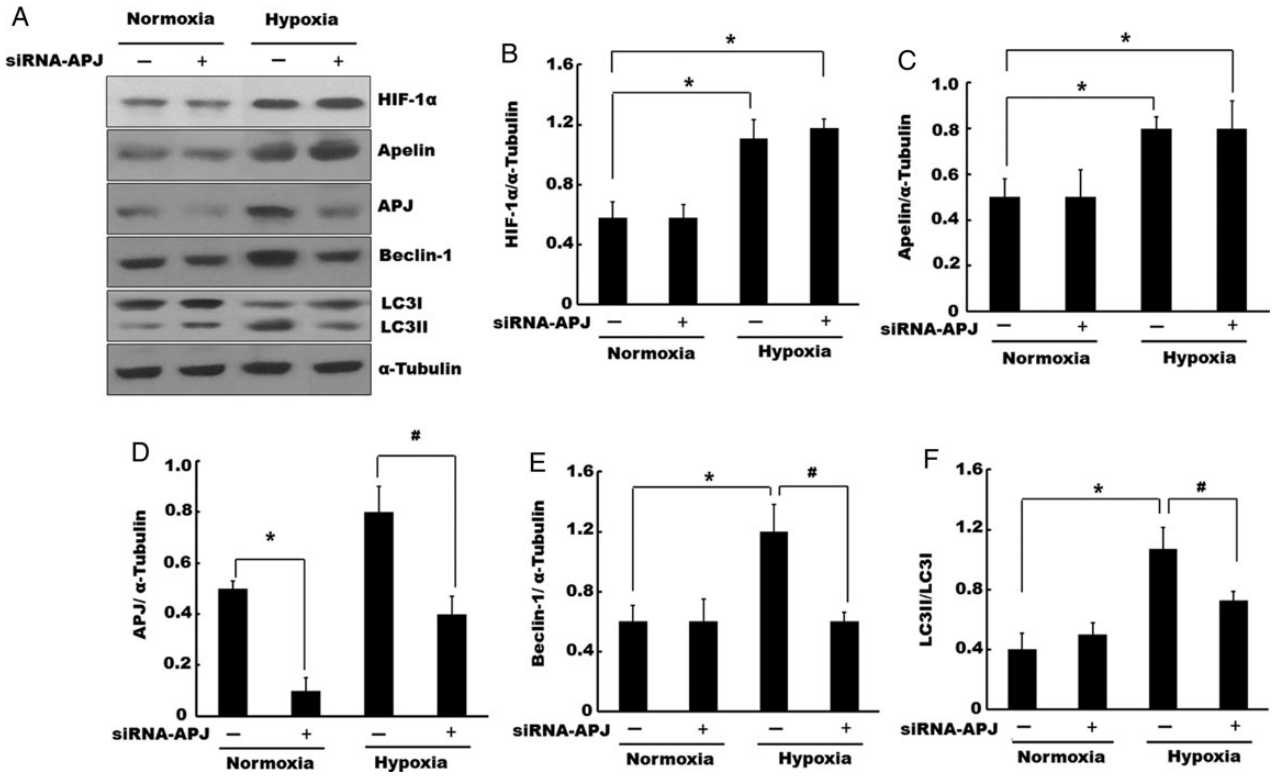

Figure 4. siRNA-APJ abolished the hypoxia-induced expression of Beclin-1 BMSCs were pretreated with siRNA-APJ for 24 h then cultured in the presence of normoxia or hypoxia for 24 h. Western blot results indicated hypoxia induced the expression of HIF-1α, apelin, APJ, Beclin-1, and LC3II/LC3I, but siRNA-APJ had no impact of the level of HIF-1α and apelin (A–C). siRNA-APJ inhibited APJ expression under both normoxia and hypoxia conditions (A,D) and reversed the expression of Beclin-1 and LC3II/LC3I induced by hypoxia (A,E,F). Data are presented as the mean ± SEM. $n = 4$. $^{*}P < 0.05$ vs. control under normoxia condition and $^{\#}P < 0.05$ vs. control under hypoxia condition.

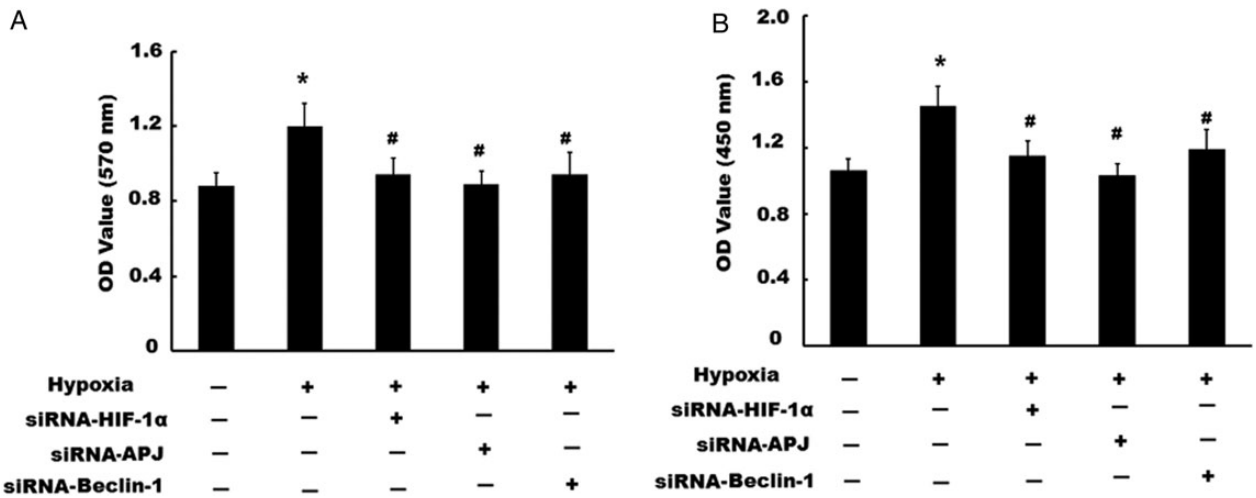

Figure 5. siRNA-HIF-1α, siRNA-APJ, and siRNA-Beclin-1 reversed hypoxia-induced BMSC proliferation MTT (A) and BrdU (B) results indicated that hypoxia promoted BMSC proliferation, and siRNA-HIF-1α, siRNA-APJ, and siRNA-Beclin-1 inhibited BMSC proliferation induced by hypoxia. Data are presented as the mean ± SEM ($n = 6$). $^{*}P < 0.05$ vs. control under normoxia condition and $^{\#}P < 0.05$ vs. control under hypoxia condition.

Recently, we have found that apelin plays a crucial role in processes of BMSC proliferation [10]. To further explore the functional significance of HIF-1α, apelin, and APJ expressions, we knocked down the expression of HIF-1α and APJ by transfecting small interfering RNA and found that both siRNA-HIF-1α and siRNA-APJ inhibited BMSC proliferation induced by hypoxia.

Autophagy, a catabolic digestion process of cellular macromolecules or even whole organelles, is induced under conditions of stress

such as starvation, hypoxia, heat, and drug treatment. Autophagy plays an important role in the process of hypoxia. Recent studies have indicated that HIF-1 regulates the autophagy under hypoxia [20]. On the contrary, it was reported that oxygen deprivation-induced autophagy does not require HIF-1 activity. Therefore, to further identify whether autophagy could be induced after hypoxia treatment, the expressions of Beclin-1 and LC3II/LC3I induced by hypoxia were investigated. The results showed that hypoxia induced a significant increase of Beclin-1 and LC3II/LC3I, and knock-down of HIF-1α by siRNA inhibited the expressions of hypoxia-induced Beclin-1 and LC3II/LC3I.

Autophagy has been reported to play a protective role in the heart following myocardial ischemia/reperfusion. In our previous study, we showed that treatment of post-MI mice with apelin-BMCs increased the expressions of autophagy gene LC3-II/I and Beclin-1 [21]. Some other researchers also reported that apelin/APJ might be related to the process of autophagy. Xie *et al*. [22] indicated that static pressure up-regulated APJ expression to promote cardiomyocyte hypertrophy by autophagy pathway. Yang *et al*. [7] also confirmed that apelin-13 promote autophagy via ERK1/2 signaling pathway. In the present study, we observed the effects of apelin/APJ on the autophagy. Our results indicated that hypoxia also promoted the expressions of Beclin-1 and LC3II/LC3I, and siRNA-APJ reduced the expression of Beclin-1 and LC3II/LC3I induced by hypoxia. These findings showed that hypoxia may induce the autophagy via apelin/APJ signaling pathway.

In summary, a preliminary understanding of the biological functions and novel mechanistic insights of the role of apelin/APJ/autophagy signaling pathway during hypoxia-induced BMSC proliferation was presented in this study. The effects of hypoxia on promoting BMSC proliferation have a close relationship with the apelin/APJ/autophagy signal pathway. Our results demonstrated that hypoxia might induce the proliferation of BMSCs through the activation of apelin/APJ and the activation of downstream autophagy pathway. However, further studies are needed to verify whether it is effective to use hypoxia induced- or apelin-overexpressing MSCs for therapeutic interventions in diseases and conditions.

Funding

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References

1. Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, Kawamata Y, *et al*. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem Biophys Res Commun* 1998, 251: 471–476.
2. Masri B, Lahlou H, Mazarguil H, Knibiehler B, Audigier Y. Apelin (65–77) activates extracellular signal-regulated kinases via a PTX-sensitive G protein. *Biochem Biophys Res Commun* 2002, 290: 539–545.
3. Charo DN, Ho M, Fajardo G, Kawana M, Kundu RK, Sheikh AY, Finsterbach TP, *et al*. Endogenous regulation of cardiovascular function by apelin-APJ. *Am J Physiol Heart Circ Physiol* 2009, 297: H1904–H1913.
4. Li F, Li L, Qin X, Pan W, Feng F, Chen F, Zhu B, *et al*. Apelin-induced vascular smooth muscle cell proliferation: the regulation of cyclin D1. *Front Biosci* 2008, 13: 3786–3792.
5. Lv D, Li H, Chen L. Apelin and APJ, novel critical factor and therapeutic target for atherosclerosis. *Acta Biochim Biophys Sin* 2013, 45: 527–534.
6. Li L, Li L, Xie F, Zhang Z, Guo Y, Tang G, Lv D, *et al*. Jagged-1/Notch3 signaling transduction pathway is involved in Apelin-13-induced vascular smooth muscle cells proliferation. *Acta Biochim Biophys Sin* 2013, 45: 875–881.
7. Yang L, Su T, Lv D, Xie F, Liu W, Cao J, Sheikh I, *et al*. ERK1/2 mediates lung adenocarcinoma cell proliferation and autophagy induced by Apelin-13. *Acta Biochim Biophys Sin* 2014, 46: 100–111.
8. Liu C, Su T, Li F, Li L, Qin X, Pan W, Feng F, *et al*. PI3K/AKT signaling transduction pathway is involved in rat vascular smooth muscle cell proliferation induced by Apelin-13. *Acta Biochim Biophys Sin* 2010, 42: 376–402.
9. Wang G, Anini Y, Wei W, Qi X, Am OC, Mochizuki T, Wang HQ, *et al*. Apelin, a new enteric peptide: localization in the gastrointestinal tract, ontogeny, and stimulation of gastric cell proliferation and of cholecystokinin secretion. *Endocrinology* 2004, 145: 1342–1348.
10. Li L, Li L, He L, Zhang Z, Xiao J, Guo Y, Chen L, *et al*. Effects of Apelin-13 on rat bone marrow-derived mesenchymal stem cell proliferation through the AKT/GSK3b/CyclinD1 pathway. *Int J Pept Res Ther* 2014, doi:10.1007 /s10989-014-9404-1.
11. Eyries M, Siegfried G, Ciumas M, Montagne K, Agrapart M, Lebrin F, Soubrier F. Hypoxia-induced apelin expression regulates endothelial cell proliferation and regenerative angiogenesis. *Circ Res* 2008, 103: 432–440.
12. Farini A, Sitzia C, Erratico S, Meregalli M, Torrente Y. Clinical applications of mesenchymal stem cells in chronic diseases. *Stem Cells Int* 2014, 2014: 306573.
13. Haque N, Rahman MT, Abu Kasim NH, Alabsi AM. Hypoxic culture conditions as a solution for mesenchymal stem cell based regenerative therapy. *Scientific World J* 2013, 27: 632972.
14. Fehrer C, Brunauer R, Laschober G, Unterluggauer H, Reitinger S, Kloss F, Gülly C, *et al*. Reduced oxygen tension attenuates differentiation capacity of human mesenchymal stem cells and prolongs their lifespan. *Aging Cell* 2007, 6: 745–757.
15. Heyman SN, Rosen S, Rosenberger C. Hypoxia-inducible factors and the prevention of acute organ injury. *Crit Care* 2011, 15: 209.
16. Geiger K, Muendlein A, Stark N, Saely CH, Wabitsch M, Fraunberger P, Drexel H. Hypoxia induces apelin expression in human adipocytes. *Horm Metab Res* 2011, 43: 380–385.
17. Han S, Wang G, Qi X, Lee HM, Englander EW, Greeley GH Jr. A possible role for hypoxia-induced apelin expression in enteric cell proliferation. *Am J Physiol Regul Integr Comp Physiol* 2008, 294: R1832–R1839.
18. Li L, Li F, Li F, Mao X, Yang L, Huang H, Guo Y, *et al*. NOX4-derived reactive oxygen species drive apelin-13-induced vascular smooth muscle cell proliferation via the ERK pathway. *Int J Pept Res Ther* 2011, 17: 307–315.
19. Li L, Zeng H, Chen JX. Apelin-13 increases myocardial progenitor cells and improves repair postmyocardial infarction. *Am J Physiol Heart Circ Physiol* 2012, 303: H605–H618.
20. Liu XW, Cai TY, Zhu H, Cao J, Su Y, Hu YZ, He QJ, *et al*. Q6, a novel hypoxia-targeted drug, regulates hypoxia-inducible factor signaling via an autophagy-dependent mechanism in hepatocellular carcinoma. *Autophagy* 2014, 10: 111–122.
21. Li L, Zeng H, Hou X, Chen JX. Myocardial injection of Apelin-overexpressing bone marrow cells improves cardiac repair via upregulation of Sirt3 after myocardial infarction. *PLoS One* 2013, 8: e71041.
22. Xie F, Liu W, Feng F, Li X, Yang L, Lv D, Qin X, *et al*. A static pressure sensitive receptor APJ promotes H9c2 cardiomyocyte hypertrophy via PI3K-autophagy pathway. *Acta Biochim Biophys Sin* 2014, 46: 699–708.