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Apelin promotes mesenchymal stem cells survival and vascularization under hypoxic-ischemic condition in vitro involving the upregulation of vascular endothelial growth factor☆

Jingying Hou [a,b,1], Tingting Zhong [a,b,1], Tianzhu Guo [a,b], Changqing Miao [c], Changqing Zhou [a,b], Huibao Long [a,b], Hao Wu [a,b], Shaoxin Zheng [a,d], Lei Wang [a,b], Tong Wang [a,b,d,*]

[a] Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetics and Gene Regulation, Sun Yat-sen Memorial Hospital of Sun Yat-sen University, 107 Yanjiang Xi Road, Guangzhou, Guangdong 510120, China
[b] Department of Emergency, Sun Yat-sen Memorial Hospital of Sun Yat-sen University, 107 Yanjiang Xi Road, Guangzhou, Guangdong 510120, China
[c] The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shanxi 710061, China
[d] Guangdong Province Key Laboratory of Arrhythmia and Electrophysiology, 107 Yanjiang Xi Road, Guangzhou, Guangdong, China

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ABSTRACT

Background: Mesenchymal stem cells (MSCs) transplantation has been regarded as an optimal therapeutic approach for cardiovascular disease. However, the inferior survival and low vascularization potential of these cells in the local infarct site reduce the therapeutic efficacy. In this study, we investigated the influence of apelin on MSCs survival and vascularization under hypoxic-ischemic condition in vitro and explored the relevant mechanism.

Methods: MSCs were obtained from C57BL/6 mice and cultured in vitro. Cells of the third passage were divided into MSCs and MSCs + apelin groups. In the MSCs + apelin group, MSCs were stimulated with apelin-13 (5 μM). The two groups experienced exposure to hypoxia (1% O_2) and serum deprivation for 24 h, using normoxia (20% O_2) as a negative control during the process. Human umbilical vein endothelial cells (HUVECs) were used and incubated with conditioned media from both groups to promote vascularization for another 6 h. Vascular densities were assessed and relevant biomarkers were detected thereafter.

Results: Compared with MSCs group, MSCs + apelin group presented more rapid growth. The proliferation rate was much higher. Cells apoptosis percentage was significantly declined both under normoxic and hypoxic conditions. Media produced from MSCs + apelin group triggered HUVECs to form a larger number of vascular branches on matrigel. The expression and secretion of vascular endothelial growth factor (VEGF) were significantly increased.

Conclusion: Apelin could effectively promote MSCs survival and vascularization under hypoxic-ischemic condition in vitro, and this procedure was associated with the upregulation of VEGF. This study provides a new perspective for exploring novel approaches to enhance MSCs survival and vascularization potential.

1. Introduction

The bone marrow mesenchymal stem cells (MSCs) have great potential of proliferation and differentiation. They are considered as a suitable source for the treatment of cardiovascular illness (Sanina and Hare, 2015; Karantalis and Hare, 2015; Wen et al., 2011). MSCs transplantation can ameliorate ventricular remodeling and improve cardiac function in patients with MI and chronic heart failure (Guijarro et al., 2016; Hou et al., 2015; Wang et al., 2009; Montanari et al., 2015). However, inferior viability and low transdifferentiation efficiency of these cells curtail their therapeutic efficacy to a large extent (Xing et al., 2014). There was sparse newly formed vascular like structures in the local infarct tissues (Huang et al., 2010; Park et al., 2015). In view of this, how to promote MSCs survival and vascularization becomes an

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* Corresponding author at: Department of Emergency, Sun Yat-sen Memorial Hospital of Sun Yat-sen University, 107 Yanjiang Xi Road, Guangzhou, Guangdong, China; Guangdong Province Key Laboratory of Arrhythmia and Electrophysiology, 107 Yanjiang Xi Road, Guangzhou, Guangdong, China.
E-mail address: tongwang316@163.com (T. Wang).

[1] Dr. Jingying Hou and Dr. Tingting Zhong were co-first authors and played equally important roles in the development of the experimental protocol, in the interpretation of the results, and in the texture of the present article.

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0014-4800/

Table 1
List of primers for qRT-PCR.

Genes	Forward	Reverse
VEGFA	5′ATGCCGGTTCCAACCAGAA 3′	5′GTGGAGGAGCGAGCTGAA3′
β-Actin	5′GCTTCTAGGCGGACTGTTAC3′	5′CCATGCCAATGTTGTCTCTT3′

issue that needs to be imperatively addressed (Buravkova et al., 2014; McGinley et al., 2013).

Putative receptor protein related to the angiotensin receptor AT1 endogenous ligand (apelin) is involved in MSCs proliferation, survival and differentiation in hypoxic-ischemic condition (Li et al., 2015; Zeng et al., 2012; Liang et al., 2016; Mottaghi et al., 2012). It promotes MSCs proliferation and survival and reduces their apoptosis. Additionally, apelin also vividly participates in the regulation of vascular function (Narayanan et al., 2015). It has been discovered that apelin facilitates the vascularization of several progenitor cells (Tempel et al., 2012). In spite of this, the underlying mechanism has not been fully elucidated.

In this study, we investigated the influence of apelin on MSCs survival and vascularization in hypoxic-ischemic condition in vitro and explored the relevant mechanism.

2. Materials and methods

2.1. Ethics statement

3 weeks old C57BL/6 mice were obtained from the Animal Experimental Center of the Sun Yat-sen University. All animal handling and procedures were performed in accordance with protocols approved by the Animal Ethics Committee of Sun Yat-sen University (201411023).

2.2. Isolation and culture of bone marrow mesenchymal stem cells

Bone marrow cells were collected from C57BL/6 ($n = 10$) mice by flushing femurs and tibias under aseptic conditions. Cells were cultured (37 °C, 5% CO_2) in 25 cm^2 culture flasks with complete culture medium (Hyclone, USA) supplemented with 10% fetal bovine serum (Hyclone, USA), L-Glutamine(4.0 mM), penicillin (100 IU/mL) and streptomycin (100 μg/mL). On the third day of culture, the medium was replaced and non-adherent cells were removed. The adherent cells were washed two times gently with PBS to reduce the degree of hematopoietic lineage cell contamination. The cells were cultured in complete culture medium and the medium was changed every 3 to 4 days for 3–4 weeks. Adherent cells gaining 90% of confluence cultures were trypsinized with 0.25% trypsin–ethylenediaminetetraacetic acid (Mediatech, USA) and passaged into new flasks for further expansion. Characteristics of MSCs were identified by fluorescence-activated cell sorting (FASC) as previously reported (Chen et al., 2014; Wang et al., 2009). Cell markers used in FACS were CD34 (rat anti-mouse CD34 FITC; Clone: RAM34; Cat No: 11-0341-82; eBioscience, USA), CD44 (rat anti-mouse CD44 FITC; Clone: IM7; Cat No: 553133; BD Biosciences, USA) and CD29 (PE hamster anti-mouse CD29; Clone: HM β1; Cat No: 562801; BD Biosciences, USA). The third passage MSCs were used for all the experiments.

2.3. Hypoxia-ischemia treatment of MSCs

MSCs were stimulated with apelin-13 (Cat No: ab141010, Abcam, UK) at a concentration of 5 μM (unpublished data provided by the preliminary studies) in the MSCs + apelin group for 24 h. Cells in the MSCs and MSCs + apelin group were both incubated in serum-free media with 1% O_2 in Galaxy® 48 R incubator (Eppendorf/Galaxy Corporation, USA) at 37 °C for 24 h. Normoxia (20% O_2) was used as a negative control during the whole experiments for the two groups.

2.4. Cell viability and apoptosis assays

MSCs of different groups were collected and suspended in complete culture medium (Hyclone, USA). Growth curve and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) cell viability assay (cellTiter96AQ, one solution cell proliferation assay, Cat No: G3582, Promega, USA) were adopted to evaluate survival and proliferation ability of MSCs. A total of 1×10^5 cells were equally seeded into each well on 96-well plates after 24 h of preconditioning, MTS was added to the medium at a final concentration of 0.5 mg/mL for 4 h. The optimal density values (OD490) were read from cellTiter96AQ at different time points (0 h, 24 h, 48 h, 72 h) respectively, Proliferation rate was calculated as previously described (Xing et al., 2014): proliferation rate = OD values at other time points divided by OD value at the beginning × 100% (the same sample).

Terminal deoxynucleotidyl transferase biotin-dUPT nick end labeling (TUNEL) assay was applied for assessing MSCs death and apoptosis in vitro after 24 h of exposure to normoxic or hypoxic-ischemic conditions. Cell slices were fixed in 10% formaldehyde for 15 min and

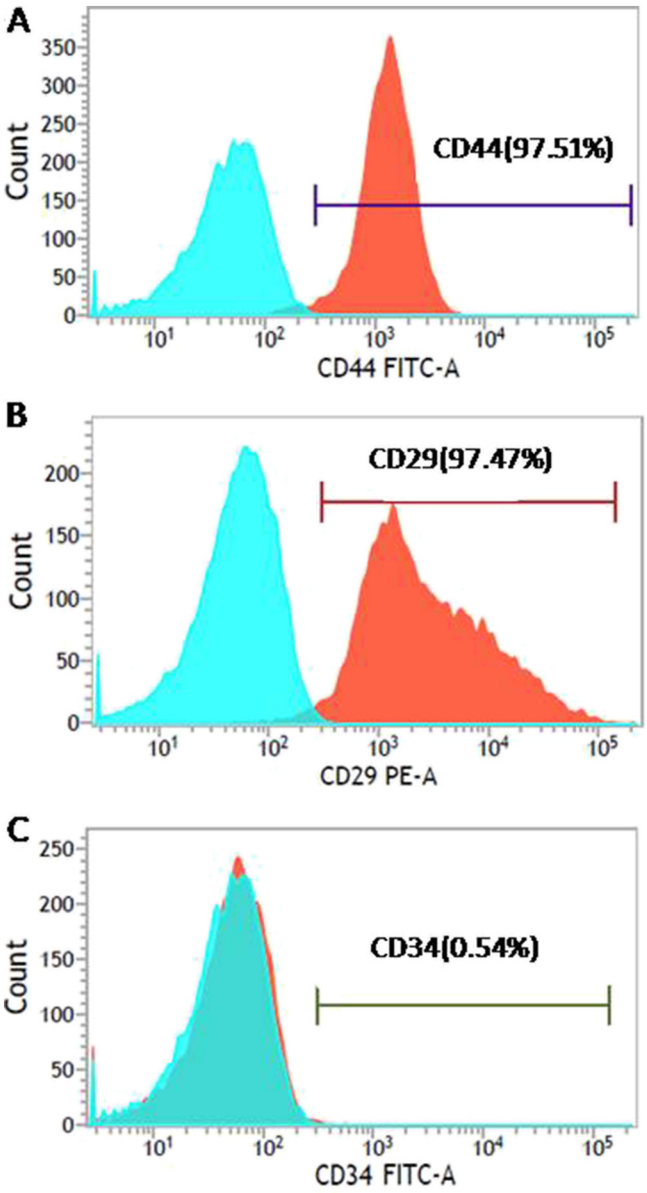

Fig. 1. Characteristics of MSCs. Representative images of MSCs cells markers were shown by FACS analysis. (A), (B) and (C) represented CD44, CD29 and CD34 respectively.