Biochemical and Biophysical Research Communications xxx (xxxx) xxx

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Impaired Vps34 complex activity-mediated autophagy inhibition contributes to endothelial progenitor cells damage in the ischemic conditions

Ru-chao Jiang [b, 1], Xiao-ling Zhang [d, 1], Qi-ai Zhang [b], Xue-ying Zheng [b], Hai-jie Shi [b], Yuan Qin [a, b], Gui-ping Zhang [a, b], Qing Xiao [a, b, c, *], Jian-dong Luo [a, b, c, **]

[a] Key Laboratory of Molecular Target & Clinical Pharmacology, School of Pharmaceutical Sciences & the Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou, Guangdong, 511436, PR China
[b] Department of Pharmacology, Guangzhou Medical University, Guangzhou, Guangdong, 511436, PR China
[c] Guangzhou Institute of Cardiovascular Disease, Guangzhou Key Laboratory of Cardiovascular Disease, The Second Affiliated Hospital, Guangzhou Medical University, Guangzhou, Guangdong, 511436, PR China
[d] Maternal and Children Hospital of Guangdong Province, Guangzhou, Guangdong, 510260, PR China

ARTICLE INFO

Article history:
Received 20 January 2020
Accepted 23 January 2020
Available online xxx

Keywords:
Endothelial progenitor cells
Ischemia conditions
Autophagy
Vps34 complex

ABSTRACT

Aims: Endothelial progenitor cells (EPCs) are widely accepted to be applied in ischemic diseases. However, the therapeutic potency is largely impeded because of its inviability in these ischemic conditions. Autophagy is recognized to be vital in cell activity. Therefore, we explore the role and the mechanism of autophagy in ischemic EPCs.

Methods and results: We applied 7d-cultured bone marrow EPCs to investigate the autophagy status under the oxygen and glucose deprivation (OGD) conditions in vitro, mimicking the in-vivo harsh ischemia and anoxia microenvironment. We found increased EPC apoptosis, accompanied by an impaired autophagy activation. Intriguingly, mTOR inhibitor Rapamycin was incapable to reverse this damped autophagy and EPC damage. We further found that autophagy pathway downstream Vps34-Beclin1-Atg14 complex assembly and activity were impaired in OGD conditions, and an autophagy-inducing peptide Tat-Beclin1 largely recovered the impaired complex activity and attenuated OGD-stimulated EPC injury through restoring autophagy activation.

Conclusions: The present study discovered that autophagy activation is inhibited when EPCs located in the ischemia and anoxia conditions. Restoration of Vps34 complex activity obtains sufficient autophagy, thus promoting EPC survival, which will provide a potential target and advance our understanding of autophagy manipulation in stem cell transplantation.

1. Introduction

Ischemic diseases are prevalent in recent years. Endothelial progenitor cells (EPCs), a population of cells that located in bone marrow, peripheral blood and cord blood, can be mobilized into the local ischemic injury to participate in repair [1,2]. EPC-based therapies are proved to be effective through several pre-clinical and clinical trials [3,4]. Unfortunately, most of the cells (more than 80%) die a few days (<3 days) after delivery [5–7], thus hindering the therapeutic effect.

To improve these outcomes, it is necessary to elucidate the mechanism of EPC survival after transplantation and to find new ways for improving the survival of implanted cells.

Autophagy is an intracellular catabolic mechanism by which cell components, including proteins, lipids and whole organelles, are degraded and recycled inside lysosomes [8]. Autophagy is closely related to cell viability in most studies [9]. Previous studies have shown that autophagy formation in EPCs is activated and plays a

* Corresponding author. Key Laboratory of Molecular Target & Clinical Pharmacology, School of Pharmaceutical Sciences & the Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou, Guangdong 511436, PR China.
** Corresponding author. , Department of Pharmacology, Guangzhou Medical University, Xinzao, Panyu District, Guangzhou, 511436, PR China
E-mail addresses: xiaoqingxiaomeng@163.com (Q. Xiao), jiandongluo@hotmail.com (J.-d. Luo).
[1] Ru-chao Jiang and Xiao-ling Zhang contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2020.01.133
0006-291X/

protective role in a transient hypoxia stimulation (1% O2 and DMEM plus 10% FBS and growth factors, within 2 h), mimicking the hypoxic preconditioning [10,11]. However, exogenous EPCs in vivo are persistently damaged in a harsh microenvironment when transplanted in the ischemic zone. The autophagy performance is not clear during this sustained ischemia conditions. Therefore, we established the in vitro oxygen-glucose deprivation (OGD) model to mimic the vivo severe microenvironment, and investigate the role and the mechanism of EPC autophagy in this persist injury (OGD 2–12 h).

2. Methods

2.1. Animal study protocol

Ethics statement: This study was carried out in accordance with the recommendations of the Institutional Animal Ethics Committee of Guangzhou Medical University (Guangzhou, China). The protocol was approved by the Institutional Animal Ethics Committee of Guangzhou Medical University. All animal experiments conformed to the National Institutes of Health guidelines (Guide for the care and use of laboratory animals).

Euthanasia: Adult male C57/B6 mice were intraperitoneal injected with an overdose of sodium pentobarbital (100 mg/kg), followed by cervical dislocation.

2.2. BM-EPCs culture and characterization

In order to culture bone marrow-endothelial progenitor cells (BM-EPCs), single BM–nucleated cells were isolated from the bone marrow from femurs and tibias of male C57BL/6 J mice by density gradient centrifugation with Histopaque-1083 (Sigma-Aldrich), and were plated on human plasma fibronectin–coated (Corning) dishes with endothelial cells basal medium (EBM-2, Lonza) supplemented with 5% FBS, antibiotics, and cytokine cocktail (Lonza) containing hydrocortisone, hFGF-B, VEGF, R3-IGF-1, Ascorbic Acid, hEGF, GA-1000, Heparin. Nonadherent cells were removed and changed into fresh EGM-2 after 3 days. Confirmation of BM-EPCs phenotype was performed at day 7. Adherent cells were stained with DiI-acLDL (1 μg/mL, Invitrogen) for 1 h and then incubated with FITC-UEA-1(1 μg/mL, Sigma) for another 1 h. Adherent cells stained both FITC–UEA-1 and DiI-acLDL were identified as EPCs. In addition, stem cell marker CD34 (1: 100, Abcam) and endothelial cell marker CD31 (1:100, BD Pharmingen) were also used to identify endothelial progenitor cells [12,13].

2.3. Treatment of BM-EPCs

BM-EPCs were pretreated with Rapamycin (200, 400 nM, Sigma) for 2 h, or with Tat-Beclin1 (5 μm, Selleck) or 3-MA (5 mM, Sigma) for 4 h. After that, BM-EPCs were washed thrice with PBS, and then put into serum-free, glucose-free and pyruvate-free DMEM (D5030, Sigma) to establish OGD model [14], mimicking the ischemic anoxic microenvironments [15]. OGD model were performed in an anoxia chamber (InVivo 500, Ruskinn Life Science) at 37 °C under hypoxic (1.0% O2, 5.0% CO2, and 94.0% N2) conditions. After OGD treatment, BM-EPCs were harvested and used for further analysis.

2.4. Western blot analysis

Procedures are stated in Supplementary material [16].

2.5. Immunofluorescence analysis

Procedures are stated in Supplementary material [17].

2.6. TUNEL staining

The apoptotic cardiomyocytes are stained by TUNEL dye. Procedures are stated in Supplementary data.

2.7. MDC staining

Procedures are stated in Supplementary material [18].

2.8. P62 and aggresomes colocalization assay

Procedures are stated in Supplementary material.

2.9. Statistical analysis

All variables were presented as means ± standard error of the mean. Comparisons between two groups were analyzed using the student's *t*-test for continuous variables, and one-way analysis of variance(ANOVA)were used to compare multiple groups. Statistical significance in this study was set at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. All statistical analyses and graph were performed using GraphPad Prism 5.

3. Results

3.1. Characterization of EPCs

Although EPCs are heterogeneous in nature, they possess stem cell characteristics and can be differentiated into endothelial cells. To characterize BM-derived EPCs, the expression of the stem cell marker CD34 and the endothelial cell marker CD31 was examined by immunofluorescence (Fig. S1A). The 7 day-cultured EPCs were also confirmed by Dil-ac-LDL and FITC- lectin double staining, a previously defined characteristic of EPCs (Fig. S1B). Fig. 1C displays the growth of EPCs for 7 days. Spindle cells and colonies occurred from day 3, several cobblestone cells appeared from day 5, and the density of cobblestone cells increased on day 7.

3.2. Autophagy activation is dampened in OGD-stimulated EPC

In order to investigate the autophagy status during the in vivo ischemic conditions, 7 day-EPCs were cultured in DMEM free of glucose and serum, with 1% O2 for 2–12 h. First, we estimate EPC injury by apoptosis. Cleaved caspase-3 in western blotting was increased during 2–6 h, and further increased during 8–12 h (Fig. 1A); the numbers of TUNEL-positive EPCs also confirmed the results in OGD 6 h and 10 h (Fig. 1B).

Then, autophagy performance was assessed. Western blotting assay showed that the ratio of LC3 II/I was significantly reduced from OGD 2 h–12 h, and p62 protein was significantly accumulated after OGD 6 h, and consistently increased during OGD 8–12 h (Fig. 1A). MDC staining and p62/aggresomes assay were applied to further assess the autophagy process. MDC, labeled in autophagic structures, was significantly decreased at OGD 6 h and 10 h (Fig. 1C); p62/aggresomes, presenting autophagic substrate clearance, was significantly increased at OGD 6 h and 10 h (Fig. 1D).

Moreover, Baf (35 nM) was delivered to inhibit the autophagy degradation, and then LC3 II expression was detected. Data showed that LC3 II level was still lower in OGD group than in control group, in the presence of Baf (Fig. 1E), indicating that the decreased LC3 II level in OGD status was induced by the impaired autophagy activation rather than the acceleration of autophagy clearance.

All above results suggest that autophagy activation is impeded, followed by the whole stumble autophagy flux.

Please cite this article as: R.-c. Jiang et al., Impaired Vps34 complex activity-mediated autophagy inhibition contributes to endothelial progenitor cells damage in the ischemic conditions, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2020.01.133

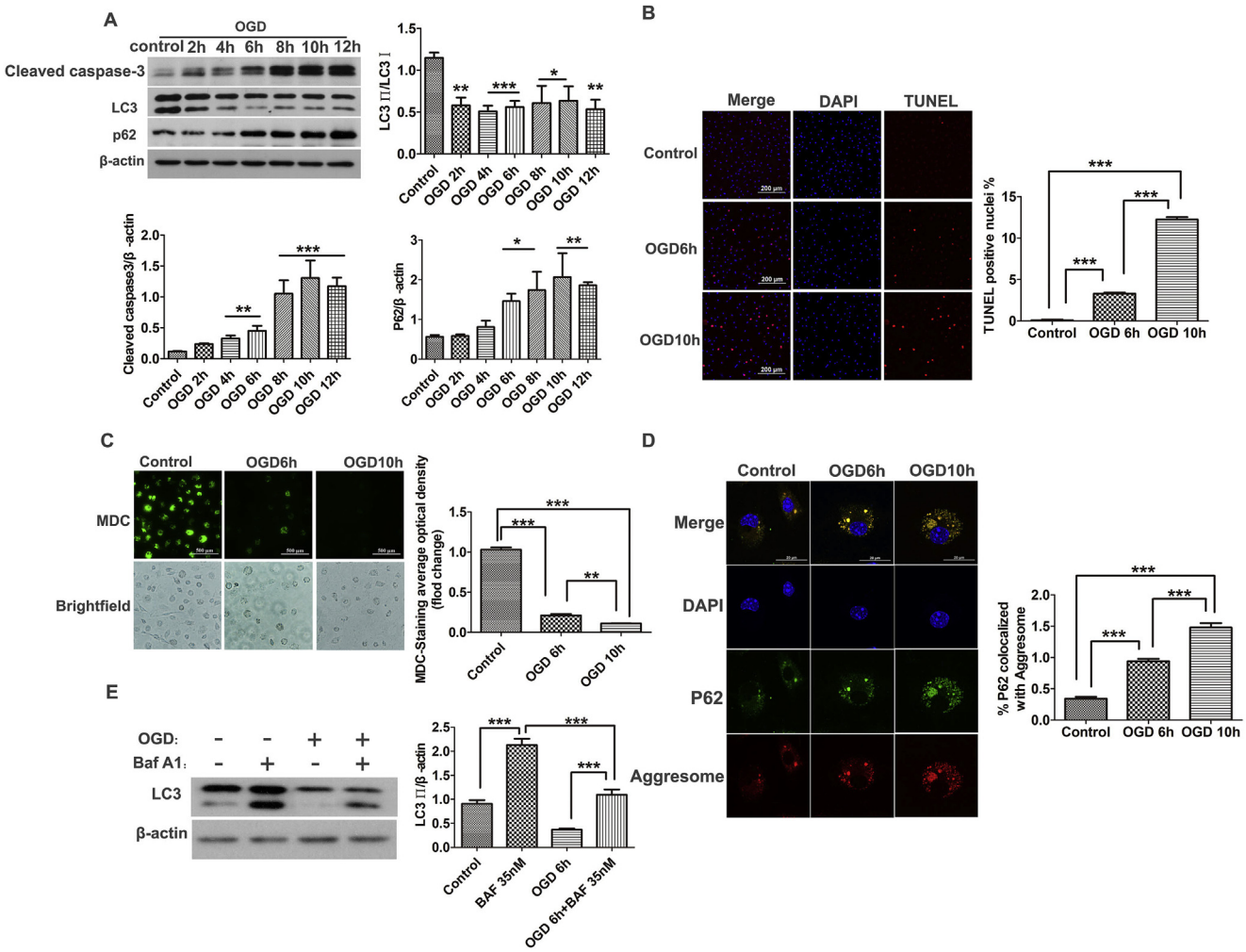

Fig. 1. Autophagy activation is inhibited in OGD-stimulated EPCs. (A) Western blotting analysis of apoptotic indicator Cleaved caspase-3, autophagy protein markers LC3 and p62 in EPCs under OGD stimulation (n = 4), *P < 0.05, **P < 0.01, ***P < 0.001 *vs* control. (B) Representative images and quantitive analysis of TUNEL-positive cells (red) and nuclear cells (DAPI, blue) (n = 5). (C) Representative images and quantitive analysis of autophagosomes stained by MDC (n = 4). (D) Representative images and quantitive analysis of p62/SQSTM1 (green) and ProteoStat® Aggresome detection reagent (red) colocalization (n = 4). (E) Western blotting analysis of LC3 II level after Baf A1 treatment (35 nM) for 4 h under OGD stimulation (n = 4). *P < 0.05, **P < 0.01, ***P < 0.001. Data were analyzed by one-way ANOVA, followed by a Bonferroni post-hoc test, or analyzed by unpaired student's *t*-test. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Rapamycin slightly activates autophagy and fails to alleviate EPC damage under OGD conditions

Then, we explored the molecular mechanism in the impaired autophagy activation. The classic autophagy activation upstream factors are the first to be assessed. Western blotting assay displayed the upregulated p-AMPK, p-mTOR, p-p70S6K, p-Ulk555 and p-Ulk757, and the hardly unchanged Beclin1 at OGD 6 h and 10 h (Fig. 2A). Among these molecules, the change of p-mTOR, p-p70S6K and p-Ulk757 are consistent with the endogenous impaired autophagy activation. Therefore, we applied mTOR inhibitor Rapamycin (200, 400 nM) to rescue the depressed autophagy activation. Western blotting showed that Rapamycin significantly reduced the ratio of mTOR pathway downstream factors p-p70S6K/p70S6K and p-4E-BP1/4E-BP1, suggesting the effectiveness of Rapamycin treatment. However, the LC3 II/I ratio was slightly increased and the p62 level was slightly reduced, yet the Cleaved caspase-3 had no significant changes in the presence of Rapamycin (200, 400 nM) (Fig. 2B). All these data indicate that the classic upstream molecules may not be the specific target that induces the impaired autophagy activation.

3.4. The Vps34 complex in EPCs is impaired during OGD status

We supposed that other molecules might lead to this impaired autophagy activation. PI3K-III complex (also named as Vps34 complex) functions at the downstream of the above molecules in autophagy activation. Vps34 always acts in a dimer with a pseudo-kinase Vps15, Vps34-Vps15 dimer needs Beclin1 and Atg14 to form the tetramer to participate in autophagy process [19]. Therefore, we applied Vps34 antibody to pull down Beclin1 and Atg14 to detect their assembly. Co-IP assay showed that the binding of Vps34-Beclin1 and Vps34-Atg14 are decreased in OGD 6 h (Fig. 3A), indicating an impaired protein assembly of Vps34 complex.

More importantly, the Vps34 complex activity, i.e. the production of phosphatidylinositol 3-phosphate (PI [3] P) through activating Vps34 kinase, is the ultimate determinant in Vps34 complex-induced autophagy activation [20]. By means of producing PI [3] P, Vps34 complex recruits other proteins to participate in the autophagosome formation [21]. Therefore, WIPI2, a critical molecule that binds to PI [3] P at the site of early autophagosomal structures [22,23], was applied to estimate the Vps34 complex activity [24]. Compared to control group, we observed a significant

Please cite this article as: R.-c. Jiang et al., Impaired Vps34 complex activity-mediated autophagy inhibition contributes to endothelial progenitor cells damage in the ischemic conditions, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2020.01.133

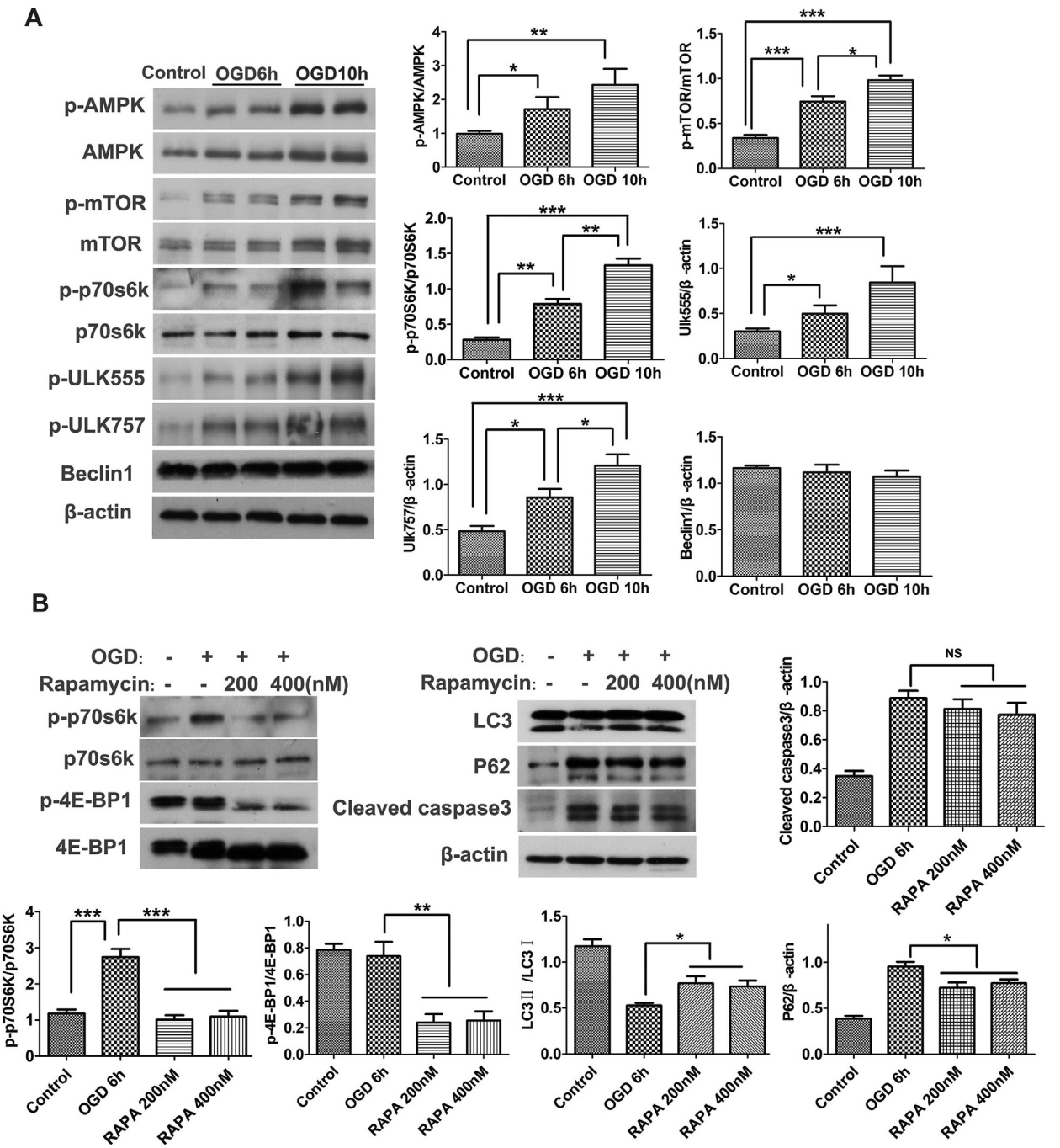

Fig. 2. Rapamycin slightly activates autophagy and fails to reduce EPCs apoptosis under OGD conditions. Rapamycin (200, 400 nM) was pretreated for 2 h, and then OGD was applied for 6 h, co-treating with Rapamycin (200, 400 nM) for 6 h. (A) Representative images of p-AMPK, AMPK, p-mTOR, mTOR, p-p70S6K, p70S6K, p-ULK555, p-ULK757 and Beclin1 by Western blotting analysis (n = 4). (B)After treatment with mTOR inhibitor Rapamycin, mTOR pathway downstream factors p-p70S6K/p70S6K and p-4E-BP1/4E-BP1, autophagy protein markers LC3, p62 and apoptosis indicator Cleaved caspase-3 were detected by Western blotting assay (n = 4). *P < 0.05, **P < 0.01, ***P < 0.001. Data were analyzed by one-way ANOVA, followed by a Bonferroni post-hoc test, or analyzed by unpaired student's *t*-test.

decrease in the numbers of punctate structures containing WIPI2 in OGD 6 h and 10 h (Fig. 3B), indicating an impaired Vps34 complex activity in autophagy activation.

3.5. Tat-Beclin1 boosts autophagy activation to resist EPC injury via enhancing the complex activity

Then we applied Tat-Beclin1, an autophagy-inducing peptide that specifically enhances Vps34 complex activity while having no effect on complex assembly [24]. Tat-Beclin1 (5 μM) was delivered 4 h before OGD in Opti-MEM, and then the condition was changed into OGD for 6 h, in the presence of Tat-Beclin1. Data showed that Tat-Beclin1 plus OGD group had more WIPI2 puncta than OGD group did (Fig. 4A), indicating the recovery of Vps34 kinase activity. Moreover, autophagy activation had a remarkable enhancement, as confirmed by the increased ratio of LC3 II/I and MDC dots, and the decreased p62 level and p62/aggresomes dots (Fig. 4B–D). Besides, the apoptosis of EPCs was significantly reduced after treatment

Please cite this article as: R.-c. Jiang et al., Impaired Vps34 complex activity-mediated autophagy inhibition contributes to endothelial progenitor cells damage in the ischemic conditions, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2020.01.133

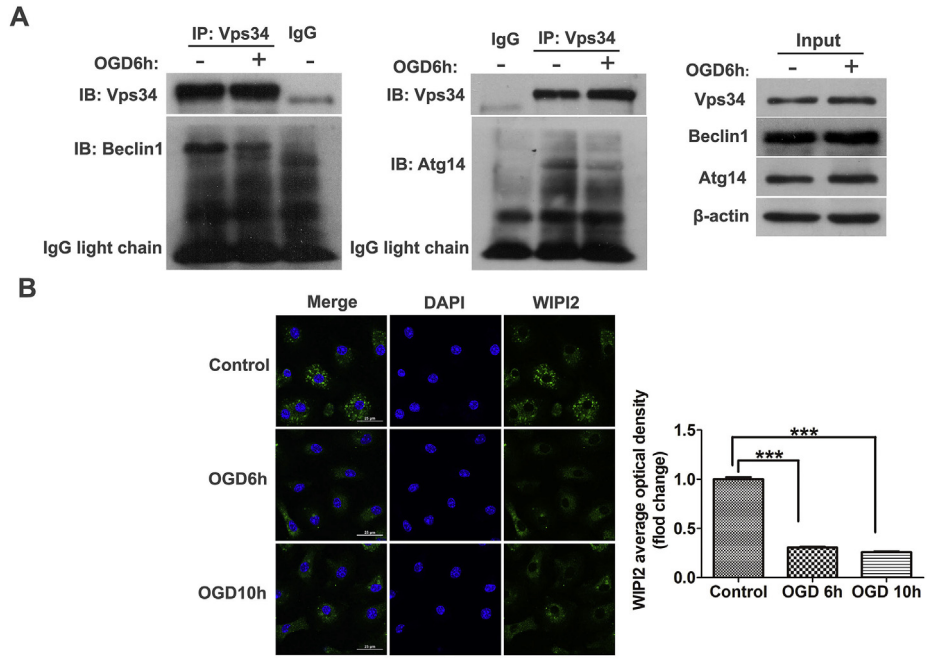

Fig. 3. The Vps34 complex is impaired under continuous OGD status. (A) Endogenous Vps34 complex in EPCs. Vps34 was IP, and followed by IB for Beclin1 and Atg14. (B) Representative immunofluorescent images of WIPI2 (green) and DAPI (blue), a marker of Vps34 kinase activity (n = 5). *P < 0.05, **P < 0.01, ***P < 0.001. Data were analyzed by one-way ANOVA, followed by a Bonferroni post-hoc test, or analyzed by unpaired student's *t*-test. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

with Tat-Beclin1, as indicated by the decreased level of Cleaved caspase-3 (Fig. 4B) and TUNEL-positive EPCs (Fig. 4E). When autophagy inhibitor 3-MA (5 mM) was delivered, the autophagy activation and the decreased apoptosis were reversed in the Tat-Beclin1 group, as shown by reduced MDC dots (Fig. 4C), accumulated p62/aggresomes (Fig. 4D) and increased TUNEL-positive EPCs (Fig. 4E). Taken together, our data indicate that applying Vps34 complex activator Tat-Beclin1 indeed alleviates EPC damage through autophagy activation.

4. Discussion

In the present study, we investigated the autophagy status and its role in endothelial progenitor cells survival that suffer from sustained oxygen-glucose deprivation (OGD) conditions, mimic to cell-transplantation to the ischemic zone in clinics. Our significant findings in this study were (i) Autophagy activation is impeded, leading to the whole impaired autophagy flux in the persistent OGD conditions; (ii) the classic autophagy activator Rapamycin is only able to slightly activate autophagy, which is insufficient to mitigate OGD-induced EPCs injury; and (iii) Vps34 complex is impaired during OGD stimulation, which can be ameliorated by Tat-Beclin1 through activating complex kinase activity, thus alleviating EPCs injury via autophagy activation.

Stem cell therapy has evolved into a promising treatment for ischemic heart disease. EPC-based therapy continues to gain consent and appeal because of the large preclinical evidence supporting highly cardio-reparative potential [25,26]. However, poor survival of the donated EPCs in the damaged organ occurs within the first days after delivery, posing a significant challenge in the field [27,28]. A complete understanding in the mechanisms of EPCs damage under the microenvironment of the injured myocardium is imperative to improve EPC therapeutic application.

Autophagy is fundamental for the vital activity of organism, maintaining the process of cellular homeostasis and stress tolerance [29]. However, the autophagy status of EPCs in the persist ischemic status is still unknown. Our present study demonstrates that autophagy is downregulated in the OGD-stimulated EPCs, as indicated not only by the classic marker LC3, p62 but also by the BAF delivery, suggesting that autophagy activation is impaired, which leads to the dampened autophagy flux in the persistent OGD conditions, mimic to the ischemic microenvironment in clinical practice.

Rapamycin is a classic autophagy inducer, but it isn't fit for any circumstance. In the present study, we apply Rapamycin (200, 400 nM) to compensate the inadequate autophagy in OGD-stimulated EPCs. However, it slightly enhances the autophagy, and present no significant change in the EPCs apoptosis. We further discover that although the endogenous autophagy activation in OGD status is impaired, the changes in the classic upstream molecules (p-AMPK, p-ULK555) are beneficial to autophagy activation. Combined to the results of Rapamycin, we consider that these upstream molecules may not contribute to the impaired autophagy activation in OGD-induced EPCs.

Vps34 (vacuolar protein sorting 34) is a member of the phosphoinositide 3-kinase (PI3K) family of lipid kinases, which combines Beclin1 and Atg14 to form a complex and signals to downstream effectors through the production of phosphatidylinositol 3-phosphate (PI [3]P) [30]. Our present data demonstrate that the Vps34-Beclin1-Atg14 assembly is reduced and the Vps34 complex activity (PI [3]P/WIPI2 in autophagosomes) is greatly declined in OGD-stimulated EPCs, which may be involved in the impaired autophagy activation and EPC damage. After applying Tat-Beclin1 to restore the impaired Vps34 complex activity [24,31], autophagy activation is remarkable enhanced, resulting in the alleviation of EPCs damage. This is consistent with other studies: Grieco G et al. reported that Vps34/PI3KC3 deletion in kidney proximal tubules impairs apical trafficking and blocks autophagic flux, causing a Fanconi-like syndrome and renal insufficiency [32]; He F et al. observed that a conditional Vps34 knockout mouse line

Please cite this article as: R.-c. Jiang et al., Impaired Vps34 complex activity-mediated autophagy inhibition contributes to endothelial progenitor cells damage in the ischemic conditions, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2020.01.133

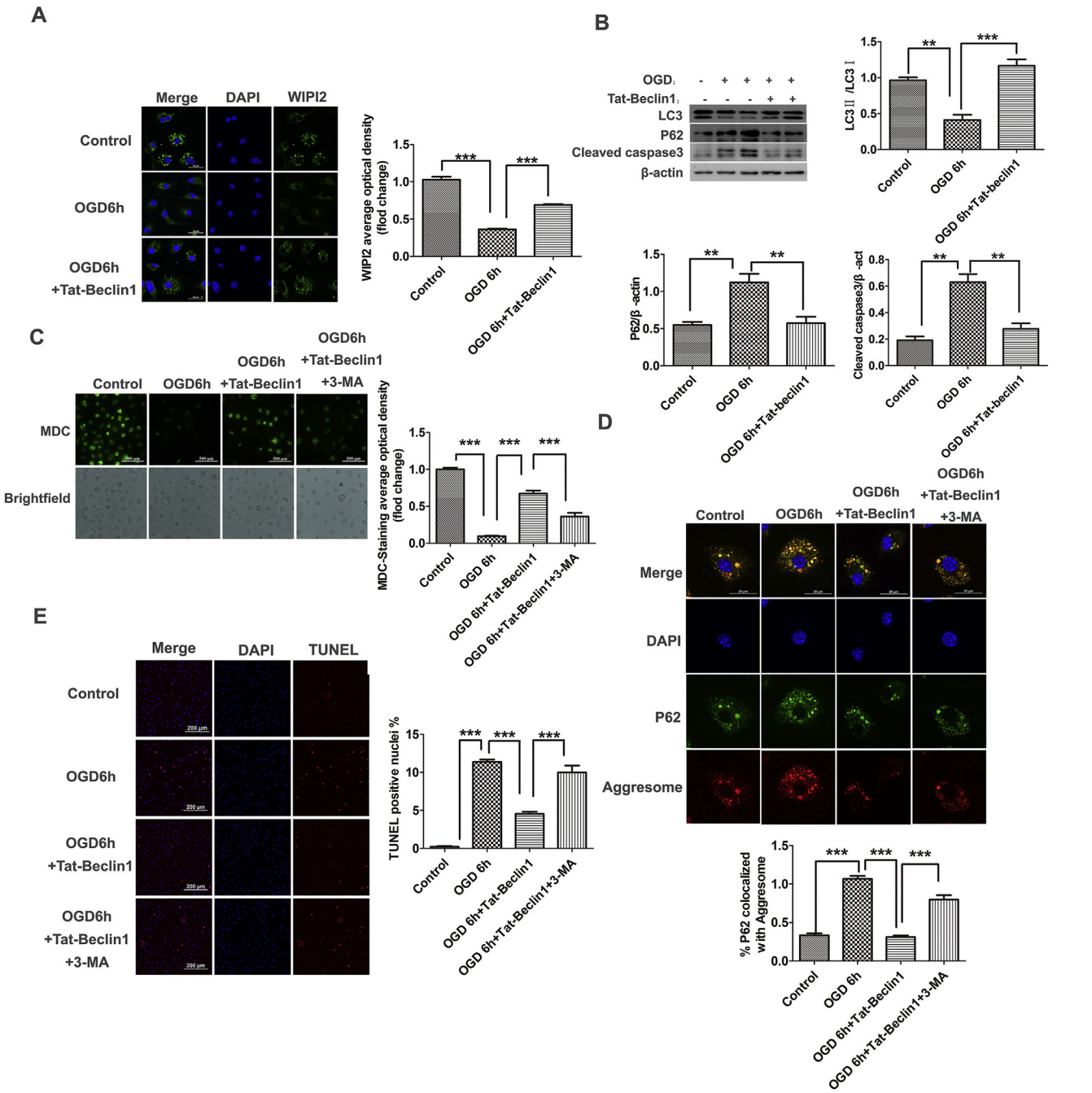

Fig. 4. Restoration of Vps34 complex activity by Tat-Beclin1 reduces EPC apoptosis via autophagy activation. Tat-Beclin1 (5 μM) and 3-MA (5 mM) were pretreated for 4 h in Opti-MEM, and then OGD was applied for 6 h, co-treating with Tat-Beclin1 (5 μM) and 3-MA (5 mM) for 6 h. (A) Representative immunofluorescent images of WIPI2 (green) and DAPI (blue) (n = 5). (B) Western blotting analysis of autophagy protein markers LC3, p62 and apoptosis indicator Cleaved caspase-3 (n = 4). (C) Representative images and quantitive analysis of autophagosomes stained by MDC (n = 6). (D) Representative immunofluorescent images of p62/SQSTM1 (green), aggresomes (red) and DAPI (blue) colocalization (n = 4). (E) Representative images and quantitive analysis of TUNEL-positive cells (red) and nuclear cells (DAPI, blue) (n = 5). *P < 0.05, **P < 0.01, ***P < 0.001. Data were analyzed by one-way ANOVA, followed by a Bonferroni post-hoc test, or analyzed by unpaired student's *t*-test. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

exhibits impaired autophagy and injured ON-bipolar cells and purkinje cells in the cerebellum, which leads to severe and progressive ataxia [33]. Hence, Vps34 complex is indispensable in the autophagy process, and manipulation of autophagy through promoting or inhibiting this complex is closely related to various diseases.

There are still several limitations in our research. Firstly, in vivo study is needed for further investigation. Secondly, the mechanism in the impaired assembly of Vps34-Beclin1-Atg14 under ischemic conditions hasn't been deeply explored, maybe the microenvironment factors such as oxidative stress and inflammation are involved.

In summary, persistent ischemia condition-induced autophagy inhibition in EPCs is firstly discovered in our present study, which may be attributed to the impaired Vps34 complex rather than the classic autophagy activation upstream factors. Recovery of this

Please cite this article as: R.-c. Jiang et al., Impaired Vps34 complex activity-mediated autophagy inhibition contributes to endothelial progenitor cells damage in the ischemic conditions, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2020.01.133

complex activity by Tat-Beclin1 effectively resist OGD-induced EPC injury. These observations state the specific target in EPC autophagy regulation and advance our understanding of autophagy manipulation in stem cell transplantation.

Author contributions

R.C.J., X.L.Z., Q.A.Z., X.Y.Z., H.J.S., Y.Q., G.P.Z. helped in collection and/or assembly of data; R.C.J., X.L.Z. contributed to data analysis and manuscript writing; J.D.L. and Q.X helped in design, data analysis, manuscript writing and final approval of the manuscript.

Declaration of competing interest

All authors declare no potential conflict of interest.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (grant no. 81302767 to Dr Qing Xiao, and grant no. 81573433 to Dr Jian-dong Luo), the Science and Technology Planning Project of Guangdong Province (grant no. 2017A020215194 to Dr Qing Xiao), the Medical Scientific Research Foundation of Guangdong Province (grant no. B2018054 to Dr Xiao-ling Zhang).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2020.01.133.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrc.2020.01.133.

References

[1] U. Bayraktutan, Endothelial progenitor cells: potential novel therapeutics for ischaemic stroke, Pharmacol. Res. 144 (2019) 181–191, https://doi.org/10.1016/j.phrs.2019.04.017.
[2] D.M. Smadja, A.J. Cornet, M. Aiach, P. Gaussem, Endothelial progenitor cells: characterization, in vitro expansion, and prospects for autologous cell therapy, Cell Biol. Toxicol. 23 (2007) 223–239.
[3] S.K. Sanganalmath, R. Bolli, Cell therapy for heart failure: a comprehensive overview of experimental and clinical studies, current challenges, and future directions, Circ. Res. 113 (2013) 810–834, https://doi.org/10.1161/CIRCRESAHA.113.300219.
[4] Z. Raval, D.W. Losordo, Cell therapy of peripheral arterial disease: from experimental findings to clinical trials, Circ. Res. 112 (2013) 1288–1302, https://doi.org/10.1161/CIRCRESAHA.113.300565.
[5] D.C.S. Pedroso, T. Ana, M. Liane, F.C. Isabel, D. Jo?O, C. Eugénia, F. Lino, Improved survival, vascular differentiation and wound healing potential of stem cells co-cultured with endothelial cells, PloS One 6 (2011), e16114.
[6] T. Frank, V.H. Filip, D.S. Magda, R. Robrecht, P. Frank, M.L. Buyzere, De, T.C. Gillebert, P. Jean, V. Bart, Endothelial outgrowth cells are not derived from CD133+ cells or CD45+ hematopoietic precursors, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 1572–1579.
[7] H. Bompais, J. Chagraoui, X. Canron, M. Crisan, X.H. Liu, A. Anjo, C. Tolla-Le Port, M. Leboeuf, P. Charbord, A. Bikfalvi, G. Uzan, Human endothelial cells derived from circulating progenitors display specific functional properties compared with mature vessel wall endothelial cells, Blood 103 (2004) 2577–2584, https://doi.org/10.1182/blood-2003-08-2770.
[8] C.F. Bento, M. Renna, G. Ghislat, C. Puri, A. Ashkenazi, M. Vicinanza, F.M. Menzies, D.C. Rubinsztein, Mammalian autophagy: How Does It Work? Annu. Rev. Biochem. 85 (2016) 685.
[9] Y.G. Zhao, H. Zhang, Core autophagy genes and human diseases, Curr. Opin. Cell Biol. 61 (2019) 117–125, https://doi.org/10.1016/j.ceb.2019.08.003.
[10] W. Hai-Jie, Z. Dan, T. Yu-Zhen, L. Ting, Autophagy in endothelial progenitor cells is cytoprotective in hypoxic conditions, Am. J. Physiol. Cell Physiol. 304 (2013) C617–C626.
[11] P. Zhou, Y.Z. Tan, H.J. Wang, G.D. Wang, Hypoxic preconditioning-induced autophagy enhances survival of engrafted endothelial progenitor cells in ischaemic limb, J. Cell Mol. Med. 21 (2017) 2452–2464, https://doi.org/10.1111/jcmm.13167.
[12] E.J. Marrotte, C. Dan-Dan, J.S. Hakim, A.F. Chen, Manganese Superoxide Dismutase Expression in Endothelial Progenitor Cells Accelerates Wound Healing in Diabetic Mice, 120, J. Clin. Invest., 2010, pp. 4207–4219.
[13] Q. Xiao, X.Y. Zhao, R.C. Jiang, X.H. Chen, X. Zhu, K.F. Chen, S.Y. Chen, X.L. Zhang, Y. Qin, Y.H. Liu, J.D. Luo, Increased expression of Sonic hedgehog restores diabetic endothelial progenitor cells and improves cardiac repair after acute myocardial infarction in diabetic mice, Int. J. Mol. Med. 44 (2019) 1091–1105, https://doi.org/10.3892/ijmm.2019.4277.
[14] W.J. Lu, H.B. Liang, Y.F. Li, X.Q. Tu, J.R. He, K.Q. Ding, G.Y. Yang, X.Y. Xin, L.L. Zeng, MicroRNA-210-3p targets RGMA to enhance the angiogenic functions of endothelial progenitor cells under hypoxic conditions, Front. Cell. Neurosci. 13 (2019) 223, https://doi.org/10.3389/fncel.2019.00223.
[15] X. Wu, Y. Qin, X. Zhu, D. Liu, F. Chen, S. Xu, D. Zheng, Y. Zhou, J. Luo, Increased expression of DRAM1 confers myocardial protection against ischemia via restoring autophagy flux, J. Mol. Cell. Cardiol. 124 (2018) 70–82, https://doi.org/10.1016/j.yjmcc.2018.08.018.
[16] X. Qing, H. Ning, W. Yan-Ping, H. Li-Shan, H. Yan-Hua, Z. Gui-Ping, Y. Quan, L. Shi-Ming, C. Min-Sheng, L. Jian-Dong, Impaired Sonic Hedgehog Pathway Contributes to Cardiac Dysfunction in Type 1 Diabetic Mice with Myocardial Infarction, 95, Cardiovasc. Res., 2012, pp. 507–516.
[17] W. Yan, Y. Guo, L. Tao, W.B. Lau, L. Gan, Z. Yan, R. Guo, E. Gao, G.W. Wong, W.L. Koch, Y. Wang, X.L. Ma, C1q/Tumor necrosis factor–related protein-9 regulates the fate of implanted mesenchymal stem cells and mobilizes their protective effects against ischemic heart injury via multiple novel signaling pathways, Circulation 136 (2017) 2162.
[18] Q. Xiao, Y. Yang, Y. Qin, Y.H. He, K.X. Chen, J.W. Zhu, G.P. Zhang, J.D. Luo, AMP-activated protein kinase-dependent autophagy mediated the protective effect of sonic hedgehog pathway on oxygen glucose deprivation-induced injury of cardiomyocytes, Biochem. Biophys. Res. Commun. 457 (2015) 419–425, https://doi.org/10.1016/j.bbrc.2015.01.006.
[19] J.M. Backer, The intricate regulation and complex functions of the Class III phosphoinositide 3-kinase Vps34, Biochem. J. 473 (2016) 2251–2271.
[20] R. Ksenia, S. Nicolas, O. Yohei, Z. Lufei, P. Els, J.E. Burke, G.R. Masson, J. Chris, S. Jan, N.T. Ktistakis, Structure and flexibility of the endosomal Vps34 complex reveals the basis of its function on membranes, Science 350 (2015) aac7365-aac7365.
[21] S.F. Funderburk, Q.J. Wang, Z. Yue, The Beclin 1-VPS34 complex–at the crossroads of autophagy and beyond, Trends Cell Biol. 20 (2010) 355–362, https://doi.org/10.1016/j.tcb.2010.03.002.
[22] H.E.J. Polson, D.L. Jane, D.J. Rigden, R. Marco, U. Sylvie, M.J. Clague, S.A. Tooze, Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation, Autophagy 6 (2010) 506–522.
[23] H.C. Dooley, M. Razi, H.E. Polson, S.E. Girardin, M.I. Wilson, S.A. Tooze, WIPI2 links LC3 conjugation with PI3P, autophagosome formation, and pathogen clearance by recruiting Atg12-5-16L1, Mol. Cell. 55 (2014) 238–252, https://doi.org/10.1016/j.molcel.2014.05.021.
[24] S.K. Sanae, S. Rhea, L. Matthew, G.R. Campbell, Z. Zhongju, K. Lisa, A.D. Wilkins, S. Qihua, P. Kathrin, M.D. Donna, Identification of a candidate therapeutic autophagy-inducing peptide, Nature 494 (2013) 201–206.
[25] K. Jujo, M. Ii, D.W. Losordo, Endothelial progenitor cells in neovascularization of infarcted myocardium, J. Mol. Cell. Cardiol. 45 (2008) 530–544.
[26] D. Dah-Ching, S. Woei-Cherng, L. Shinn-Zong, L. Hung, The role of endothelial progenitor cells in ischemic cerebral and heart diseases, Cell Transplant. 16 (2006) 273–284.
[27] J.V. Terrovitis, R.R. Smith, E. Marban, Assessment and optimization of cell engraftment after transplantation into the heart, Circ. Res. 106 (2010) 479–494, https://doi.org/10.1161/CIRCRESAHA.109.208991.
[28] P. Lipiec, M. Krzeminska-Pakula, M. Plewka, J. Kusmierek, A. Plachcinska, R. Szuminski, T. Robak, A. Korycka, J.D. Kasprzak, Impact of intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction on left ventricular perfusion and function: a 6-month follow-up gated 99mTc-MIBI single-photon emission computed tomography study, Eur. J. Nucl. Med. Mol. Imag. 36 (2009) 587–593, https://doi.org/10.1007/s00259-008-0988-6.
[29] R. Singh, A.M. Cuervo, Autophagy in the cellular energetic balance, Cell Metabol. 13 (2011) 495–504, https://doi.org/10.1016/j.cmet.2011.04.004.
[30] S. Misra, G.J. Miller, J.H. Hurley, Recognizing phosphatidylinositol 3-phosphate, Cell 107 (2001) 559–562, https://doi.org/10.1016/s0092-8674(01)00594-3.
[31] C. Chang, L.N. Young, K.L. Morris, S. von Bulow, J. Schoneberg, H. Yamamoto-Imoto, Y. Oe, K. Yamamoto, S. Nakamura, G. Stjepanovic, G. Hummer, T. Yoshimori, J.H. Hurley, Bidirectional control of autophagy by BECN1 BARA domain dynamics, Mol. Cell. 73 (2019) 339–353, https://doi.org/10.1016/j.molcel.2018.10.035, e336.
[32] G. Grieco, V. Janssens, H.P. Gaide Chevronnay, F. N'Kuli, P. Van Der Smissen, T. Wang, J. Shan, S. Vainio, B. Bilanges, F. Jouret, B. Vanhaesebroeck, C.E. Pierreux, P.J. Courtoy, Vps34/PI3KC3 deletion in kidney proximal tubules impairs apical trafficking and blocks autophagic flux, causing a Fanconi-like syndrome and renal insufficiency, Sci. Rep. 8 (2018) 14133, https://doi.org/10.1038/s41598-018-32389-z.
[33] F. He, R.M. Nichols, L. Kailasam, T.G. Wensel, M.A. Agosto, Critical role for phosphatidylinositol-3 kinase Vps34/PIK3C3 in ON-bipolar cells, Invest. Ophthalmol. Vis. Sci. 60 (2019) 2861–2874, https://doi.org/10.1167/iovs.19-26586.

Please cite this article as: R.-c. Jiang et al., Impaired Vps34 complex activity-mediated autophagy inhibition contributes to endothelial progenitor cells damage in the ischemic conditions, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2020.01.133