Am J Physiol Cell Physiol 304: C614–C616, 2013;
doi:10.1152/ajpcell.00046.2013.

Autophagy - the friendly fire in endothelial cell regeneration. Focus on "Autophagy in endothelial progenitor cells is cytoprotective in hypoxic conditions"

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CARDIOVASCULAR DISEASE remains the number one killer in the Western world and is growing rapidly in developing countries in Asia and South America (4). While significant progress has been made in understanding the pathogenic process responsible for acute ischemic events such as myocardial infarction and stroke, the cellular response mechanism to hypoxia has remained largely elusive despite extensive investigations in the past three decades. In this issue, Wang et al. (11) identified an essential role of autophagy to protect the survival of endothelial progenitor cells (EPCs) undergoing hypoxia, raising the prospect that modulation of autophagic response in EPCs may provide significant therapeutic advantage following EPCs transplantation.

In the quest to find effective treatments for cardiovascular diseases, regenerative therapies utilizing adult EPCs have emerged as a potential breakthrough. These rare cells, which can differentiate into the endothelial cells lining blood vessels, travel the bloodstream until specific cytokines instruct them to settle in a specific region of the body and initiate blood vessel formation (10). EPCs also catalyze angiogenesis during tumor formation, and thus, represent a prospective cellular target for cancer therapy (9). Additionally, particular interest has been given to EPC-based transplantation therapies for their potential to restore vascular function following instances of ischemia. Recent clinical studies have raised the prospect that EPC-based transplantation therapies may improve vascular function in

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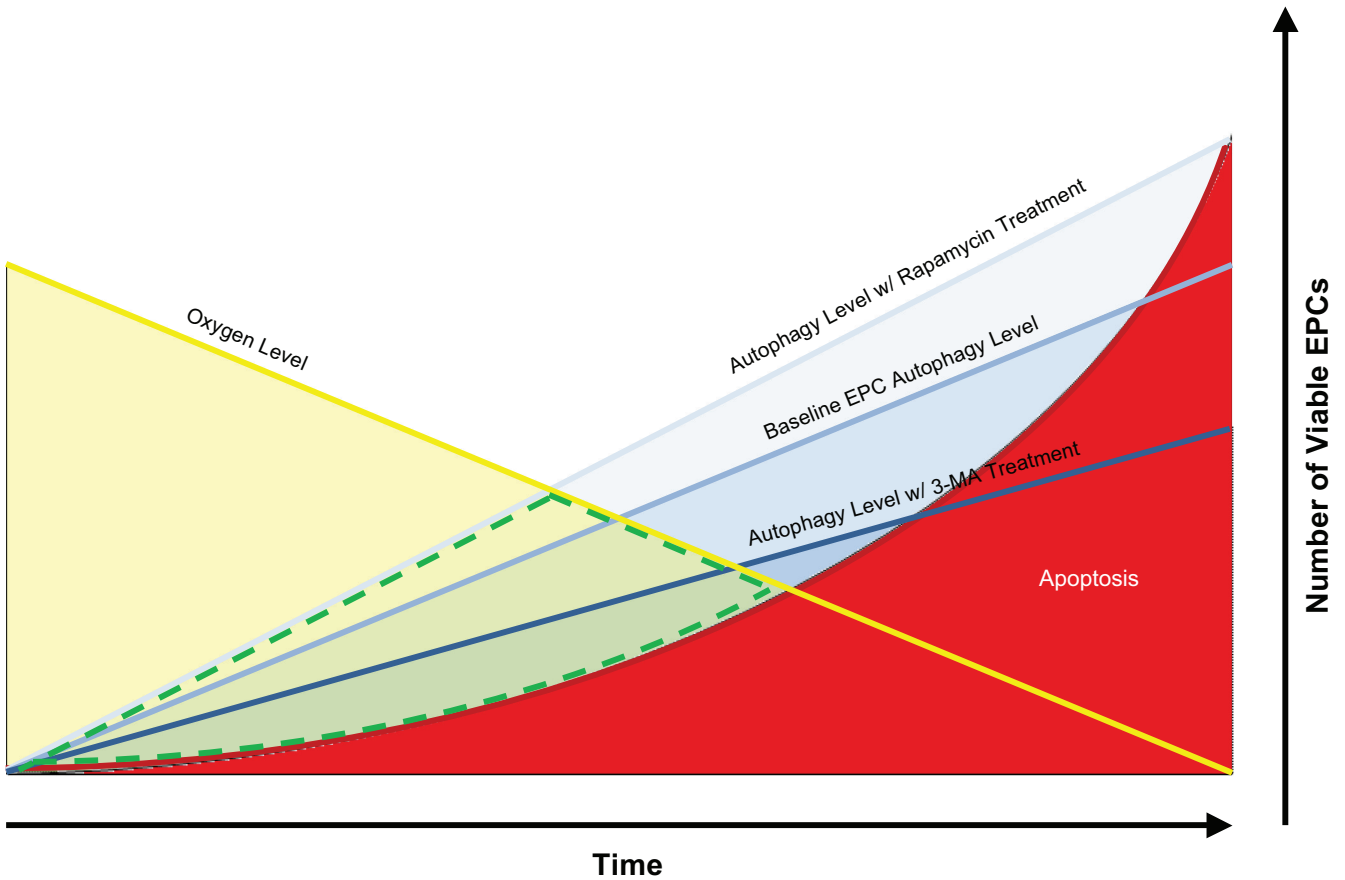

Fig. 1. Hypoxia upregulates autophagy and apoptosis in a time-dependent manner. Small-molecule treatment can alter EPC autophagy levels. The area outlined in green represents a "goldilocks" region where hypoxia induces the maximal level of autophagy while minimizing apoptosis.

patients with ischemic disorders such as peripheral artery disease or in individuals having a recent, acute ischemic event such as myocardial infarction (1, 5). However, high rates of cell death and ineffective cell engraftment have plagued these otherwise promising transplantation trials (3). Thus, identifying the mechanisms regulating EPC survival after transplantation is essential for improving EPC-based therapy for patients with chronic or acute ischemia.

Along this line, the determination of optimal conditions at which EPCs can grow, proliferate, and differentiate is crucial for the survival and engraftment of EPCs after transplantation. Previous studies have shown that hypoxic conditions are beneficial to stem and progenitor cell growth and survival because the embryonic environment in which these cells are often found are typically under low oxygen tension (2, 8). Additionally, the self-degradation of damaged intracellular components, or autophagy, has been shown to have a "protective" effect during times of cellular stress (7). Loos et al. (6) demonstrated that a low-oxygen environment induces this protective autophagy and moderate apoptosis at early time points but promotes major apoptosis and destructive necrosis after longer exposure to hypoxic conditions. Thus there appears to be a critical time period following hypoxic exposure that leads to activation of the autophagic response without significant cell death (Fig. 1). In this issue, Wang et al. illustrate that in EPCs, hypoxia enhances autophagy in a time-dependent manner. The authors first isolated EPCs from human umbilical cord blood and then, using flow cytometry, sorted for $CD34^+$ $VEGFR\text{-}2^+$ cell populations. EPCs concurrently express CD34, a cell-cell adhesion protein found in mesodermally derived progenitor cells, and vascular endothelial growth factor receptor-2 (VEGFR-2), a marker for vascular endothelium. These EPCs were able to proliferate and differentiate into endothelial cells after 10 days of stimulation with VEGF ligand, a driver of endothelial cell development. The differentiated cells exhibit positive immunostain for the endothelial-specific protein CD31 also known as PECAM-1 (platelet endothelial cell adhesion molecule-1), confirming their fate.

Notably, hypoxic conditions enhance autophagy in EPCs in a time-dependent manner. The authors demonstrate that subjecting EPCs for up to two hours of extended hypoxic conditions increases the expression of Beclin-1, a critical protein for autophagosome formation, and LC3, a specific marker for autophagosome structures. For Beclin-1, hypoxia upregulates mRNA and protein expression levels of Beclin-1 as demonstrated by quantitative PCR analysis and immunofluorescence staining. Transmission electron microscopy demonstrated a hypoxia-driven increase in autophagosome cross-sectional area and the number of visible autophagosomes and lysosomes. Since lysosomal degradation represents the final destination for all autophagic recycling pathways, the upregulation of lysosome quantity is consistent with the increase in autophagosomes shown in this study.

Interestingly, while the autophagic response is considered protective to normal EPCs during hypoxic challenge, the inhibition of autophagosome formation with 3-methyladenine (3-MA), an inhibitor of the P13K signaling pathway, significantly reduces EPC proliferation and long-term viability. This suggests that signaling pathways that are downstream of autophagy are critical to cell survival. Pretreatment of EPCs with 3-MA markedly reduces Beclin-1 expression and the size and number of autosome and lysosome and abrogates the hypoxia-induced upregulation of autophagosome and lysosome formation. Additionally, 3-MA pretreatment prior to placement in hypoxic conditions significantly enhances EPC apoptosis, implying that autophagy plays a protective role against cell death when EPCs are subjected to a low-oxygen environment. To support this claim, the authors pretreated EPCs with rapamycin, an inhibitor of the mTOR pathway and an inducer of autophagy. In contrast to 3-MA, rapamycin pretreatment reduces EPC apoptosis in hypoxic conditions, suggesting that enhancing autophagy may improve cell survival when EPCs are cultured under hypoxic conditions. This is provided that the hypoxic condition is only temporary since it is well known that prolonged hypoxia promotes apoptosis and necrosis in a number of cell types (6). It will be important to fine-tune the balance between the induction of autophagy to promote EPC survival under low O_2 tension and the excessive hypoxia that leads to cellular stress and subsequent apoptosis such that the most optimal protocol for enhancing EPC survival following transplantation can be developed ex vivo.

While the modulation of autophagic pathways for clinical application of EPCs in cell-based therapy will require further refinement and validation, the findings here offer new insights into key signaling pathways that may potentially improve EPC integrity under hypoxic conditions. Hopefully, this will also translate into improvement in patient outcomes in cell transplantation studies.

GRANTS

This work was supported in part by National Institutes of Health (NIH)/National Heart, Lung, and Blood Institute and an NIH Director's Innovator Award (to S. M. Wu).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

A.S. prepared the figure; A.S. and S.M.W. drafted the manuscript; A.S. and S.M.W. edited and revised the manuscript; S.M.W. approved the final version of the manuscript.

REFERENCES

1. **Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, Grunwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, Zeiher AM.** Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). *Circulation* 106: 3009–3017, 2002.
2. **Basciano L, Nemos C, Foliguet B, de Isla N, de Carvalho M, Tran N, Dalloul A.** Long term culture of mesenchymal stem cells in hypoxia promotes a genetic program maintaining their undifferentiated and multipotent status. *BMC Cell Biol* 12: 12, 2011.
3. **Chavakis E, Koyanagi M, Dimmeler S.** Enhancing the outcome of cell therapy for cardiac repair: progress from bench to bedside and back. *Circulation* 121: 325–335, 2010.
4. **Gaziano TA.** Cardiovascular disease in the developing world and its cost-effective management. *Circulation* 112: 3547–3553, 2005.
5. **Lawall H, Bramlage P, Amann B.** Treatment of peripheral arterial disease using stem and progenitor cell therapy. *J Vasc Surg* 53: 445–453, 2011.
6. **Loos B, Genade S, Ellis B, Lochner A, Engelbrecht AM.** At the core of survival: autophagy delays the onset of both apoptotic and necrotic cell death in a model of ischemic cell injury. *Exp Cell Res* 317: 1437–1453, 2011.
7. **Mizushima N, Levine B, Cuervo AM, Klionsky DJ.** Autophagy fights disease through cellular self-digestion. *Nature* 451: 1069–1075, 2008.

8. **Prado-Lopez S, Conesa A, Arminan A, Martinez-Losa M, Escobedo-Lucea C, Gandia C, Tarazona S, Melguizo D, Blesa D, Montaner D, Sanz-González S, Sepúlveda P, Götz S, O'Connor JE, Moreno R, Dopazo J, Burks DJ, Stojkovic M.** Hypoxia promotes efficient differentiation of human embryonic stem cells to functional endothelium. *Stem Cells* 28: 407–418, 2010.
9. **Shaked Y, Henke E, Roodhart JM, Mancuso P, Langenberg MH, Colleoni M, Daenen LG, Man S, Xu P, Emmenegger U** Rapid chemotherapy-induced acute endothelial progenitor cell mobilization: implications for antiangiogenic drugs as chemosensitizing agents. *Cancer Cell* 14: 263–273, 2008.
10. **Urbich C, Dimmeler S.** Endothelial progenitor cells: characterization and role in vascular biology. *Circ Res* 95: 343–353, 2004.
11. **Wang HJ, Zhang D, Tan YZ, Li T.** Autophagy in endothelial progenitor cells is cytoprotective in hypoxic conditions. *Am J Physiol Cell Physiol* (December 26, 2012). doi:10.1152/ajpcell.00296.2012.