Scientific Paper:

Am J Physiol Cell Physiol 298, 1527 – 1537, 2010

Adaptation to oxygen deprivation in cultures of human pluripotent stem cells, endothelial progenitor cells, and umbilical vein endothelial cells

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Abstract:

Hypoxia plays an important role in vascular development through hypoxia-inducible factor-1α (HIF-1α) accumulation and downstream pathway activation. We sought to explore the in vitro response of cultures of human embryonic stem cells (hESCs), induced pluripotent stem cells (iPSCs), human endothelial progenitor cells (hEPCs), and human umbilical cord vein endothelial cells (HUVECs) to normoxic and hypoxic oxygen tensions. We first measured dissolved oxygen (DO) in the media of adherent cultures in atmospheric (21 % O_2), physiological (5 % O_2), and hypoxic oxygen conditions (1% O_2). In cultures of both hEPCs and HUVECs lower oxygen consumption was observed when cultured in 1 % O_2. At each oxygen tension, feeder-free cultured hESCs and iPSCs were found to consume comparable amounts of oxygen. Transport analysis revealed that the oxygen uptake rate (OUR) of hESCs and iPSCs decreased distinctly as DO availability decreased, whereas the OUR of all cell types was found to be low when cultured in 1 % O_2, demonstrating cell adaptation to lower oxygen tensions by limiting oxygen consumption. Next, we examined HIF-1α accumulation and the expression of target genes, including VEGF and angiopoietins (ANGPT; angiogenic response), GLUT-1 (glucose transport), BNIP3, and BNIP3L (autophagy and apoptosis). Accumulations of HIF-1α were detected in all four cell lines cultured in 1 % O_2. Corresponding upregulation of VEGF, ANGPT2, and GLUT-1 was observed in response to HIF-1α accumulation, whereas upregulation of ANGPT1 was detected only in hESCs and iPSCs. Upregulation of BNIP3 and BNIP3L was detected in all cells after 24-h culture in hypoxic conditions, whereas apoptosis was not detectable using flow cytometry analysis, suggesting that BNIP3 and BNIP3L can lead to cell autophagy rather than apoptosis. These results demonstrate adaptation of all cell types to hypoxia but different cellular responses, suggesting that continuous measurements and control over oxygen environments will enable us to guide cellular responses.

Key-words: oxygen, embryonic stem cells, induced pluripotent stem cells, human umbilical cord vein endothelial cells, angiogenesis, hypoxia