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Commentary

Commentary on "Bach1 differentially regulates distinct Nrf2-dependent genes in human venous and coronary artery endothelial cells adapted to physiological oxygen levels" by Chapple et al.

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Physiologists have long recognized that what are often referred to as hypoxia and normoxia in cell culture studies are actually normoxia and hyperoxia, except for cells from the few tissues that are directly exposed to the atmosphere (*e.g.* the cornea, alveolus, *etc.*). In this outstanding original research article, Chapple and coworkers [1] have demonstrated the significance of getting this right. They have provided important mechanistic understanding of the differential roles of Bach1, a suppressor of Nrf2, on antioxidant genes, NQO1 and HO-1 and others, in normoxia and hyperoxia. Furthermore, they have shown how Bach1 independent regulation of glutathione biosynthesis in normoxia involves induction of the modulatory subunit of glutamate cysteine ligase, which increases the rate of catalysis for the first step in glutathione biosynthesis, and induction of xCT, the transport protein responsible for mediating uptake of cystine and glutamate. Cystine is reduced to cysteine that, along with glutamate and glycine, is used in *de novo* glutathione biosynthesis.

Although studies on Nrf2 regulated genes is the primary focus of this paper, analysis of cell ultrastructure, quantification of cytosolic oxygen concentration using a cell-penetrating phosphorescent oxygen-sensitive nanoparticle probe, indices of cell proliferation and adherence using impedance, and a picture of differences in oxidation in mitochondria using MitoSox, places these findings into a wider physiological context. Adaptation of cells to physiological oxygen levels provides a more appropriate *in vitro* model to characterize intracellular signaling pathways.

One curiosity is that the authors tell us that the HUVEC cells have been "adapted to physiological oxygen levels" when it is actually their normal state. Of course, the cells would have to have made many adaptations to return to something approaching the 3–5% levels of oxygen that most cells experience *in vivo*. Nevertheless, both the experiment, and the terminology remind us that the field has largely succumbed to the idea that "normal" is culture in 5% CO_2 and the approximately 20–21% oxygen found in room air. Indeed, findings presented by Chapple et al. demonstrate the adaptive plasticity of Nrf2-dependent gene expression following *adaptation* or *readaptation* to physiological oxygen or hyperoxia. In fact, one of the great difficulties encountered in the early days of cell culturing was that many/most cell types simply could not survive the high oxygen levels in our air. The cells that survived and flourished, and those we largely culture to this day, were those with strong defense/repair mechanisms, and those that could adapt to unusually high oxygen. Indeed, when we first demonstrated adaptation to oxidative stress in mammalian cells, the greatest problem we had was in finding cells that had not already adapted to stress because of the high oxygen levels in our culture system [2,3]. It also became quickly apparent that cells cultured in normoxic 3–5% oxygen had far greater adaptive capacities than did their counterparts cultured in 20–21% oxygen.

Campisi and colleagues have shown that that the phenotypes of senescent human fibroblasts and mouse embryonic fibroblasts (MEFs) are strongly affected by culturing in 3% oxygen versus 20% oxygen [4]. This is one of a series of studies from the Campisi group demonstrating the importance of oxygen levels in cell culture studies trying to unravel the mechanisms of aging and senescence. These are interesting findings, but as highlighted by phosphorescent nanoparticle measurements of cytosolic oxygen content in the study by Chapple et al. (see Fig. 1B) (1), culture of MEFs under 3% oxygen would most likely have resulted in an intracellular oxygen content ~1.5–2% oxygen and stabilization of HIF-1α. Given the growing appreciation of the importance of Nrf2 in age-related processes, the new findings of Chapple et al. highlighted in this Commentary will hopefully be a stimulus to more studies at physiologically relevant oxygen tension.

A very interesting finding for us is that Bach1 plays a major suppressive role in normoxia but a lesser role in hyperoxia. We have reported that Bach1 increases in aging (from studies with young and old animals) and likely accounts for the part of the suppression of Nrf2 signaling that occurs with age [5,6]. Thus, it would be interesting to compare HUVEC cells with endothelial cells from the mother's microvasculature to ascertain whether this oxygen plasticity is affected by lifespan known to affect the expression of Nrf2 regulators such as Bach1.

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