Redox and metal profiling in ischemic stroke and endothelial cells adapted to physiological oxygen levels

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In vivo, vascular and other cell types are exposed to physiological oxygen levels ranging from to 2 – 13 kPa O_2 while cells cultured in vitro in standard CO_2 gassed incubators are routinely exposed to hyperoxic oxygen levels (18 kPa O_2). Although recent evidence highlights the importance of studying cellular redox signaling under physiological O_2 levels, few studies have examined the effects of short- or long-term adaptation of cells to different pericellular O_2 levels (see Keeley & Mann, Physiol. Reviews 2019; 99:161-234). As molecular mechanisms regulating NRF2 mediated redox signaling have primarily been studied in cells exposed to hyperoxia, we characterised NRF2 gene targets in endothelial cells following 5 d adaptation to hyperoxia (18 kPa), physiological normoxia (5 kPa) or hypoxia (1 kPa) in an O_2 regulated Scitive workstation. Gene profiling established that activation of NRF2 and induction of GSH related genes were insensitive to alterations in O2 whereas upregulation of HO-1 and NQO1 in response to electrophiles or NO was diminished under 5 kPa O_2 due to an upregulation of the NRF2 repressor Bach 1 (Chapple et al. Free Radic Biol Med 2016; 92: 152-62. We further established that a PP2A mediated feedback mechanism regulates Ca^{2+} dependent endothelial NO synthesis (Keeley et al. FASEB J 2017; 31: 5172-5183) and NO bioavailability (Sevimli et al. Redox Biol. 2022; 53: 102319) under 5 kPa O_2 and that enhanced SERCA activity under 5 kPa O_2 protects endothelial cells against calcium overload (Keeley et al. FASEB J 2018; 32: 2531-2538). We recently employed ICP-MS and LA-ICP-MS to measure changes in total metal content in human coronary artery endothelial cells cultured long-term under 18, 5 kPa or 1 kPa O_2 and then subjected to ischemia reoxygenation injury. In a recent Expert Recommendation (Sies et al. Nature Rev Mol Cell Biol 2022; 23: 499-515), we emphasize the importance of maintaining physiologically relevant O_2 levels in cell culture to mimic redox reactions associated with specific cell types in vivo.

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