

# Crosstalk between Nrf2 Signalling and Zinc in Human Coronary Artery Cells under Hyperoxia, Physiological Normoxia and Hypoxia

F. Yang<sup>1</sup>, M. Smith<sup>1</sup>, A. Morrell<sup>1</sup>, T. Stewart<sup>1</sup>, W. Maret<sup>1</sup>, G. Mann<sup>1</sup>

<sup>1</sup>King's College London, London, United Kingdom

Zinc is an important component of the cellular antioxidant defence and dysregulation of zinc homeostasis is a risk factor for coronary heart disease and is associated with oxidative damage in ischemia-reperfusion injury. This study aimed to (i) characterise the metallomics and redox phenotype of human coronary artery smooth muscle cells (HCASMC) and human coronary artery endothelial cells (HCAEC) adapted long-term (5 days) to hyperoxia (18 kPa O<sub>2</sub>), physiological normoxia (5 kPa O<sub>2</sub>) or hypoxia (1 kPa O<sub>2</sub>) and (ii) investigate crosstalk between Zn and Nrf2 signalling under 18 or 5 kPa O<sub>2</sub>. When HCASMC and HCAEC were adapted to 18, 5 or 1 kPa O<sub>2</sub>, HIF-1 $\alpha$  stabilisation was only observed in cells under 1 kPa O<sub>2</sub>. The redox phenotype of HCASMC adapted long-term to 5 kPa O<sub>2</sub>, was affected negligibly as evidenced by negligible changes in intracellular GSH levels and Nrf2-targeted HO-1, whilst both were significantly lower in HCAEC adapted to 5 and 1 kPa compared to 18 kPa O<sub>2</sub>. Total Zn66 levels determined by ICP-MS analysis were similar in HCASMC under 18, 5 kPa or 1 kPa O<sub>2</sub> (18 kPa =  $0.345 \pm 0.090$  ng/ $\mu$ g protein, 5 kPa =  $0.298 \pm 0.020$  ng/ $\mu$ g protein, 1 kPa =  $0.441 \pm 0.058$  ng/ $\mu$ g protein) but decreased as pericellular O<sub>2</sub> decreased in HCAEC (18 kPa =  $0.345 \pm 0.056$  ng/ $\mu$ g protein, 5 kPa =  $0.267 \pm 0.032$  ng/ $\mu$ g protein, 1 kPa =  $0.177 \pm 0.020$  ng/ $\mu$ g protein). The effects of pericellular O<sub>2</sub> levels on redox phenotype and total Zn66 content are thus cell-type specific. Notably, Zn supplementation induced Nrf2 nuclear accumulation in HCASMC not in HCAEC under 18 or 5 kPa O<sub>2</sub>, and whilst Nrf2 siRNA silencing did not significantly alter Zn66 content in HCASMC it led to a significant decrease in HCAEC under 18 kPa O<sub>2</sub>. Our study highlights the critical importance of adapting cells in vitro to physiological O<sub>2</sub> levels and provides the first insights into the crosstalk between Nrf2 and Zn in human coronary artery cells.