PROTEINS REVERSIBLY OXIDIZED IN CYSTEINES IN ENDOTHELIAL CELLS IN RESPONE TO ACUTE HYPOXIA

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Cells and living organisms have developed different systems to sense the availability of oxygen (O_2), allowing them to promote specific answers to adapt to hypoxic environments. In metazoans, cells respond to hypoxia exposition by activating transcriptional programs induced by a set of hypoxia-inducible factors (HIF). Examples of these adaptations include activation of the angiogenic response by overexpressing VEGF, or metabolic adaptations that occur in tissues of high oxygen demand, such as cardiac and skeletal muscle. There are already enough evidences showing that there is an increase in hypoxic ROS production, especially from the mitochondria, which is involved in the activation of the HIF pathway. ROS, in addition to being able to alter the cellular redox balance, leading to a situation of stress, are used by the cell in signal sense and transmition by reversibly modifying the redox state of cysteine residues of proteins.

We have used a novel redox proteomics methodology for fluorescently labelling reversibly oxidized cysteines to evaluate this kind of modification in endothelial cells subjected to short (2 h) hypoxia and reoxygenation. We found a general increase in cysteine oxidation during hypoxia, which was reverted in reoxygenation. When coupled to two-dimensional electrophoresis, a pattern of specific proteins was shown to be differentially oxidized, unlike the unspecific pattern obtained with a general oxidizing agent such as diamide. We have carried out the identification of specific targets in order to get further insight into the possible mechanism of ROS signalling in hypoxic conditions.

These kind of techniques have been frequently used to identify proteins that are differentially oxidized after strong oxidative treatments, but we show that it can be useful for physiological treatments producing lower oxidation but in specific proteins.