

Proteome analysis for protein expression of cultured human dermal microvascular endothelial cells by hydrogen peroxide

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Reactive oxygen species (ROS) have been traditionally regarded as toxic byproducts of aerobic metabolism. However, ROS can also act as intracellular signaling molecules in vascular cells and they can mediate phenotypes in vascular endothelial cells that may be considered both physiological and pathological. To clarify the molecular mechanisms of ROS signaling, we examined the H₂O₂-responsive proteins using proteomics tools. Protein expression of endothelium was studied in cultured human dermal microvascular endothelial cells exposed to low- and high-level hydrogen peroxide at various periods. We also examined the intracellular ROS production using flow cytometry and spectrophotometer. We separated the proteins from dose dependent and time course series experimental conditions by two-dimensional electrophoresis, and they were visualized by silver staining and quantified by image processing. The proteins of interest were subjected to in-gel digestion with trypsin, and the masses of resulting peptides were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. This tryptic mass map was used to identify the proteins through the search of a protein-sequence database. At least 117 proteins were stimulated by H₂O₂, whereas 46 other proteins were repressed by this treatment. The majority of changes occurred within the first 15 min of cell stimulation. Oxidative stress challenge results in a dramatic change of genomic response proteins and their

mapping into cellular process give a global view of the ubiquitous cellular changes elicited by H_2O_2 . The results could provide the framework for understanding the mechanisms of cellular redox homeostasis and H_2O_2 metabolism.