ADVANCES IN QUANTITATIVE PROTEOMICS: APPLICATIONS TO CARDIOVASCULAR RESEARCH

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In spite of the unprecedented protein depth and quantification accuracy attained by current stable isotope labeling (SIL) techniques, important issues remain unresolved, including the absence of suitable protocols and statistical models of general applicability and the difficulty of making meaningful interpretations of global proteome alterations. We recently described a model for the analysis of ^{18}O labeling data that decomposes variance at the spectrum, peptide and protein levels, and a statistically-validated protocol for sample preparation and labeling suitable for any kind of proteomes. In a large-scale collaborative project involving several laboratories, we have further developed the model and demonstrate that this statistical theory provides a general framework for the analysis of any kind of SIL results. Furthermore, it allows statistical analysis at upper layers of complexity, including coherent integration of quantitative data from different experiments and threshold-free onthological analysis of global proteome behavior.

This general statistical theory has proved extremely successful to interpret results obtained in several research projects in the cardiovascular area. The increase in statistical power achieved at the uppermost levels is shown in several studies, including elucidation of molecular mechanisms underlying cardioprotection and the effects of ischemia-reperfusion in rat heart and in mouse models of heart preconditioning. Analysis of variance at the protein level was also crucial to determine a pattern of consistent alterations in the human high-density lipoprotein proteome after angioplasty within a background of strong inter-individual variation, as well as the specific subset of proteins secreted by human T-cells upon activation. Furthermore, analysis of variance at the peptide level was essential to develop GELSILOX, a novel, high-throughput technique that has allowed determination of Cys sites that increase their oxidized state upon oxygen peroxide stimulation in endothelial cells, as well as the effect of oxidative stress upon mitochondrial proteome of cardiomyocites in a model of ischemia-reperfusion.