

Proteomic analysis of atherosclerosis: What are the challenges for analyzing the data and where are the opportunities for bioinformatics to help us understand our results?

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There are two general approaches to proteomics experiments, experiments that use electrophoresis to separate and quantify the proteins (so-called top-down approaches) and multidimensional chromatography experiments with protein digests (bottom-up approaches). The common part of each approach is the use of tandem mass spectrometry to sequence peptides and identify proteins. One role for bioinformatics has been to develop robust methods to relate the mass spectrometry data to sequence database information to identify the proteins. These efforts have resulted in a number of search programs, such as Sequest and Mascot, which are now mature, robust, and reliable tools. The new challenges that have emerged are in the areas of results organization, analysis, and (in some settings) communication.

Our laboratory has been using a standard top-down approach to the proteomics experiments in several systems. These experiments have used 2D electrophoresis, tryptic digestion, and tandem mass spectrometry to detect and identify a group of differentially expressed proteins for additional studies. In our studies related to atherosclerosis, for example, we developed a model of chronic oxLDL-exposure under cell culture conditions to determine if the hallmark parameters of oxLDL-uptake and cytotoxicity are altered during foam cell formation, and to determine if the changes in protein expression that may accompany acute and chronic oxLDL-exposure. In the cell system, the extent of oxLDL-uptake did not change but a resistance to oxLDL-induced cytotoxicity was observed in the chronically-exposed model. Macrophages subjected to chronic oxLDL-exposure required a 40% increase in oxLDL concentration to achieve 50% survival after 48 hours, in comparison to macrophages subjected to a single exposure to oxLDL. A main feature of the differential proteome was a statistically significant increase in the antioxidant response of the chronically-exposed macrophages. This increased protective response may contribute to the survival of the foam cell, which assumes a pro-atherogenic role by promoting a continued inflammatory response.

The results of these studies also highlight two issues in the handling and analysis of the proteomics data; the need to organize the data in a manner that makes subsequent experiments sequentially easier because the protein identifications have already been made, and the desire to utilize the immediate results to rationally expand into a secondary set of targets. These issues represent opportunities for bioinformatics solutions to maximize the power of proteomic experiments.