53a Plenary: Prefractionation of Serum Proteins Using Microscale Solution Isoelectrofocusing to Enhance Detection of Low Abundance Proteins for Biomarker Discovery

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Systematic identification of low abundance proteins in human plasma and serum is problematic due to the extreme sample complexity and wide range of protein concentrations. In addition, a few major serum proteins severely limit sample loading capacities for most separation methods, thereby making detection of potential disease biomarkers in the ng/ml to pg/ml ranges difficult. To enable detection of these low abundance proteins, reduction of proteome complexity by prefractionation is essential. Microscale solution isoelectrofocusing (MicroSol-IEF) prefractionation using the ZOOM IEF Fractionator provides a simple convenient method for high resolution separation of complex proteomes based upon their isoelectric points. We recently incorporated MicroSol-IEF prefractionation into a novel 4-dimensional separation strategy that is highly effective in reducing sample complexity and allows greater detection of lower abundance proteins. This strategy consists of three orthogonal protein separations: major protein immunodepletion, MicroSol-IEF, and 1-D SDS PAGE. The result is a 2-dimensional array of pixels or gel slices that is equivalent to a low resolution 2-D gel, since each pixel in the array contains a group of proteins with a known pI and molecular weight range. Each pixel is then digested with trypsin followed by nanocapillary reversed phase tryptic peptide separation prior to tandem mass spectrometry analysis. When human serum was analyzed, more than 2,700 proteins spanning up to nine-orders-of-magnitude were identified using HUPO criteria for high confidence assignments. More importantly, a substantial number of low abundance proteins (< 100 ng/ml to pg/ml range) were identified. We are currently using this multi-dimensional profiling strategy to analyze sera from SCID mice harboring human melanoma, lung, breast or colorectal carcinomas for potential human cancer biomarkers. Preliminary results indicate that a substantial number of human proteins, representing potential biomarkers secreted by the tumors, have been identified. Therefore, the methods described here provide an efficient proteomics platform for comprehensive protein profiling and cancer biomarker discovery.