Global Proteomic Analysis of Mouse Serum

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Mouse models, either transgenic or xenograft, represent invaluable experimental systems for understanding cancer pathogenesis. An obvious advantage in using mouse models over human subjects to investigate the proteomic changes in serum/plasma that indicate disease onset is that issues related to genetic background and lifestyle can be controlled, minimizing the large background variability seen within samples acquired from human patients, thereby streamlining the discovery process. Proteomic analysis of these systems will enable more precise comparisons between serum/plasma from control and cancer mouse models.

A multi-dimensional peptide separation strategy utilizing conventional separation techniques combined with tandem mass spectrometry (MS/MS) was employed for a proteome analysis of mouse serum (Figure 1.). A mouse serum proteome sample was fractionated at the intact protein level by weak anion exchange (WAX) and weak cation exchange (WCX) chromatography. Each of the resolved fractions (seven WAX and five WCX fractions) was digested with trypsin and further resolved by SCX chromatography. The SCX fractions were analyzed by microcapillary reversed-phase liquid chromatography (μRPLC) coupled online with MS/MS analysis.

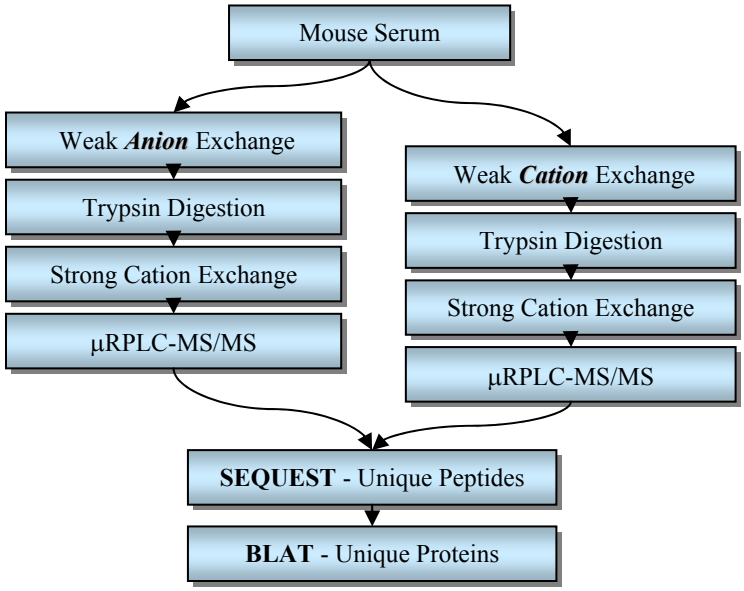

Figure 1. Global Mouse Proteome Analysis Strategy

This investigation resulted in the identification of over 12,000 unique peptides. A modified **B**last-**L**ike **A**lignment **T**ool (BLAT) search was utilized to remove redundant protein identifications by using unique protein identifiers to accurately establish the presence of a specific protein instead of those identified based on protein class, family or similarity with the result of 4,338 unique proteins being identified in mouse serum. Proteins from all functional classes, cellular localization, and abundance levels were identified, including several low abundance proteins. Interestingly, 45% of the proteins identified have membrane-associations, lending support to the presence of shed species in serum (Figure 2.). This investigation provides the foundation for functional serum/plasma analyses in mouse model systems of cancer.

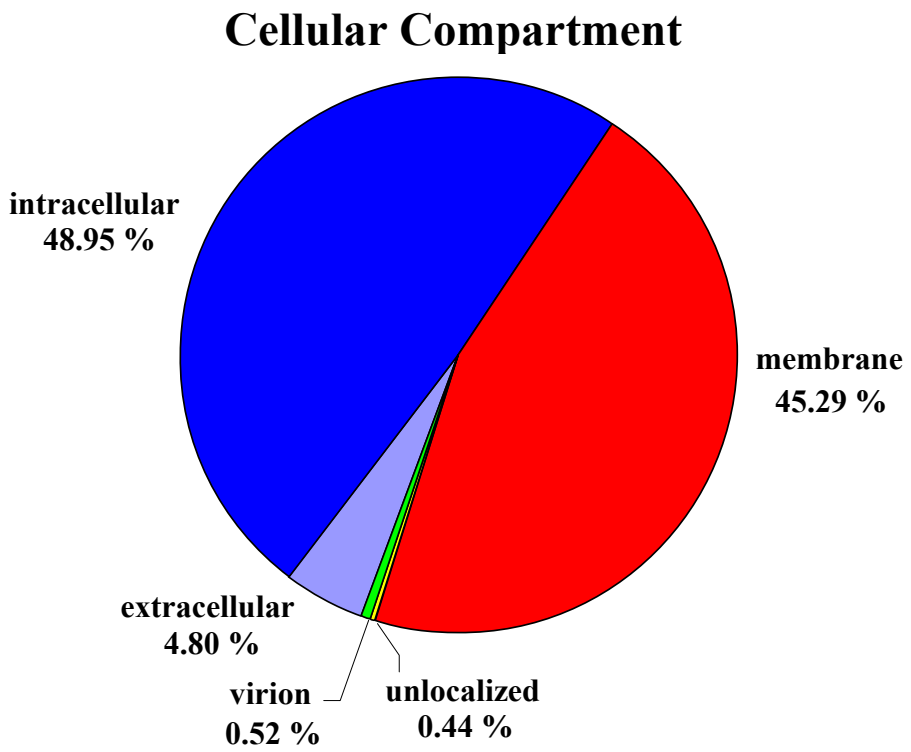

Figure 2. Mouse Serum Proteome and Gene Ontology Analysis. Unique proteins and peptides identified in global mouse serum proteome analysis. The pie chart illustrates the classification of the proteins identified in mouse serum according to cellular compartment, with a significant portion identified associated with the cell membranes.

These findings show that the protein content of serum is quite reflective of the overall profile of an organism and a conventional multi-dimensional fractionation strategy combined with MS/MS is entirely capable of characterizing a significant fraction of the serum proteome without the need to deplete high abundant species, such as albumin. This analysis provides a foundation for the detection of the presence, or absence, of various proteins/peptides in mouse cancer model systems.

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