Identification of growth factors in mouse plasma by immunoaffinity depletion, low molecular weight protein enrichment, and LC-MS/MS analysis

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Overview

- IgY7 and SuperMix columns were applied online to separate mouse plasma proteins, which provided a nearly 2-fold improvement in the overall proteome coverage, as well as more than 2-fold increase in the coverage of cytokines, growth factors, and other low abundance proteins compared to single column depletion using IgY7.

- Mouse plasma was filtered by 10 kDa MWCO to collect the low molecular weight fraction, and analyzed by high resolution LC-MS/MS to identify plasma peptidome.

- The IgY7-SuperMix and MWCO systems were combined to achieve deeper profiling of growth factors.

Introduction

- The discovery of novel circulatory growth factors in plasma has significant potential for advancing our understanding and therapeutic treatment of metabolic disorders such as obesity, insulin resistance, and diabetes.

- The relative low abundance of these factors and the enormity of the plasma proteome make it challenging for proteins identification and quantification.

- In this study, we explored multiple protein fractionation techniques to enrich the low molecular weight growth factors to facilitate the effective detection of these proteins by LC-MS/MS.

Methods

Online IgY7-SuperMix separation

- Plasma proteome flow through the IgY7 column were used as antigens to generate antibodies against SuperMix.

- The IgY7 and SuperMix columns were applied online to separate mouse plasma proteins.

- 125 µL of mouse plasma per injection was used for tandem depletion.

- Parameters were optimized through a series of experiments.

Low molecular weight (LMW) protein enrichment

- IgY7 and SuperMix columns were applied online to separate mouse plasma proteins.

- 125 µL of mouse plasma per injection was used for tandem depletion.

- Parameters were optimized through a series of experiments.

Results

IgY7-SuperMix depletion

- The proteome coverage by IgY7-SuperMix depletion was improved compared to IgY7 alone.

- The coverage of cytokines, growth factors, and other low abundance proteins was significantly improved.

- The IgY7-SuperMix and wavelengths systems were combined to achieve deeper profiling of growth factors.

Detection of LMW proteins

- LC-MS/MS analysis of IgY7-SuperMix depleted plasma revealed a nearly 2-fold improvement in proteome coverage.

- Only peptides with ≥2 unique peptides were counted.

- Data analysis was performed using the SEQUEST software against the mouse protein database.

- Protein groups were identified by searching the MS/MS spectra against the mouse protein database.

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Conclusions

- Our initial global characterization of the mouse plasma proteome using SuperMix depletion and IgY7-SuperMix resulted in identification of ~1000 unique proteins, with 472 proteins identified by more than two unique peptides.

- More than 30 known cytokines and growth factors and many other putative factors in mouse plasma, such as insulin, leptin, adiponectin, insulin growth factor 1, granulin, and prosaposin, were identified.

- Different protocols for further enriching the LMW fractions are being explored and are anticipated to significantly expand the coverage of putative growth factors.

- Currently we are integrating the IgY7-SuperMix depletion and LMW fractionation methods to achieve deeper profiling of low abundance plasma actors.

- We anticipate this integrative methodology will greatly facilitate the discovery of novel growth factors related to different disease conditions.

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References


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