

Small Molecular Weight Serum Protein Profiles in Growth Hormone Transgenic Mice and Growth Hormone Receptor/Binding Protein Knock-Out Mice

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Growth hormone (GH) is a serum protein that is important in control of growth and metabolism. GH transgenic mice are giant, suffer from hyperinsulinemia, and die prematurely due to liver and kidney diseases. On the other hand, GH receptor gene disrupted or 'knock-out' mice are small, have low insulin like growth factor 1 (IGF-1) and insulin levels and live significantly longer than wild type mice.

It is interesting to study the effect of GH on the circulating proteins, which are important indicators of metabolism state and growth condition of the body. Among them the small molecular weight proteins (<20 kD) are of particular interest, because of their highly active role in regulating whole body metabolism and their vulnerability to oxidative stress. We hypothesize that a subset of serum proteins in these two types of mice will be different in terms of both the level and the post translational modifications.

Proteomics is the study at the level of whole organ proteins. One common approach is to isolate and identify proteins by 2 D gel electrophoresis (2D GEL). Using this procedure, proteins are separated first by their isoelectric points (pI), then by molecular weights. The isolated spots are then quantified and the proteins that are up or down regulated determined are removed from the gel and analyzed by Mass spectrometry (MS) or tandem MS/MS. Data derived from these analyses yield tryptic peptide peaks characteristic for each protein. These data are used to retrieve information on protein identity, protein modification and so on by blasting the various protein databases. Since 'normal' 2D GEL does not resolve protein less than 5 kD, many proteins/peptides that change in relation to GH action are not analyzed

We have modified the second dimension electrophoresis by using tricine buffer system instead of glycine. The modified 2 D GEL can resolve small molecular proteins between 1 and 20 kD. Using this tool, we can study the small molecular serum proteins of GH transgenic and GHR/BP knock-out mice. Of the 72 protein spots resolved using this gel system, we have found that 19 are down-regulated two fold or more and 23 are up-regulated two fold or more in GH transgenic mice versus controls. MS and tandem MS analysis give out the preliminary identification of these proteins. They contain identified peptides found in transthyretin, kallikreins, protease inhibitors, apolipoprotein A, major urinary proteins, immunoglobulin G heavy chain variable regions, plasminogen, hemopexin, hemoglobin B, etc. Some of these proteins may be the modified, degraded or cleaved products of the above known proteins. The bioactivity of these 'cleaved', small peptides can be intriguing.

The result of this study could be of great interest in understanding the physiological effect of GH on small molecular circulating proteins in mice. The tool will also be applied to human serum samples from acromegalic patients (GH excess) and GH deficient patients. We anticipate discovering a variety of small molecular weight proteins that may be diagnostic of GH action and may ultimately lead to therapeutic targets.

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