18.1
DIGE Analyses Indicates Phenotypic Changes between Glucose-responsive and Glucose-non-responsive MIN-6 Beta Cells

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Cell replacement therapies are potential alternatives to the insulin injections currently employed to control blood glucose levels in type 1 diabetics. The long-term potential to routinely use replacement beta cells/islets as a cell therapy relies on our ability to culture such cells/islets, in vitro, while maintaining their functional status. Previous beta cells studies have indicated that the glucose-stimulated insulin-secretion (GSIS) phenotype is relatively unstable, in long-term culture.

Insulin content and glucose responsiveness/non-responsive nature of MIN-6 cells at low (p18) and high (p40) passages were investigated using ELISA (Mercodia) assays. To establish associated protein expression changes, 2-D Fluorescence Difference Gel Electrophoresis (DIGE) studies were performed on these cell populations. Subsequent bioinformatics analyses together with protein identification included the use of Decyder software and mass spectrometry.

Long-term culture was found to be associated with many phenotypic changes, including changes in growth rate and cellular morphology, as well as loss of GSIS. DIGE analyses indicated many proteins, including many involved in glucose regulation, oxidation, adhesion, and proliferation - to be significantly affected by passaging/long-term culture.

18.2
Proteomic Analysis of Strain-specific Liver Proteins in Inbred Mice

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It has been known that the genetic background of inbred mice can make an influence on physiological response and general biological characteristics such as susceptibility and resistant to high fat feeding, radiation and even viral infection. Also same genetic alterations (transgenic or knock-out) may represent diverse phenotype based on genetic background in genetically modified mice(GEM). However little was known about proteins leading to diverse phenotype according to genetic background of mice.

Recently we reported the changes of liver proteome in high fat diet-induced atherosclerosis-susceptible(C57BL/6J) and resistant mice(C3H/HeJ). We found the differences of liver proteome regardless of high-fat feeding in two inbred strains of mice. Our previous results suggest a clear distinction in differential expression of oxidative stress proteins and lipid metabolism related proteins between the two strains in response to atherogenic diet feeding, which might account for their difference in susceptibility to atherogenesis. Now we reported strain-specific liver proteome in several inbred strains of mice(C57BL/6J, KK/HJ, A/J, DBA/2J, SJL/J, and C3H/HeJ). They were known as high fat diet-fedInterestingly many proteins which we found were known as oxidative stress-related proteins(AOP-2, glutathione peroxidase and SOD), and lipid metabolism-related proteins(Acyl Co A, APO A-1, and APO-E) were differentially expressed in liver based on inbred strains of mice. These results support that the different responses for high-fat induced atherosclerosis among inbred strains of mice may result from differences of the normally expressed liver proteomes and interactions of different variants of lipid metabolism and anti-oxidative stress-responsive protein family.

18.3
Quantitative Profiles of Secreted Factors during Adipocyte Differentiation

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Adipose tissue represents the largest secretion organ in the body. Factors secreted by adipocytes (adipokines) exert their function in auto-, para- and endocrine fashion. Adipokines are involved in regulation of different processes such as appetite control, insulin action, angiogenesis, cell growth and differentiation. Therefore, identification of known as well as novel adipokines and quantitative profiling of their expression changes during differentiation is a key step towards understanding how adipose tissue is able to influence total body homeostasis. In addition this can provide a possible clarification of the missing links between obesity and obesity-associated disorders such as diabetes type II, hypertension and cardiovascular diseases. Using a quantitative mass spectrometry technique called triple encoding SILAC (Stable Isotope Labeling by Amino acids in Cell culture) we followed the changes in expression levels of secreted proteins during the conversion of cells from fibroblastic state into mature adipocytes. Preadipocytes were grown in SILAC media containing either normal or “heavy” forms of Arg and Lys for five population doublings. Cells adapted in media containing the “heavy” forms were induced to differentiate into adipocytes using a defined induction mixture. The normal Arg and Lys encoded cells were serum starved at day 0 of the differentiation protocol and the media was collected. At the terminal stage of differentiation media from the “heavy” isolate encoded cells was collected as well. The samples were combined, concentrated and resolved by one-dimensional SDS-PAGE. Excised gel slices were digested with trypsin and analysed by LC-MS/MS using a hybrid linear ion trap Fourier Transform mass spectrometer LTQ-FT. We identified and quantified a large number of known adipokines that are up or down regulated during the differentiation. We also found several secreted molecules that were not previously described to be differentially expressed during the process of adipogenesis. Using this strategy, we also followed changes in the adipocyte secretion profile as a result of treatment of the cells with anti-diabetic drugs.
18.4 Identification and Characterization of DNA-binding Proteins Using Affinity Purification and Mass Spectrometry

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DNA-binding proteins are traditionally studied by using mobility shift assays where the DNA-protein complex can be detected by nature electrophoresis. However, such methods do not provide any information on the identity of the bound proteins. A powerful method for purification of DNA-binding proteins is to immobilize the DNA of interest to a chromatographic matrix and to isolate DNA-binding proteins by their affinity. Combined with a very sensitive method for protein identification, e.g., mass spectrometry, this method has been proven useful for identification of DNA-binding proteins (Nordhoff, E. et al., Nat. Biotechnol. (1999) 1, 884–888).

Diabetes Mellitus (DM) is one of the most common endocrine diseases that present a serious challenge to health care worldwide. Two different types of DM exists; insulin dependent (Type 1, T1DM) and non-insulin dependent (Type 2, T2DM). The pathogenesis of T1DM is characterized by immune-mediated destruction specific to the insulin-producing beta-cells in the pancreatic islets of Langerhans, but the mechanisms directly involved in beta-cell dysfunction have not yet been elucidated. Secretagogin is a novel Ca2+ -binding protein which is rather pancreatic beta-cell specific. It was demonstrated by Wagner et al. (2000) to increase insulin secretion therefore, it is proposed to play a role in the T1DM. Promoters of secretagogin immobilized to magnetic beads were used to affinity-purify proteins from INS-1e nuclear extract. After eluted from the beads, the proteins were separated by SDS-PAGE and identified by mass spectrometry. Stable isotope labeling (SILAC) was used to get a differential expression profile by mass spectrometry of proteins binding to the secretagogin promoter in stimulated (by Interleukin-1β) and non-stimulated conditions.

18.5 Proteomic Analysis of Cytokine-induced Dysfunction and Death in Insulin-producing INS-1E Cells

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Objective: Locally released cytokines contribute to b-cell dysfunction and death in type 1 diabetes. Previously performed microarray analysis of insulin producing INS-1E cells identified 698 genes as cytokine modified, many of which are related to apoptotic pathways. The aim of this study was to investigate the relevance of these findings at the protein level. For this purpose quantitative changes in protein expression upon cytokine treatment were investigated in INS-1E cells using 2-dimensional difference gel electrophoresis (2D-DIGE).

Methods and results: INS-1E cells were incubated during 24 hours with IFNg(500U/ml) and IL1b(10U/ml). As expected, these cytokines induced nitric oxide (96 pmol/10^6 cells in cytokine-induced versus not detectable in control condition; n = 3) and apoptosis (Annexin/PI staining; 35.2% increase compared to control condition; n = 3). Differential protein expression was analyzed by 2D-DIGE, using 3 different fluorescent dyes (Cy2, Cy3 and Cy5). A total of 1662 spots were detected, of which 112 were changed in expression upon cytokine-treatment. Of these, 45 protein spots were induced, while 67 were down-regulated.

Conclusion: Cytokine-treatment of INS-1E cells induces marked changes in protein expression profiles. The identification of these proteins and the pathways involved will contribute to a better understanding of the mechanisms involved in the destruction of β-cells in type 1 diabetes.
A Fully Automated and Miniaturized Immunoassay for Insulin Quantification within a CD Microlaboratory

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Diabetes, a disorder affecting insulin production in the body, is increasingly common and research into its mechanisms and treatment is a growing research area. Insulin is one of the most extensively studied metabolic factors in diabetes. Current techniques for insulin quantification, such as ELISA and radioimmunoassays (RIA), have limitations in terms of assay performance and throughput. In particular, when using small animal models, where sample volumes are very small, and in patient screening and population studies, where large numbers of samples have to be analyzed. For such studies, conventional techniques are labor intensive, and consume large quantities of reagents and sample. To overcome these limitations, we have developed an automated and miniaturized assay format for insulin quantification within a CD microlaboratory. The CD contains microstructures in which multiple samples are analyzed in parallel. Each microstructure contains a pre-packed column of streptavidin-coated particles. Analyte specificity of each column in the CD is determined by addition of biotinylated capture molecules. Samples are passed through the columns followed by complementary, fluorescently-labelled detection molecules. The amount of specifically-bound protein is measured by scanning each column using a laser-induced fluorescence detector integrated into the workstation that runs the CD microlaboratories. Case studies for the quantification of insulin from human, rat and mouse, with comparative data from ELISA and RIA will be presented, showing the ability of the microlaboratory to produce well-correlated results with more efficient use of sample and time.

Discovery of Biomarkers in Type 2 Diabetes Urine; Potential for a Non-invasive Early Diagnostic

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Introduction: As the number of people with diabetes grows worldwide, the disease consumes an ever-increasing proportion of national health care budgets. The World Health Organization estimate for type 2 diabetes cases rises to 285 million people worldwide by 2025. In the USA in 2003 approximately 18 million people were estimated to have type 2 diabetes, of these 13.0 million were diagnosed and an estimated 5.2 million undiagnosed. Additionally, 38 million U.S. adults have pre-diabetes. The reason that an estimated 40% of individuals remain undiagnosed, often for a period of years, is the lack of a simple and reliable way to detect diabetes and pre-diabetes.

Objective: Many proteins and other blood components may be modified in individuals with elevated blood glucose. Identification of these molecules or of identifiable correlates of hyperglycaemia would facilitate development of potential new laboratory tests for diagnosis of diabetes. Proteomic methods have been successfully used for studying complex biological problems and for the identification of disease markers.

Methods: A full Proteomics workflow was applied to urine samples from Type 2 diabetics, pre-diabetics and controls, in order to find diabetes-specific biomarkers. It involved sample preparation with the Microflow MF10, 2D electrophoresis, image analysis, spot cutting and tryptic digestion, followed by MALDI MS and LC-MS/MS.

Results: From this analysis, a number of proteins were isolated as putative diabetes biomarkers. Some of these proteins were post-translationally modified in diabetes. A number of these were selected for further validation and this is currently proceeding.

Conclusion: By using body fluids other than plasma we have developed an approach to biomarker discovery that uses a non-invasive test based on easy to collect samples, such as urine and tears.
Overexpression of Angiopoietin-like Protein 4 Alters the Protein Expression Profiles of the Liver Tissue in DB/DB Diabetic Mice

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Type 2 Diabetes Mellitus (T2DM) is one of the most common endocrine diseases worldwide. The prolonged hyperglycemia can cause many diabetic complications such as cardiovascular diseases and stroke, which are becoming the top cause of morbidity and mortality in the aging population. Recently, we have reported that angiopoietin-like protein 4 (ANGPTL4), a circulating protein predominantly produced from fat tissue and liver, can potently decrease blood glucose in db/db diabetic animal mice, possibly by acting through the liver tissue (PNAS, 2005, 102:6086–91). However, the molecular and cellular basis that underlies the liver actions of ANGPTL4 remains to be clarified. In this study, we have employed two-dimensional differential gel electrophoresis (2D-DIGE) technology to study the protein profiles in the livers of db/db diabetic mice treated with or without ANGPTL4. Our results demonstrated that, compared with those in the lean littermates, several enzymes involved in gluconeogenesis and lipid metabolism are increased while a lot of cytoskeletal proteins and chaperons are down regulated in db/db diabetic mice. Especially, several enzymes in the methionine/homocysteine metabolic cycle are significantly elevated, leading to the increased levels of S-adenosylmethionine, the methyl donor in virtually all known biological methylation reactions. On the other hand, ANGPTL4 treatment can reverse most of these protein changes in db/db mice. In addition, we have also found that ANGPTL4 can selectively modulate the expression of a lot of proteins that are involved in other biological pathways, such as inflammatory responses and cell growth, suggesting that this protein may possess other functions as well. In conclusion, our results support our previous finding that ANGPTL4, an emerging adipokine involved in energy metabolism, exert its actions through the liver tissue.