Proteomics Method for Rapid Screening of Type 1 Diabetes Markers

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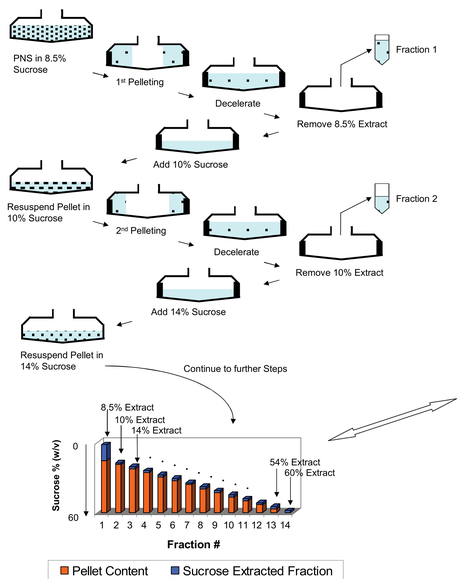
Introduction

Autoimmune type 1 diabetes (T1D) can result from an imbalance between regulatory and autoreactive T lymphocytes in genetically predisposed individuals. Recently, the differentiation of T lymphocytes into cytokine-secreting effector cells was shown to initiate an endoplasmic reticulum (ER) stress response, including the upregulation of heat shock protein (HSP) 'chaperones' found in the ER that help to fold newly synthesized proteins.

The purpose of this study was to use a recently developed proteomics sample preparation technology (Edge™Technology) to investigate the role of ER chaperones, and other heat shock protein family members, in the T cell changes that occur with the induction of T1D.

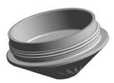
Edge Technology provides well defined and reproducible fractionation of biological samples using enhanced density gradient extraction.

Method

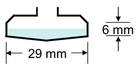


Instrumentation

Prospect Biosystems **Edge 200**



Edge Sample Rotor
(~2x actual size as shown)



Sample Container

BBDR rats (3 per group) were injected with phosphate-buffered saline (PBS, control group) or anti-ART2 mAb plus poly I:C (treatment group).

Untreated BBDR rats do not develop diabetes, whereas 100% of those treated with anti-ART2 mAb plus polyinosinic:polycytidylic acid (poly I:C) become diabetic over a time course of 14-18 days.

Rats were killed at day 3, and cervical lymph nodes were collected and snap frozen in liquid nitrogen.

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Rat Cervical Lymph Nodes (CLN)
3 CLN ~110mg

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Homogenization

⇩

Removing Nuclei

⇩

Post Nuclear Supernatant (PNS)
3ml

⇩

Fractionation by Edge Technology
Using sucrose as density extracting medium

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Western Blotting Analysis

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2D Gel and Image Analysis

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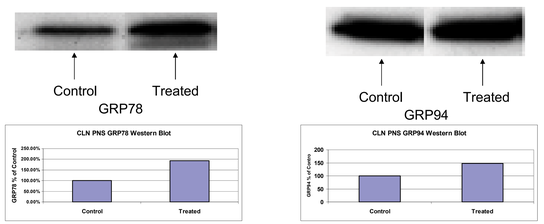
MALDI-TOF-TOF

⇩

Protein ID

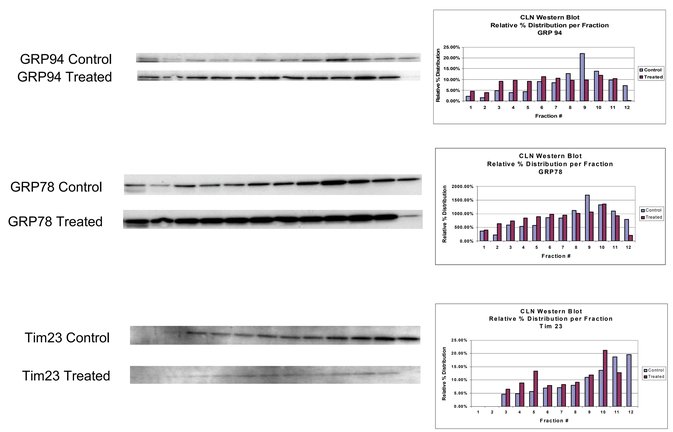
Results

Western Blotting Analysis of CLN Post Nuclear Supernatant

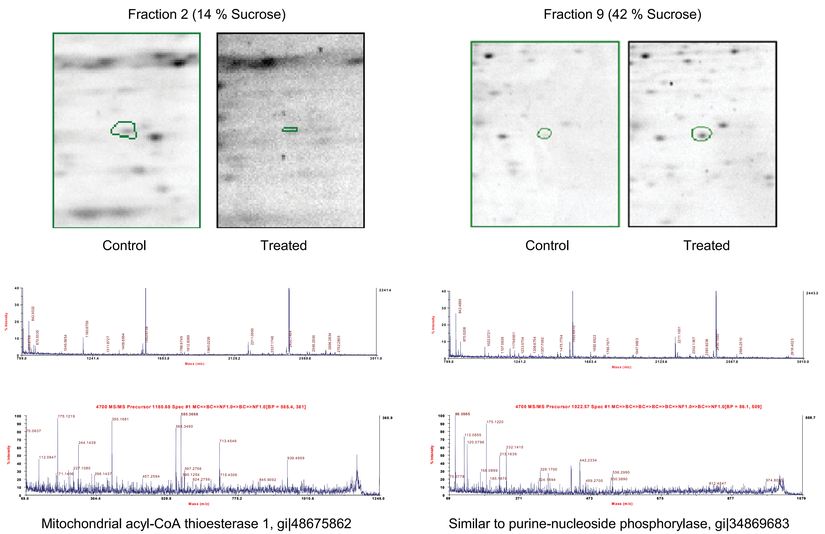


- GRP78 of PNS for treated CLN was up regulated
- GRP94 of PNS for treated CLN was up regulated
- Results are consistent with previous reports
- Actin was used as a control

Western Blotting Analysis of Fractionated CLN



2D Gel Analysis of Selected CLN Fractions



Conclusion

- Edge technology provides a rapid fractionation and screening method for various T1D markers.
- Changes in profiles of relative percentage distribution of GRPs per fraction indicate potential HSP translocation in the cell with the onset of T1D.
- Tim23 western results may be very important for understanding any biological relationship between mitochondrial proteins and HSPs.
- Further investigation needed to explore the biological roles of those unique proteins identified in 2D gel analysis.

Edge Technology Benefits

- **Edge Technology** provides a powerful, selective sample fractionation method for proteomics analysis, including low abundance protein isolation and enrichment, biomarker discovery, as well as information on subcellular location.

- Sample complexity is reduced significantly.
- Starting sample volume is less than 4 ml.
- Extraction volume is flexible and can be as low as 100 µl.
- Each extraction step takes about 5 minutes.
- Extraction may start at any density step of interest without the need for going through the whole gradient.
- The recovery yield is > 90%.
- The method DOES NOT need any density gradient medium and does not need any gradient mixer.
- The density of the extraction medium at each step is defined and no other instrument is needed for density determination.
- Fractionation conditions are non-denaturing.