A New Sample Fractionation and Enrichment Technology for the Rapid Screening and Discovery of Potential Protein Biomarkers

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Introduction

Proteomic technologies have opened new avenues in the search for clinically useful protein biomarkers of diseases, treatment responses and drug development processes. Protein biomarker discovery can be accomplished in body fluids, tissues as well as cells. Due to the complexity of the biological samples and the huge dynamic range of protein abundance, the success of any protein biomarker discovery effort will depend upon the quality of sample preparation, the ability to generate quality information on relatively low protein levels, and the ability to readily interpret the data generated. We have developed a density based fractionation technology (Edge?) for proteomics sample preparation to reduce sample complexity and to increase the possibility for low abundance protein isolation and biomarker discovery. In this study, we applied Edge technology and other proteomics techniques to perform rapid screening for disease markers using rat as a model.

Method

- BBDR rats (3 per group) were injected with phosphate-buffered saline or anti-ART2 mAb plus poly I:C to induce type 1 diabetes. Rats were killed at day 3, and then cervical lymph nodes (CLN) were collected and snap frozen in liquid nitrogen.
- Healthy Adult rats (7-8 weeks old, 4 per group) were fed with high fat diet or control diet. Rats were killed after 4 weeks, and then rat brains were collected and snap frozen in liquid nitrogen.
- Rat tissues were homogenized, fractionated using Edge 200 separation system and samples from each fraction were subjected to Western analyses. Selected fractions were analyzed by 2DE. Interesting protein spots were excised, digested and identified by MALDI-TOF/TOF.

Work Flow

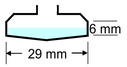
3 Rat Cervical Lymph Nodes (CLN, ~110 mg), or One rat brain (~1.6 g)
⇩
Homogenize
⇩
Remove Nuclei
⇩
Post Nuclear Supernatant (PNS) 3ml
⇩
Fractionation with Edge 200 Separation System Using sucrose as density extracting medium
⇩
Western Blotting Analysis
⇩
2D Gel and Image Analysis
⇩
MALDI-TOF-TOF
⇩
Protein ID

Instrumentation

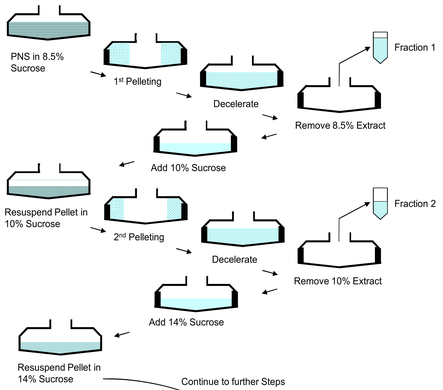
Edge 200 Separation System

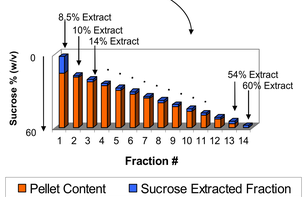
Instrument

Sample Rotor



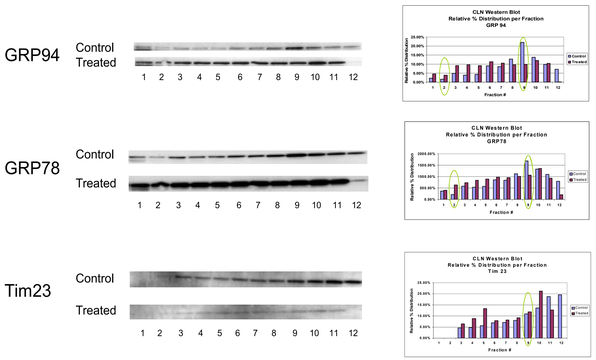
Sample Container



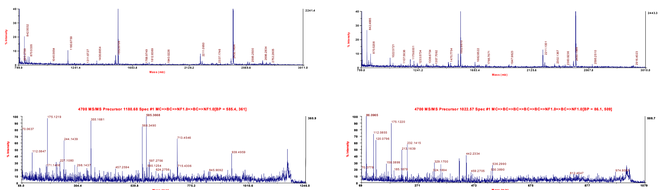


Results - Type 1 Diabetes Marker Screening

Western Blotting Analysis of Fractionated CLN



MALDI-MS/MS Analysis of Selected Spots in CLN Fractions



Mitochondrial acyl-CoA thioesterase 1, gi|48675862
Identified in Fraction 2 (14% Sucrose) and
down regulated (>2 fold) in treated rat.

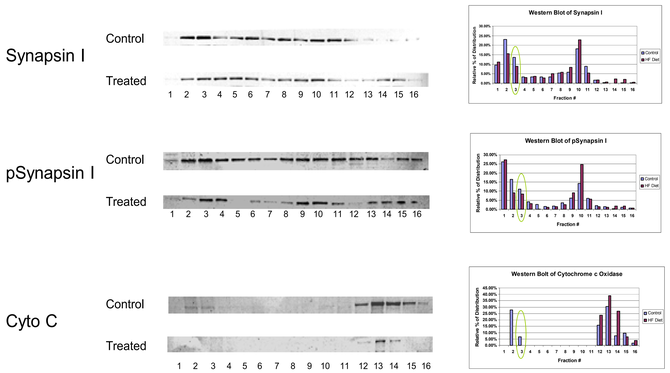
Similar to purine-nucleoside phosphorylase, gi|34869683
Identified in Fraction 9 (42% Sucrose) and
up regulated (>2 fold) in treated rat.

Conclusion

- Edge technology provides a rapid fractionation and screening method for various disease markers.
- Changes in profiles of relative percentage distribution of markers per fraction indicate their potential translocation in the cell.
- Tim23 western results in the type 1 diabetes rats may be very important for understanding any biological relationship between mitochondrial proteins and HSPs.
- Cytochrome c oxidase western results may provide validation of published reports proposing the biological relationship between high fat diet and mitochondrial dysfunction (Wu, et. al, *Eur. J. Neurosci.* **19**, 1699-1707, 2004).
- Further investigation needed to explore the biological roles of those unique proteins identified in 2D gel analysis.

Results - HF Diet Marker Screening

Western Blotting Analysis of Fractionated Rat Brain



Protein ID of Selected Spots Using MALDI-TOF/TOF (Fraction 3)

Spot	Protein	NCBI Accession #	Protein Score	Total Ion C. I.%	Relative Expression
1434	Brain lipid binding protein	gi|13540630	259	100	Up
793	Similar to gamma actin-like protein	gi|34875636	235	100	Up
1027	Similar to ClpP protease	gi|34882298	166	100	Up
323	TNF receptor-associated protein 1	gi|55741837	212	100	Up
275	N-ethylmaleimaide sensitive fusion protein	gi|13489067	51	99.291	Up
160	Unkown (proteon for MGC:94869)	gi|53734284	275	100	Up
127	Pc protein	gi|55716041	256	100	Up
343	Succinate dehydrogenase complex, subunit A flavoprotein	gi|18426858	114	100	Up
904	Syntaxin, P35A	gi|251470	150	100	Down
1041	PDGFA associated protein 1	gi|56971256	53	99.966	Down

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.

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