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Gel-based proteomics analysis of starving mouse pancreatic beta-cell insulinoma (MIN6) prior to glucose stimulation

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Abstract

Nutritional support is a crucial step for ill and starving patient management. Food consumption after starvation can induce tremendously changes in cellular machinery. The pancreas is an endocrinal organ that immediate response during food consumption. It maintains blood glucose homeostasis by secreting insulin. WHO suggested the guideline for severely malnourished children by firstly start preventing hypoglycemia. However, the amount of sugar that added to the bolus is 15 g which is an equal number of 15 percent of daily recommended sugar consumption at a time. Thus, we would like to further investigate the cellular metabolic acclimation in the pancreatic cell during starvation and after high glucose intake or refeeding stage by mimicking this condition in a culture medium. We aimed to identify novel biomarker between two groups of mouse pancreatic beta-cell line MIN6 by using proteomics approach.

Total proteins extract from MIN6 were fractionated on 2D-SDS-PAGE. This study compared protein expression profile between two groups and 356 proteins were quantitatively identified with Coomassie brilliant blue G250. ATP5H, TCTP and STMN1 proteins are suppressed in glucose stimulation group. This work provides comprehensively understanding the cellular metabolic acclimation by a high glucose condition in starving patient.

Introduction

The ability of animal to survive in deprived-food condition is an adaptive response. In the early twentieth century, starvation diet was introduced as a treatment for diabetic patient which can relief the symptom and maximize lifelong (Drenick, Swendseid et al. 1964). Doubtlessly, it can reduce blood glucose level however this can obviously be harm and lethal due to a prolonged calorie restriction. Thus, patient with long-term starvation would definitely commence with feeding and this can possibly cause refeeding syndrome. It is defined as severe electrolytes and fluid shift associated with metabolic abnormalities in starving patient (Mehanna, Moledina et al. 2008). The main causes of refeeding syndrome are related with metabolic and hormonal changes. In which, insulin plays the most important role during this stage from shifting gluconeogenesis to blood glucose reduction. Glucose-Stimulated Insulin Secretion (GSIS) (Straub and Sharp 2002). Therefore, the aim of this study was to examine differential regulation of proteome associated with starving and refeeding state of murine beta cell insulinoma (MIN6) to understand a mechanism of response of refeeding stage.

Methodology

Sample preparation for 2D-gel electrophoresis

Pancreatic beta cell line MIN6 mouse beta cells were grown in Dulbecco's High Glucose medium containing 15% fetal bovine serum, 1% Penicillin-Streptomycin, 50 uM 2-mercaptoethanol, 24 mM HEPES, pH 7.3, 1% non-essential amino acids, and 1% L-glutamate. Cells were trypsinized with 0.05% trypsin and seeded in T-25 flask with 500,000 cell/mL concentration and grown, in 5% CO_2-95% humidified air at 37°C prior to sample collection for 2-3 days without medium changing. Every experiment was done in three replicates (n=3). Total MIN6 cells were harvested by using detergent lysis buffer. The cell pallets were lysed and proteins were solubilized by a mixture of detergents including 8M Urea, 4% CHAPS, 50mM TrisHCl pH 7.4 with protease inhibitors. The protein solution was then precipitated by TCA/acetone overnight. Protein concentration was evaluated by Micro BCA™ protein assay kit (Thermo Scientific).

For 2D-gel electrophoresis, 500 ug of total protein sample was loaded onto immobilized strip gels 13 cm linear pH range (pH 4–7) (GE Healthcare). The strip was rehydrated for 14 hours at 20°C. Protein mixture was subjected to isoelectric focusing in an Ettan™ IPGphor™ 3 Isoelectric Focusing System with manufacturer protocol. Then, the strip was fractionated on 12.5% SDS-PAGE at 8°C. Protein gels were visualized by Coomassie Blue G-250 staining and the image was acquired by GS-800 Calibrated Densitometer (Bio-Rad).

Image analysis for quantitative protein expression

The 6 gels (three replicates for each of the 2 experimental conditions; starving and refeeding) were stained with colloidal Coomassie Blue (G-250) and scanned (GS-800 Imaging densitometer; Bio-Rad). The automatic alignment of the 6 gel images (according to 7 landmark spots, manually selected), spot detection, quantification, and pairing were carried out using Image Master (v5.0). Each individual spot was edited and if necessary assigned manually. The spot volume was computed with background subtraction, normalized to the total volume in the gel image and expressed in %Vn (Vilain, Cosette et al. 2004).

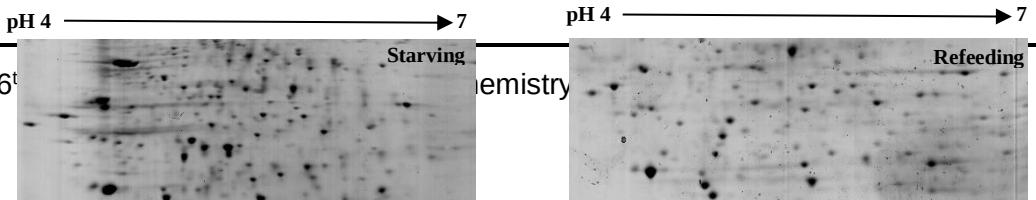
Gel-based protein identification by LC-MS/MS

Spot proteins on SDS-PAGE were excised manually into tiny pieces (approximately 1×1 mm^3). Carbamidomethyl modification (C) was performed before digestion with trypsin. The mixture peptides were protonated by 0.1% formic acid before subjected to LC-MS/MS analysis. The tryptic peptides were analyzed using an Ultimate3000 Nano/Capillary LC System (Dionex, UK) coupled to a Hybrid quadrupole Q-Tof impact II™ (Bruker Daltonics GmbH, Germany) equipped with a Nano-captive spray ion source Mass spectra (MS). The raw LC-MS/MS Spectra were carried out using ProteinScape 4.0 (Bruker Daltonics GmbH, Germany) to automatically search against in-house MASCOT 2.3 software by following parameters, Enzyme, Trypsin; Fixed modifications, Carbamidomethyl (C); Variable modifications, Oxidation (M); three missed cleavage were allowed with peptide Mass Tolerance, 1.2 Da; Fragment MassTolerance, 0.6 Da; and Instrument type, ESI-Q-TOF. Identified proteins were accepted with a total ion score of over 30 and more than 3 peptides matched.

Results and Discussion

Starving and refeeding MIN6 cell protein profile

MIN6 cell lysates with starving and refeeding stage were identified by 2D Gel electrophoresis. A total of 403 spots were detected. The total protein content in refeeding stage is clearly higher particular with protein with neutral pI after stained with Coomassie Blue G-250. (Fig. 1)



(a)

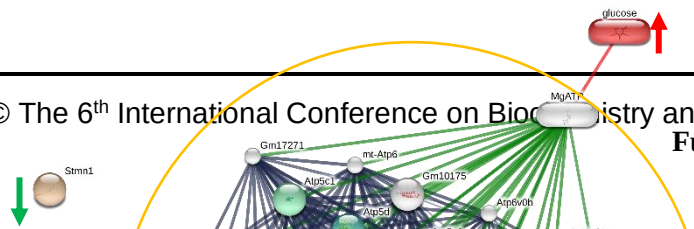
(b)

MASCOT Search Results

ATP synthase subunit d, mitochondrial OS=Mus musculus OX=10090 GN=Atp5h PE=1 SV=3

Figure 1. (a) Protein profile (2D-gel, Coomassie brilliant blue G-250 stained) of MIN6 under starving and refeeding stages. Each one out of three independent replicates was shown. In the first dimensional, total protein lysate were fractionated under pH linear range (pH 4-7), (b) MS/MS Fragmentation peptide, SCAEFVSGSQLR, that found in ATP5H_MOUSE protein.

From 2D gels, 365 proteins in starving stage and 345 proteins in refeeding stage were identified with Image Master 2D-platium version 5.0. We compared the content of each spot proteins in two different stage. Spots with their relative abundance with a *p*-value less than 0.05 between six SDS-PAGE were considered as differentially expressed. Spots volumes were normalized to total intensities and volumes on other gels to eliminate variations that occur from protein concentration determination, protein loading, transferring efficiency from the first dimension to second dimension, staining efficiency and staining time. For spots comparing based on spots volumes over a whole experiment, each spot on a certain gel has to be merged to the corresponding spots on the other gels, spot matching. Spot matching quality based on reproducibility of each gel. ATP5H, TCTP and STMN1 proteins are highly expressed more than two folds (*p*-value <0.05) in the starving group



Functional enrichments in network interaction

- ATP synthesis coupled proton transport
- ATP biosynthesis process

Figure 2. Predicted protein-ligand interaction network by STITCH software based on experimental evidence and database evidence. 3 proteins that differentially expressed proteins from 2D-GE were used to create the interaction network, the nodes of the network represent the protein and the edges of the network represent the predicted interaction between the proteins

From 2D Gel electrophoresis, these three proteins--ATP5H, TCTP and STMN1--are suppressed in refeeding stage which have relating interaction amid one another. The relation provides the functional information of these protein.

Refeeding stage of MIN6 have majority effect on ATP production and transportation including ATP synthesis coupled proton transport, ATP biosynthesis process and ATP metabolic process. Nevertheless, hyperglycemic condition can cause ATP depletion and suppress cellular respiratory factors in both human primary mesangial (HMCs) and proximal tubular cells (HK-2) (Czajka and Malik 2016). In addition, translationally controlled tumor protein (TCTP) has been reportedly related to glucose on MIN6 particularly in cyto-protective mechanism in hyperglycemic condition (Diraison, Hayward et al. 2011). However, STMN1 has never been reported to be related with hyperglycemic condition in pancreatic cell. Thus, we require further investigation.

Conclusion

We have showed that refeeding stage can alter cellular metabolic interaction by using proteomics tools. There were three prominent proteins that were suppressed due to hyperglycemic condition. STMN1 which is important for cytoskeleton movement was newly discovered in this condition. To assure the mechanistic function of STMN1, further investigation is needed and could lead to a novel biomarker for diabetes treatment.

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