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BIOINFORMATIC CHARACTERIZATION OF DIFFERENTIAL PROTEINS IN THE HIPPOCAMPUS OF TS65DN: A MOUSE MODEL OF DOWN SYNDROME

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Abstract – Down syndrome (DS) is characterized by mental retardation and the development of Alzheimer's disease (AD). The reason for this development is not yet clear. Ts65Dn mice were used in this study. We collected their hippocampi and identified protein biomarkers using i*sobaric tags for relative and absolute quantitation* (iTRAQ). We described the biochemical characteristics of proteins and explored their complicated network using GOA, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and network analysis. There were 374 significant differential proteins in the hippocampi of Ts65Dn mice. These were mainly binding proteins, related to single-organism and cellular processes. KEGG pathway analysis revealed that the insulin-signaling pathway was enriched with DS. Using PAJEK, a network of protein interactions was constructed and the top ten hub proteins were FYN, YBX1, VIM, PRKACA, EWSR1, H2AFX, CACNA1A, PTN, TFCP2L1 and CRKL. Through preliminarily discovered differentially expressed proteins by iTRAQ and bioinformatics analysis, we concluded that these proteins could be closely related to the neurological deficits of DS.

Key words: Down syndrome; iTRAQ; bioinformatics analysis; Ts65Dn; hippocampus

INTRODUCTION

Down syndrome (DS) is one of the most common gross chromosomal abnormalities and affects approximately 1 in every 700 babies (Roizen and Patterson, 2003). It is characterized by intellectual disabilities and the development of early onset Alzheimer's disease (AD) (Nieuwenhuis-Mark, 2009). However, the reason for the phenomenon is not yet clear.

Hypotheses attempting to explain how trisomy 21 causes cognitive impairment have been offered. The main hypotheses are "dosage imbalance hypothesis", "amplified developmental instability hypothesis", and "molecular misreading concept" (Contestabile et al., 2010; Lubec and Engidawork, 2002; Ding and Li, 2009). Each of these focuses on the extra copy of chromosome 21 and its associated proteins that change nerve development and metabolism in the DS brain, thereby destroying neural circuits. Research regarding protein function may be an important avenue to reveal the mechanism of mental retardation development in DS.

In our last study, based on comparative proteomic technology and bioinformatics analysis, we

reported on the different expression of proteins in maternal serum that revealed biologic processes and functional network in DS (Yu et al., 2012). Recently, we observed differentially expressed proteins in the hippocampus of Ts65Dn mice with iTRAQ technology; 374 differentially expressed proteins were found (Yu et al., 2013).

In the present study, we describe the biochemical characteristics of proteins in the hippocampus of Ts65Dn mice, and explore their complicated network using gene ontology annotation (GOA), KEGG pathway and network analysis.

MATERIALS AND METHODS

This study was conducted at the Changzhou Women and Children's Hospital of Nanjing Medical University (Changzhou City, Jiangsu Province, China). Experimental animals were bred at the Animal Center of Jiangsu University (Zhenjiang City, Jiangsu Province, China). All efforts were made to minimize the number of animals used and their suffering.

Animals

Six Ts65Dn mice (3 males, 3 females) carrying partial trisomy of chromosome 16 were purchased from the Jackson Laboratories (Bar Harbor, ME, USA). After culture reproduction, all first-generation mice were karyotyped using PCR according to the Jackson Laboratories' protocol. We selected five Ts65Dn mice as the DS group (DS), and five normal mice as the control (CG). The mice in both groups were age-matched, and were older than 16~18 weeks. The animals' health and comfort were evaluated by the veterinary service. The animals had free access to water and food according to the Animal Center of Jiangsu University.

Sample collection

Anesthetized animals were euthanized using 10% chloral hydrate (Changzhou First People's Hospital, Changzhou, China). The brain was removed and the hippocampus isolated.

iTraq

iTraq was performed as described in our previous study (Yu et al., 2013).

Gene Ontology Annotation

Functional classifications were performed with GOA (http://www.ebi.ac.uk/GOA/) according to the accession number of proteins in IPI. Some identified proteins were mapped to at least one annotation term within the GO biological process, molecular function and cellular component catalogues. To use all IPI proteins as a reference dataset, the P value that applied hypergeometric distribution with false discovery rate (FDR) correction and z-score were calculated for obtaining significant enrichment GO catalogues.

KEGG pathway analysis

The KEGG pathway is a collection of manually drawn pathway maps. The identified proteins were mapped to KEGG pathways with the corresponding relationship between protein accessions with KEGG gene IDs. The proteins marked light green were proteins discovered by iTraq.

Network analysis

Based on the INTACT (http://www.ebi.ac.uk/IntAct/, download on 04/10/2012) and HPRD (release 9) databases, the protein-protein interaction pairs between identified proteins were abstracted with corresponding gene symbols. A graph consisting of a set of nodes and a set of edges that connect nodes was constructed by PAJEK (http://vlado.fmf.uni-lj.si/pub/networks/pajek/). The nodes were proteins; the edges represent a physical interaction between two proteins. According to protein or gene functions, the nodes were approximately classified to different categories that have been marked with a different color. The sub networks with more than two nodes were singled out and were shown with FNV.

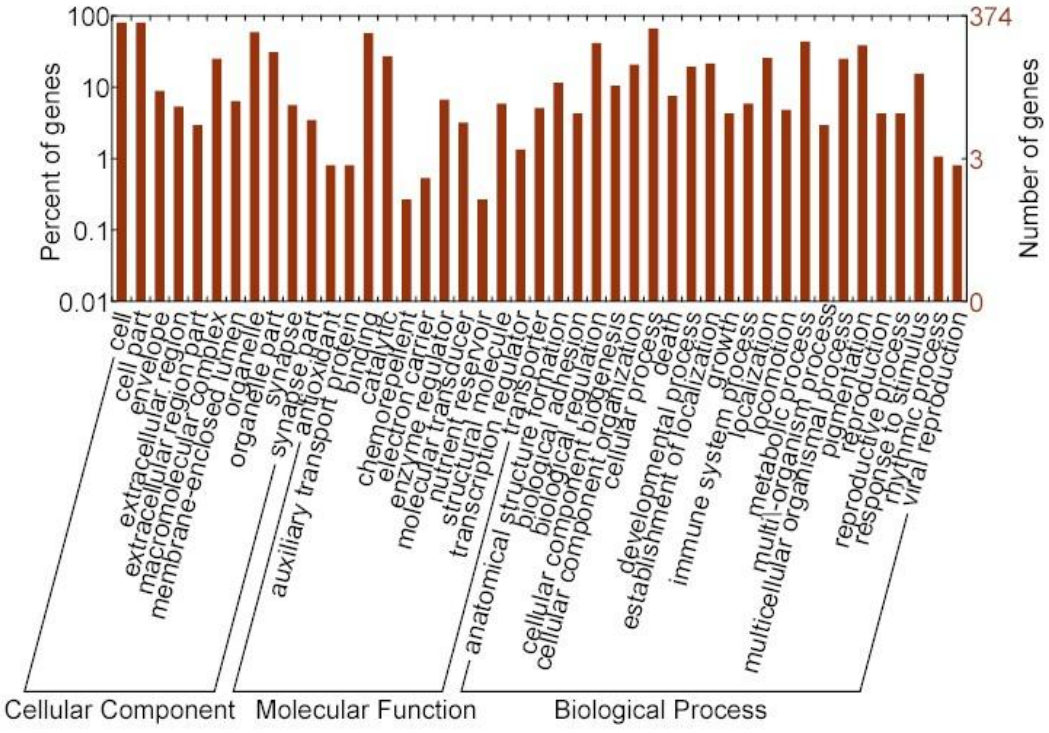

Fig. 1. Classification of the identified proteins based on GOA

Hub proteins

Hub proteins were calculated based on the INTACT and HPRD databases.

RESULTS

There were 374 significantly different proteins in the hippocampi of Ts65Dn mice, including 195 proteins that were significantly increased, while 179 proteins decreased.

By GOA, 374 proteins were mapped to at least one annotation term within the GO molecular function category. They mostly included 212 (56.7%) binding proteins, 102 (27.3%) proteins with catalytic activities, 26 (7%) proteins with enzyme regulator activities (Fig. 1). These were also mapped in the GO biological process category. The top 3 participate in single organism processes (243, 66%), cellular processes (239, 63.9%), and biological regulation (157, 42%). Most proteins belonged to cell components (284, 75.9%), including organelles (223, 59.6%) and membranes (150, 40.1%). Fig. 1 shows the classification of the identified proteins based on GOA.

Enrichment analysis consists of matching gene IDs in functional ontologies by GOA. The p value <0.05 and z-score >0 were chosen for statistically significant enrichment. The top 3 enrichments in the biological process were inflorescence meristem growth, molecular hydrogen transport and organism emergence from protective structure.

The gene symbols of these proteins were corresponded to KEGG gene ID. The first 3 enriched in the KEGG pathway were: insulin-signaling pathway, spliceosome and oxidative phosphorylation. Z-score >0 and FDR <0.05 were considered as statistically significant. Only the insulin-signaling pathway exhibited statistical significance; its z-score was 4.3057, the p value was 0.0004, and FDR 0.030 (Fig. 2). The

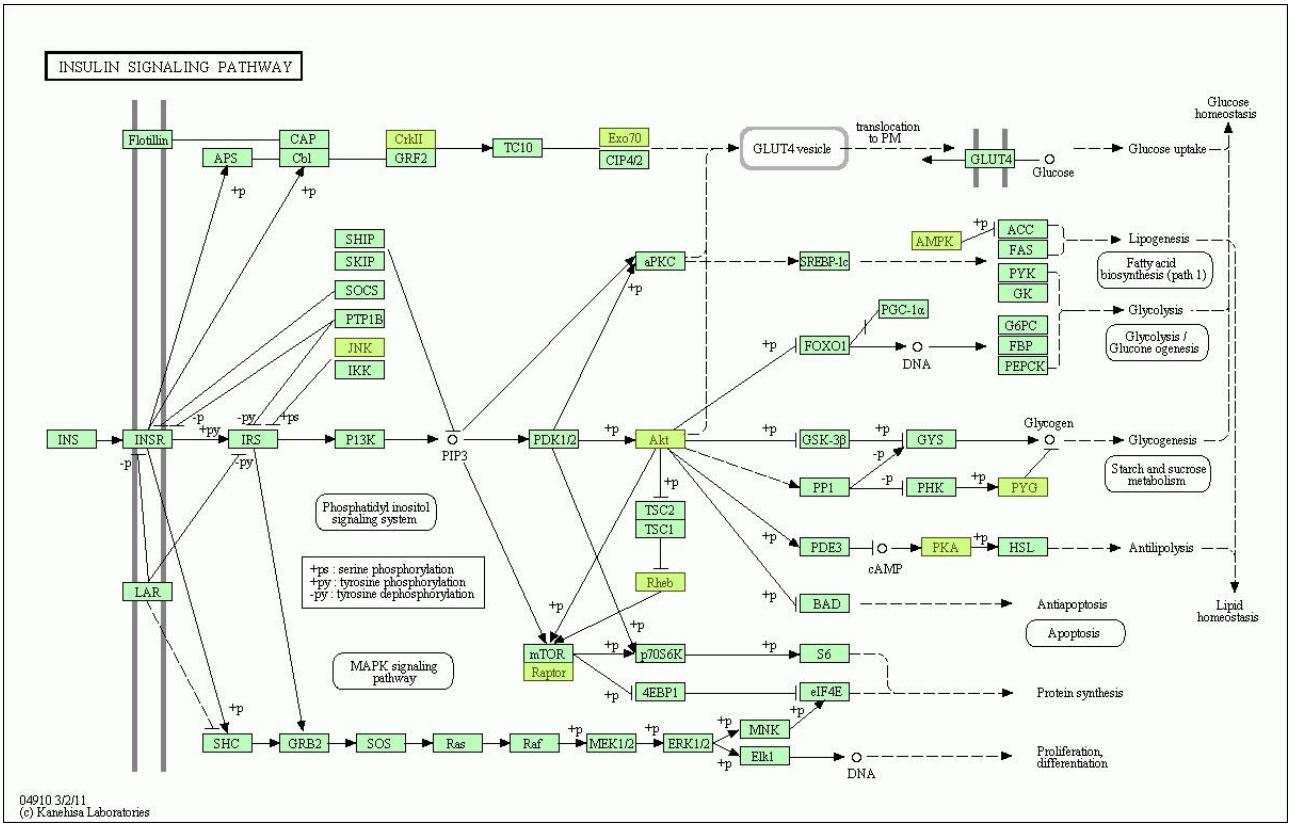

Fig. 2. Insulin-signaling pathway in KEGG
The proteins marked light green were discovered by iTRAQ.

Table 1. First ten hub proteins from the INTACT and HPRD database

Accession	Symbol	Protein Name	Degree
IPI00927961	FYN	Tyrosine-protein kinase Fyn	299
IPI00647981	YBX1	Y-box protein 1.	166
IPI00988539	VIM	Vimentin	163
IPI00227900	PRKACA	Isoform 2 of camp-dependent protein kinase catalytic subunit alpha	160
IPI00515199	EWSR1	Ewing sarcoma breakpoint region 1	153
IPI00230264	H2AFX	Histone h2a.x.	143
IPI00895403	CACNA1A	Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	95
IPI00988468	PTN	Pleiotrophin	92
IPI00120995	TFCP2L1	Transcription factor cp2-like protein 1.	91
IPI00113362	CRKL	Crk-like protein	75

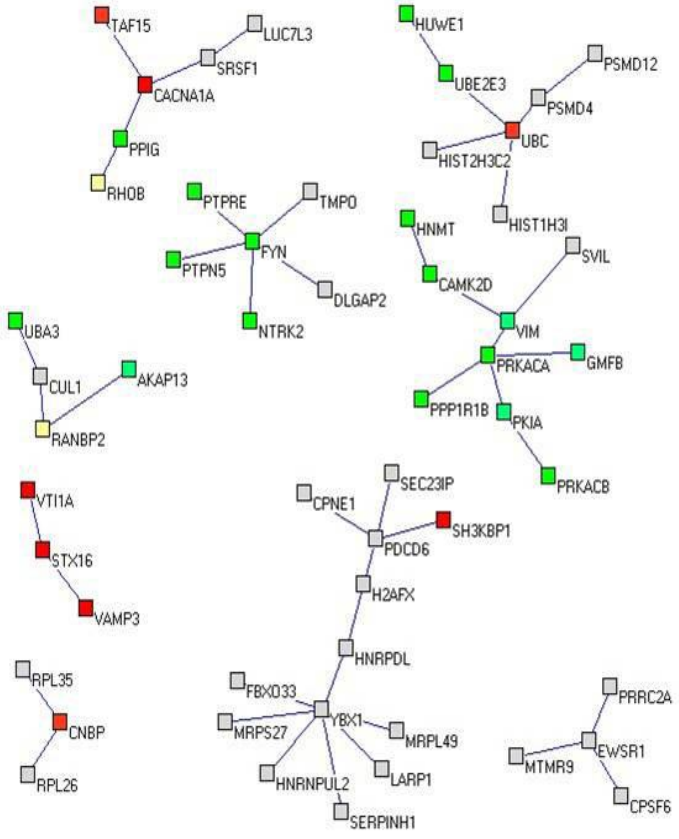

Fig. 3. Protein interaction network by Pajek

proteins marked light green were the proteins discovered by iTraq.

A network of protein interaction was constructed with PAJEK (Fig. 3). The first ten hub proteins based on the INTACT and HPRD database are show in Table 1.

DISCUSSION

How the extra chromosome 21 causes mental retardation is not clear. Previously, we applied a new proteomics platform termed iTRAQ (isobaric tags for relative and absolute quantitation) to screen differentially expressed proteins in the hippocampi of Ts65Dn mice. This technology is quantitative, accurate, reliable and reproducible, and is applicable to multiple parallel quantification analyses (Choe et al., 2005; Wu et al., 2006). Our results indicate that iTRAQ could reliably screen differentially expressed proteins. Thus, 374 differentially expressed proteins were found in the hippocampal tissue of Ts65Dn mice. In the present study, we described the biochemical characteristics of different proteins and explored their complicated network using bioinformatics analysis.

With GOA, we found that 63.9% of differentially expressed proteins in the hippocampus of Ts65Dn mice discovered by iTRAQ are related to cellular processes, including cell communication, cell death, cell growth, cell differentiation, cell division, cell organization and biogenesis, cell proliferation, cellular homeostasis, development, regulation of biological processes, reproduction, response to stimuli, and transport. Studies have suggested that enhanced ap-

optosis could play a role in the mental deficiencies and neurodegeneration in the brain of DS (Engidawork et al., 2001). Additionally, it was reported that disruption of neurogenesis and apoptosis, the two fundamental processes underlying brain development, could reduce neuron numbers in the DS hippocampal region (Guidi et al., 2008). We hypothesize that the differentially expressed proteins play an important role in the neurological impairments observed in DS. Future studies need to examine this assumption.

KEGG pathway analysis showed that the insulin-signaling pathway was statistically significant in DS. Insulin receptors in the cerebral cortex and hippocampus are closely related to cognition (Unger et al., 1991); Chiu et al., 2008).

Recent research has shown that deficits in the insulin-signaling pathway are related to Alzheimer's disease (Hoyer, 2002; Steen et al., 2005), and it was established that insulin plays an important role in regulating proliferation, differentiation and apoptosis in the central nervous system (Garcia-de Lacoba et al., 1999). We suggest that the insulin-signaling pathway is involved in the neurological impairment in DS.

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