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Differential proteomic and genomic profiling of mouse striatal cell model of Huntington's disease and control; probable implications to the disease biology

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ABSTRACT

Huntington's disease (HD) is an autosomal dominant disorder of central nervous system caused by expansion of CAG repeats in exon1 of the huntingtin gene (*Htt*). Among various dysfunctions originated from the mutation in *Htt* gene, transcriptional deregulation has been considered to be one of the most important abnormalities. Large numbers of investigations identified altered expressions of genes in brains of HD patients and many models of HD. In this study we employed 2D SDS-PAGE/MALDI-MS coupled with 2D-DIGE and real-time PCR experiments of an array of genes focused to HD pathway to determine altered protein and gene expressions in $STHdh^{Q111}/Hdh^{Q111}$ cells, a cell model of HD and compared with $STHdh^{Q7}/Hdh^{Q7}$ cells, its wild type counterpart. We annotated 76 proteins from these cells and observed differential expressions of 31 proteins (by 2D-DIGE) involved in processes like unfolded protein binding, negative regulation of neuron apoptosis, response to superoxides etc. Our PCR array experiments identified altered expressions of 47 genes. Altogether significant alteration of 77 genes/proteins could be identified in this HD cell line with potential relevance to HD biology.

Biological significance: In this study we intended to find out differential proteomic and genomic profiles in HD condition. We used the ST*Hdh* cells, a cellular model for HD and control. These are mouse striatal neuronal cell lines harboring 7 and 111 knock-in CAG repeats in their two alleles. The 111Q containing cell line ($STHdh^{Q111}/Hdh^{Q111}$) mimics diseased condition, whereas the 7Q containing ones ($STHdh^{Q7}/Hdh^{Q7}$), serves as the proper control cell line. Proteomic experiments were performed earlier to obtain differential expressions of proteins in R6/2 mice models, Hdh^{Q} knock-in mice and in plasma and CSF from HD patients. However, no earlier report on proteomic alterations in these two HD cell lines and control was available in literature. It was, therefore, an important objective to find out differential expressions of proteins in these two cell lines. In this study, we annotated 76 proteins from $STHdh^{Q7}/Hdh^{Q7}$ and $STHdh^{Q111}/Hdh^{Q111}$ cells using 2D-gel/mass spectrometry. Next, by performing 2D-DIGE, we observed differential expressions of 31 proteins (16 upregulated and 15 downregulated) between these two cell lines. We also performed customized qRT-PCR array focused to HD pathway and found differential expressions of 47 genes (8 gene expressions increased and 39 genes were decreased significantly). A total of 77 genes/proteins (Htt downregulated in both the studies) were found to be significantly altered from both the experimental paradigms. We validated the differential expressions of Vim, Hypk, Ran, Dstn, Hspa5 and Sod2 either by qRT-PCR or Western blot analysis or both. Out of these 77, similar trends in alteration of 19 out of 31 and 38 out of 47 proteins/genes were reported in earlier studies. Thus our study confirmed earlier observations on differential gene/protein expressions in HD and are really useful. Additionally, we observed differential expression of some novel genes/proteins. One of this was Hypk, a Htt-interacting chaperone protein with the ability to solubilize mHtt aggregated structures in cell lines. We propose that downregulation of Hypk in $STHdh^{Q111}/Hdh^{Q111}$ has a causal effect towards HD pathogenesis. Thus the novel findings from our study need further research and might be helpful to understand the molecular mechanism behind HD pathogenesis.

1. Introduction

Huntington's disease (HD, OMIM ID 143100) is an autosomal dominant progressive neurodegenerative disease caused by expansion of normally polymorphic CAG repeats beyond 36 at exon1 of the gene *Huntingtin* (*Htt*), also known as IT15 [1]. Various cellular processes and pathways like enhanced apoptosis, compromised mitochondrial

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functions, increased oxidative stress, ubiquitin-proteasomal dysfunctions, altered autophagy etc. are observed in HD [2–4]. Among the various dysfunctions originated from the mutation of Htt protein, transcriptional deregulation has been considered as one of the most important abnormalities. The gene expression alterations observed in HD might be contributed by several mechanisms including aberrant interaction of transcription factors with mutant Htt (mHtt) and sequestration of these transcription factors in mutant Htt aggregates, non-specific binding of mHtt with chromosomal/genomic DNA, global changes in histone modifications, DNA methylation and deregulation of many microRNAs [5–10]. Many high-throughput studies using microarray or RNA sequencing revealed alteration of thousands of genes in various models as well as in brains of HD patients [8].

Two-dimensional (2D) gel/LC/mass spectrometry (MS)-based proteomic approaches were utilized earlier by other investigators to identify altered proteins in HD models. Alterations in 6% of the proteome in early stage of the disease (before the onset of symptoms) were identified in R6/2 mice model of HD. Proteins increased in the early stage were involved in glycolysis or gluconeogenesis, while downregulated proteins were mainly found to be actin cytoskeleton [11]. Deregulations of nearly 50 proteins were identified using R6/2 mice models of HD. Among these proteins, many are associated with neurotransmission and are likely to be involved in HD etiology [12]. Perluigi et al. identified increased expression of dihydrolipoamide S-succinyltransferase and aspartate aminotransferase in 10-week old R6/2 mice, but expression of pyruvate dehydrogenase was decreased in 10-week old mice compared to the 4-week old. Six oxidized proteins namely alpha-enolase, gamma-enolase (neuron-specific enolase), aconitase, the voltage-dependent anion channel 1, heat shock protein 90 and creatine kinase were also identified [13]. In cerebrospinal fluid (CSF) of 6 pairs of HD patients and controls, increased expressions of prothrombin and apolipoprotein A-IV (Apo A-IV) were identified [14]. Alterations of proteins have been identified using SELDI-TOF and complemented by difference in-gel electrophoresis (DIGE) in brains of HD mouse models (HdhQ150 and HdhQ92). The population of altered proteins in HD was significantly enriched with proteins having mitochondrial functions [15]. Even though thousands of deregulated genes have been identified by microarray (reviewed in [8]) or RNA sequencing [10], altered expressions of only about hundred proteins have been identified by proteomics.

To identify proteomic and genomic deregulations in HD, we utilized STHdh^{Q7}/Hdh^{Q7} and STHdh^{Q111}/Hdh^{Q111} cells. These cells were derived from striatal primordia of wild type and homozygous mutant Hdh knock-in embryonic mice. ST*Hdh* cell lines express full-length *Htt* gene with 7 (STHdh^{Q7}/Hdh^{Q7}) or 111 (STHdh^{Q111}/Hdh^{Q111}) Glu residues. In these cell lines, Htt is expressed from endogenous chromosomal promoter at physiological levels. STHdh^{Q111}/Hdh^{Q111} cells represent HD model and show many features of the disease [16]. This cell model has been widely studied for identification of gene expression abnormalities [6,10] and other molecular defects in HD. STHdh^{Q7}/Hdh^{Q7} cell lines harbor full length *Htt* gene with 7 (much lower than the pathogenic polyQ threshold, i.e. 36) CAG repeats and served as control having all other genotypic features exactly the same. In our study, we performed 2D SDS-polyacrylamide gel electrophoresis (2D SDS-PAGE), 2D-DIGE and MALDI-mass spectrometry (MALDI-MS) to identify the protein expression alterations in HD using this cell model. In addition we used PCR array to identify gene expression alterations in STHdh^{Q7}/Hdh^{Q7} and STHdh^{Q111}/Hdh^{Q111} cell lines.

2. Materials and methods

2.1. Cell culture & transfection

$STHdh^{Q7}/Hdh^{Q7}$, $STHdh^{Q111}/Hdh^{Q111}$ and Neuro2A cells were grown and transfected following the methods described earlier [17,18]. ST*Hdh* cells are mouse striatal neuronal cells harboring either 7 CAG repeats or 111 CAG repeats in homozygous conditions and express from chromosomes under endogenous promoters. When translated, these cells express 7Q and 111Q respectively [16]. HD pathogenic repeats 111Q expressing cell lines $STHdh^{Q111}/Hdh^{Q111}$ represents the HD model and 7Q expressing ones ($STHdh^{Q7}/Hdh^{Q7}$) represent the wild type condition.

2.2. Chemicals

All chemicals were procured from Sigma Chemicals if not mentioned otherwise.

2.3. Antibodies

Anti-Hypk polyclonal (catalog no. SAB1101780, Sigma, USA, dilution used 1:4000), anti-Vim monoclonal (catalog no. V6630, Sigma, USA, dilution used 1:1000), anti-Ran monoclonal (catalog no. 610341, BD Biosciences, dilution used 1:6000), anti-Coffilin/Dstn monoclonal (catalog no. 3318, Cell Signaling Technology, dilution used 1:1500), anti-Hspa5 monoclonal (catalog no. 3177, Cell Signaling Technology, dilution used 1:1000), anti-Sod2 monoclonal (catalog no. ab16956, Abcam, dilution used 1:1000) and anti-β-actin monoclonal (catalog no. A2228, Sigma, USA, dilution used 1:10,000) primary antibodies were used for immunoblot analysis. Anti-mouse IgG-HRP (1:8000 dilution) and anti-rabbit IgG-HRP (1:6000 dilution) secondary antibodies were purchased from Genei, India.

2.4. Preparation of cell extracts from $STHdh^{Q7}/Hdh^{Q7}$ and $STHdh^{Q111}/Hdh^{Q111}$ cells

Whole cell extracts from exponentially growing $STHdh^{Q7}/Hdh^{Q7}$ and $STHdh^{Q111}/Hdh^{Q111}$ cells were prepared for analyzing proteins using 2D SDS-PAGE. For comparison, $STHdh^{Q7}/Hdh^{Q7}$ and $STHdh^{Q111}/Hdh^{Q111}$ cells from the same passage numbers were used for each experimental set. In brief, cells were washed and harvested in 1X ice-cold PBS upon centrifugation at 4500 rpm for 5 min at 4 °C. The harvested cell pellets were resuspended in 50 μl cell lysis buffer containing 7 M urea, 2 M thiourea, 4% CHAPS, 20 mM DTT, 1 mM PMSF and 2 mM EDTA, kept on gentle agitation at 4 °C for 1 h and centrifuged at 13,000 rpm for 15 min at 4 °C after sonication. The supernatant thus obtained was the whole cell extract from respective cell line. Protein contents were estimated by Bradford's method.

2.5. 2D SDS-PAGE

The method for 2D SDS-PAGE was similar to that published [19]. In brief, protein samples were salt depleted using acetone precipitation method. Whole cell extracts were mixed with chilled acetone 1:4 (v/v), incubated for 3 h at −20 °C with intermittent vortexing and centrifuged at 13,000 rpm for 15 min at 4 °C. After air-drying, these protein precipitates were resuspended in 300 μl of 30 mM Tris–Cl pH 8.5 rehydration buffer (containing 8 M urea, 4% CHAPS, 50 mM DTT and 2% ampholyte, pH 3–10). Two 17 cm pH 3–10 IPG strips were rehydrated overnight with 1 mg resuspended proteins from STHdh^{Q7}/Hdh^{Q7} and STHdh^{Q111}/Hdh^{Q111} cells obtained as described above. Isoelectric focusing (IEF) was carried out at 20 °C in a Protean IEF Cell (GE Healthcare, USA) at 50 μA current/strip followed by strip equilibration using methods described earlier [20]. These focused and equilibrated strips were run on 12% homogenous SDS-polyacrylamide gels followed by blue silver staining [21]. The 2D gels were scanned by Versa Doc (Bio-Rad, USA) and spots were excised from the gel(s) using Proteome Workstation Spot Cutter (Bio-Rad, USA) and processed for subsequent MALDI-MS identification. We performed four different 2D SDS-PAGE for mass spectrometric identification of proteins.