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Huntington's disease: from experimental results to interaction networks, patho-pathway construction and disease hypothesis

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Background

Protein-protein interaction networks and mechanistic pathway models are excellent tools in the drug discovery process. They can be used to identify and select targets for a given disease hypothesis. Combining information from diverse sources, like in house experiments as well as literature, allows further development of interaction networks into detailed descriptions of cellular pathways. Computerized pathway diagrams allow integrating all relevant data regarding a project into one framework by linking the different data sources. Interaction networks analysis and pathway design tools are used to support target identification and validation activities. Experimental results are incorporated in protein interaction networks, analyzed and further developed into biomolecular pathopathways including literature findings to understand the underlying modulation mechanisms. The pathway diagrams are also used as communication tools, particularly for interdisciplinary project teams, thus ensuring a common understanding and facilitating critical interrogations about disease hypotheses. The analyses of experimental results, the initial construction of an HD pathopathway are presented and two mechanistic disease hypotheses are discussed.

Materials and methods

Differential proteomics experiments (DPEs) were performed on rat PC12 cells containing either wild-type or

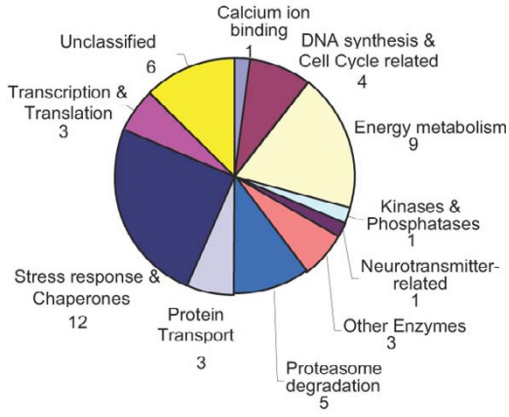

Figure 1

Functional classification by cellular process of the proteins confirmed to be modulated in the DPEs. Numeric values indicate the number of proteins in each class. The results of MS identification using partially overlapping databanks (UniProt, and rat ENSEMBL+GENSCAN) were stored in a relational database designed in-house. Redundancy was removed and the lists of genes encoding the identified proteins were mapped on networks using MetaCore http://www.genego.com/metacore.php for analysis. In agreement with the experimental design, the apoptosis process is not present amongst the functional classes, while the "Stress response & Chaperones", "Energy metabolism" and "Proteasome degradation" are the best represented processes in the final results set.

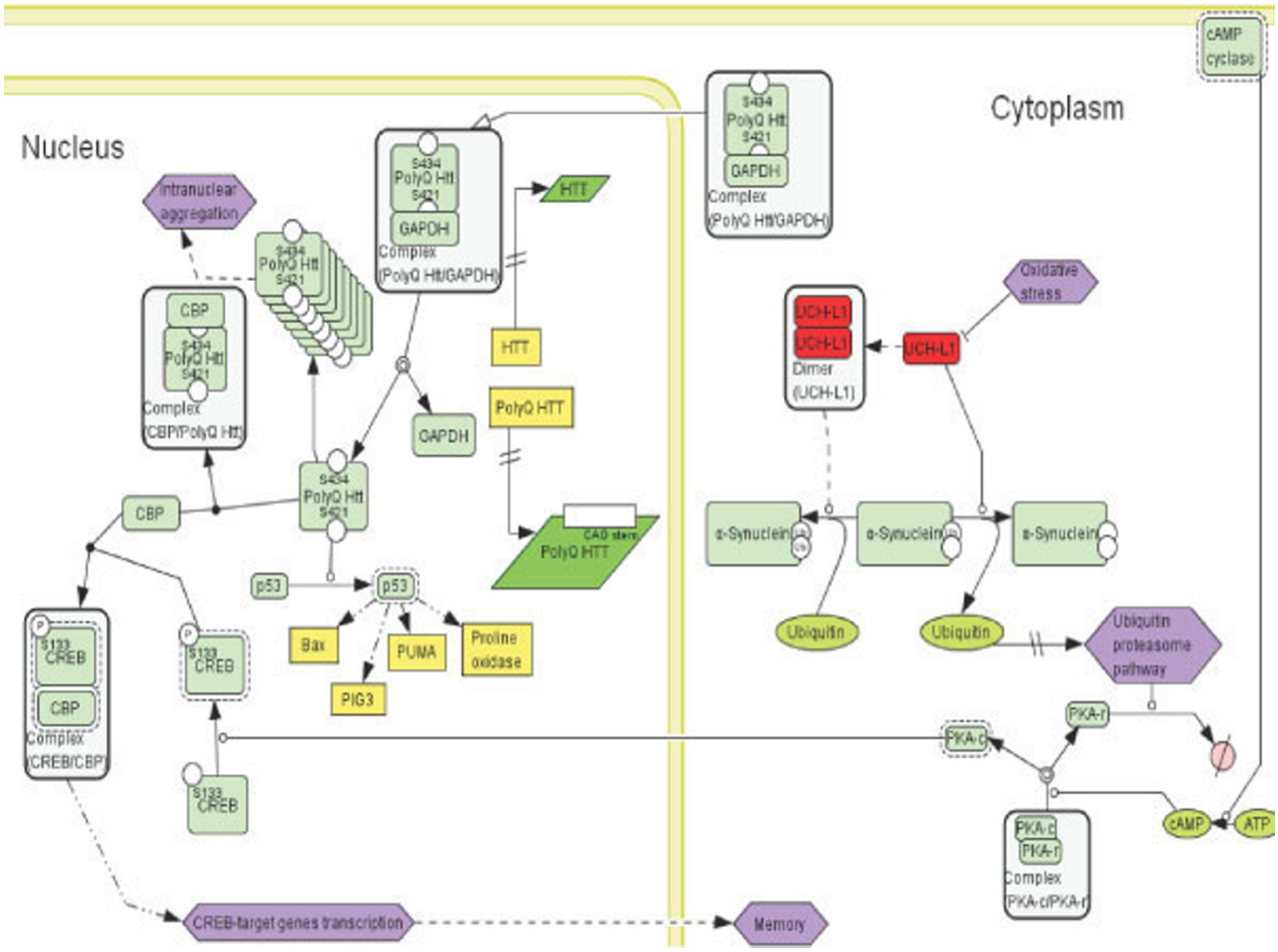

Figure 2
Mechanistic pathopathway linking UCHL1 in HD. Literature and experimental findings are integrated to link proteins modulated by the expression of PolyQ Htt with intracellular pathways. As a starting point, an HD pathway from the Panther pathway database http://www.pantherdb.org/ was used and subsequently enriched with proteins and events functionally involved in cellular processes linked to neurodegeneration using CellDesigner http://celldesigner.org/. Inside the nucleus of HD patients, the mutant PolyQ Htt gene is transcribed into a messenger RNA with a potential stem secondary structure [9]. Intranuclear aggregation of PolyQ Htt sequesters proteins binding to PolyQ Htt, including CBP, hence reducing the cAMP response element-mediated transcription of the CREB-target genes [1]. UCHL1 was reported to potentiate CREB-target genes transcription by restoring normal proteasomal degradation of the PKA-regulatory subunit II alpha (PKA-r), PKA activity (PKA-c), and CREB phosphorylation, hence resulting in contextual memory retrieval [6].

mutant full-length (PolyQ) Huntingtin (Htt) under control of a doxycycline-inducible promoter [1]. Cell extracts were prepared at 0, 12, and 48 hours post-induction to identify proteins involved in pre-apoptotic intracellular events. Protein expression modulation was measured using DIGE technology [2] followed by statistical analysis for spot selection and automated spot picking. The protein content of each picked spot was analyzed by mass spectrometry (MS).

Results

Three independent DPEs were performed and the modulated proteins, confirmed in at least two experiments, were analyzed in the context of protein networks. In a typical experiment, 121 differential spots were picked and MS identification produced 3671 entries grouped into 359 proteins, an expected average of about 3 proteins per spot [3]. In the end, 48 proteins were confirmed to be modulated by the expression of PolyQ Htt, and were

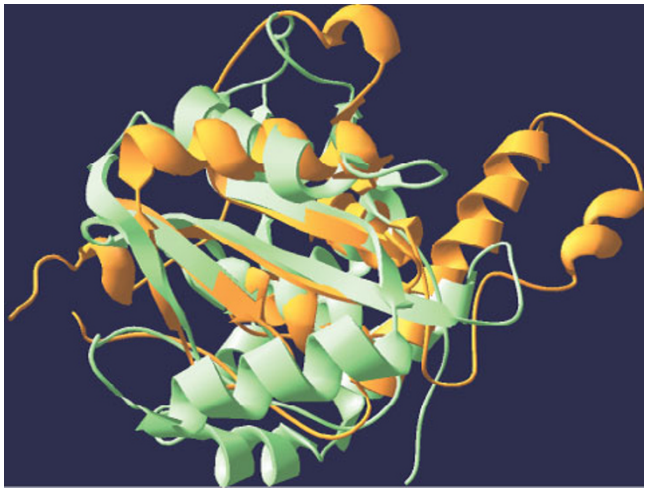

Figure 3
Common structural features of UCHL1 and of the Josephin domain of Ataxin-3. The Josephin domain of Ataxin-3 [11,12] was recently shown to initiate the aggregation of the entire protein independently of its PolyQ tract [13]. Using the DaliLite server http://www.ebi.ac.uk/DaliLite/, the superposition of UCHL1 (green, 2ETL) to the Josephin domain (orange, 1YZB) reveals a common core containing a large beta sheet surrounded by 3 superposed alpha helices.

therefore considered for bioinformatics analysis. Since the Ubiquitin-Proteasome system is a particularly important biological process shown to be involved in neurodegeneration [4], the focus was put on the "Proteasome degradation" (Figure 1) class for enrichment of the mechanistic HD pathopathway. In this class of cellular function, the Ubiquitin Carboxyl-terminal Hydrolase isozyme L1 (UCHL1) protein was of particular interest, since it is known to be associated with Parkinson [5], Alzheimer [6], and was described as a genetic modifier of the age of onset of HD [7,8]. Since modulation of UCHL1's mRNA by PolyQ Htt was confirmed by RT-PCR (data not shown), we linked UCHL1 into our HD pathopathway (Figure 2).

Divergent hypotheses can be elaborated for the role of UCHL1 in HD. First, a positive role by contributing to Ubiquitin recycling, thus maintaining normal Proteasome pathway function counteracting the accumulation of insoluble PolyQ Htt. Second, based on the recent discovery that peptide sequences can modulate the toxicity of PolyQ tracts in *cis* or *trans* [10], the transient interaction between UCHL1 and PolyQ Htt to recycle Ubiquitin could actually increase the toxicity of the extended PolyQ tract of mutant Htt by initiating the first step of the formation of aggregates. The latter mechanism can be envisaged, as UCHL1's structure can be superposed to a typical fibrillogenic domain (Figure 3).

Conclusion

DPE results were used to develop an HD pathopathway including proteins modulated by PolyQ Htt expression. In this experimental result set, the proteins from the Ubiquitin-Proteasome pathway were chosen for in depth analysis, and UCHL1 was identified as a component potentially playing opposite roles in HD.

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