Abstract

Huntington’s disease (HD) is a hereditary, progressively degenerative and fatal brain disorder classified as a rare, or ‘orphan’, disease. HD is caused by the extension of trinucleotide repeats encoding a stretch of glutamine residues at the amino-terminal end of the large huntingtin (HTT) protein. Since the discovery of the mutated HTT gene in 1993, the mechanisms by which the mutant HTT protein induces neurodegeneration remain poorly understood and no disease-modifying therapy is currently available. Several functional approaches combining different experimental models and experimental technologies have been used to shed some light on the mechanisms underlying this disease. This review presents these functional approaches, highlights their potential and limitations.

Keywords: HD; transcriptomics; proteomics; interactomics; bioinformatics; pathways

INTRODUCTION

Several neurodegenerative disorders are caused by the expansion of CAG DNA-triplet repeats coding for poly-glutamine (PolyQ) expansions [1]. The discoveries made in for Huntington’s disease (HD), the most common and probably the most actively studied of this family of diseases, may help elucidate common pathogenic mechanisms. As such, HD can be considered as a model for the family of neurodegenerative disorders caused by an extension of a PolyQ stretch. HTT is normally involved in vesicle endocytosis, regulation of calcium and inhibition of apoptosis via brain-derived neurotrophic factor (BDNF) [2], while mutant, PolyQ expanded HTT may lose these functions, as well as gain toxicity via stimulation of apoptosis, dysregulation of gene transcription, and abnormal calcium-mediated signal transduction pathways.

The functional approaches applied to the study of HD rely on the availability of several HD models. Currently, these models are cell models, yeast models, worm models, fly models, neurotoxin-based models, HTT amino-terminal transgenic mice and rats, mouse knock-in models, full-length mouse models and non-human primates [3–5]. Among these HD models, the inducible models that allow controlling the expression of the mutant HTT protein constitute tools of particular interest for the functional studies. These conditional expression models allow switching on the expression of the mutant HTT, or of an HTT fragment containing the PolyQ expansion, to study the disease onset and the early events involved in this process. They also allow switching off the mutant HTT expression to investigate which neurodegeneration phenotypes can be reverted [6].

THE FUNCTIONAL APPROACHES

Although historically the transcriptomic studies were performed earlier than the proteomics-based experiments, this review takes a functional point of view by starting with the actual cause of HD: the HTT protein. The HTT protein is known to be involved in several potentially pathogenic intracellular pathways [2], and there is currently little consensus as to the mechanism of toxicity in HD [7]. A large body of evidence indicates that the generation of aberrant proteolytic fragments from the mutant HTT protein...
produces soluble toxic species, which are inactivated as insoluble aggregates [8].

FUNCTIONAL STUDIES OF HD AT THE PROTEIN LEVEL

Since processes at the protein level, like proteolysis [9], are linked to the toxicity of mutant HTT, the mass spectrometry (MS)-based proteomics approaches are obviously deemed as particularly appropriate to study HD. Indeed, several proteomics-based studies report the identification of HD differentially expressed proteins [10, 11]. The proteins identified are functionally involved in cellular processes like the response to stress, the folding of proteins (chaperones), the proteasome degradation or the energy metabolism, which clearly constitute functions affected in HD. The limitation of the proteomics-based approaches resides in the inability to separate the causes from the effects, coupled to a dynamic range which is not sufficient to identify differences in the expression level of low abundant proteins in the brain, in which some highly abundant proteins, as for instance the protein encoded by the UCHL1 gene [12], mask the presence of rare proteins. In a disease with a slow progression like HD, it is expected that subtle imbalances will initially trigger the pathogenesis. Considering the current disease models limitations and the lack of biomarkers for early diagnosis in HD, only the latest developments in proteomics [13] might contribute to identify the early events that drive the development of the disorder.

Protein–protein interaction studies are also of particular interest to understand the molecular mechanisms underlying HD. The high-throughput Yeast Two Hybrid (Y2H) approach was applied to identify the HTT interacting partners [14, 15]. These HTT interactors are functionally important as they might mediate the toxicity of HTT and constitute better therapeutic targets than HTT itself, which is currently not tractable as drug target. Some of the interacting proteins are likely to be tractable drug targets and act as mediators of HTT’s toxicity. Actually, several validated HTT interacting proteins were reported to be modifiers of the neurodegeneration induced by HTT in a fly model [14] and might constitute better ‘druggable’ targets. The Y2H approach for finding interacting proteins of a given ‘bait’ can be generalized to many other diseases caused by a known protein. In addition, some databases allow searching for the interacting partners ‘in silico’. Public databases containing protein interaction networks like the Search Tool for the Retrieval of Interacting Genes (STRING) [16] constitute highly valuable sources of information for the bioinformatics identification of the proteins interacting with a protein involved in a disease (Figure 1). The subsequent exploration of the identified interactome ‘space’ surrounding a disease-related protein allows proposing putative disease mechanisms and generating new hypothesis for experimental studies.

Beyond the relatively crude networks of protein–protein interactions, mechanistical pathways using well-defined advanced graphical notations, like the popular Systems Biology Graphical Notation (SBGN) [17], describe in more details the succession of events driving signalling cascades or metabolic pathways and fit better the systems biology context. Actually, the most detailed and refined pathways modelled using the Systems Biology Markup Language (SBML) standard actually allow developing ‘in silico’ models and even running simulations [18]. Public databases like Panther [19] or commercial databases like Ingenuity [20] contain mechanistical pathways describing some of the molecular events involved in HD. These pathways are unique tools to integrate different levels of biological information. For instance, the HD pathway from Panther recapitulates the majority of mechanisms described in the literature and the intracellular organelles involved in the pathological processes, including the mitochondrion and the nucleus (Figure 2).
As an extension of the studies at the protein level, the mechanistical pathways describing signalling and metabolic reactions currently constitute the best tool to integrate non-protein metabolites from metabolic processes and incorporate the results from metabolomics studies [21]. The expectation is that such a systems biology integration will allow identifying the biomarkers required for preclinical and clinical testing of new therapeutic agents. The difficulty in these integrative approaches resides in the usage of extremely heterogeneous data sources: different model organisms, from yeast to primates, together with human patients [4, 5]. Even in relatively homogeneous human studies, different cell types are often used. It is thus, at present, impossible to determine if all the different models actually recapitulate the same pathological processes occurring in the individuals affected by HD.

Nevertheless, there is significant progress in the quest for the highly needed therapeutic agents to cure HD, and some of the advances in the discovery of new compounds are directly linked to the implementation of functional integrative approaches [22].

FUNCTIONAL STUDIES AT THE TRANSCRIPTION LEVEL

Another type of functional approach applied to the study of HD is associated with the evidence that expression of mutant HTT alters gene transcription [23]. The aberrant intranuclear accumulation of the cleaved HTT fragments is thought to interfere with, or cause the sequestration of, several transcription factors, hence disrupting the transcription of their target genes. This toxicity mechanism of functional impairment of gene transcription might be common in the class of polyQ disorders [1] and certainly justifies the development of transcriptomics approaches.

In the specific case of HD, one of the first large-scale studies was performed using high-throughput real-time PCR (RT-PCR) with an inducible cellular model of HD [24]. This model is constituted by stable inducible PC12 cell lines expressing an amino-terminal (exon 1) fragment of the HTT gene with various PolyQ lengths (wild type or mutant) under control of an inducible promoter. About one-quarter of the 126 differentially expressed genes identified were found to be down-regulated by
the expression of mutant HTT. This work was followed by several gene expression studies using microarrays technology (reviewed in [25]) that revealed lists of genes whose expression was down-regulated by the expression of mutant HTT. Interestingly, the changes in expression observed in several mouse models faithfully recapitulate those measured in human HD and gene expression changes measured in primary striatal neurons affected by mutant HTT are similar to those observed in human HD brain [26].

The availability of a brain atlas of gene expression [27] allows transposing the results of the transcriptomics studies in their anatomical context. For instance, amongst the mouse and human highly concordant early changes identified in microarray studies, the ADORA2A gene, which encodes the adenosine A2a receptor, is also found to be the gene with the most specific expression in the most affected area of the HD brain; namely the striatum (Figure 3).

Although the gene expression profiling of the transcriptional abnormalities in HD provides a transcriptional signature of the disease [25], which potentially constitutes a useful readout in clinical trials, there are still issues associated to this approach. First, the early reports suggesting that transcriptomic biomarkers can be revealed from human blood [28], were challenged in more recent publications [29]. Second, it is still unclear if genes displaying changes in expression actually have a role in the pathology. Answering this last question requires several validation experiments. While altered transcription is clearly a phenotype, it is not clear if these gene changes are causative or epiphenomenal, raising again the issue of causality.

LOOKING FOR A CLEAR CAUSALITY

While the results obtained with proteomics or the transcriptomics technologies do not allow separating the causes from consequences, some functional approaches do provide a clear ‘cause to effect’ link. These approaches are the genome-scale loss of function studies using either RNA-mediated interference (RNAi) or mutagenesis in HD models, from yeast to mammals [3].

Large-scale RNAi screens have emerged as a key tool in target identification and validation studies [30]. These screens allow identifying neuroprotective or
enhancers of toxicity of HTT and the pathways in which they are involved. The reagents, libraries of small interfering RNA (siRNA) and automated equipments required for performing such screens are nowadays commercially available. In the specific case of the genes protecting against neurotoxicity upon knocked down, it is particularly interesting to focus the RNAi screen on the so-called ‘druggable genome’ [31] to maximize the chances that the product of the genes identified can also be inhibited through small molecules. In the context of the hunt for therapeutic targets in HD, such a focus constitutes a pragmatic option. The focus can then be shifted to exhaustively study the genes involved the most relevant pathways and identify the limiting steps in each pathway and assess their robustness to perturbations [32].

Recent large-scale RNAi screens using a fly model of HD [33, 34] identified genetic modifiers of HTT toxicity/aggregation. These candidates fell into several functional groups including transport, protein trafficking, biogenesis, chaperones, nuclear transport and ubiquitin-proteasome system. A previous RNAi screen in the nematode worm Caenorhabditis elegans identified genes being part of the functional processes of RNA synthesis and processing, protein synthesis as modifiers of misfolded proteins production, and of protein folding, transport and degradation as modifiers of the clearance of misfolded proteins [35]. In the near future, RNAi screens performed using human cell models of HD will most likely complete this picture of the HTT toxicity gene modifiers. Hopefully, some of the proteins encoded by these genes will be amenable to therapeutic intervention.

The validity of this concept of genome-wide screens in HD model organisms is strengthened by the latest publication of a chemical mutagenesis performed in C. elegans [36]. This screen allowed the identification of an evolutionary conserved, ubiquitously expressed, modifier of aggregation involved in a previously unexplored pathway. The human orthologues of the C. elegans modifier of aggregation were also shown to suppress aggregation upon RNAi knock down, indicating that the mechanism has been conserved from worms to humans. Last, but not least, the authors verified that the aggregation and toxicity of aggregation-prone proteins involved in other pathologies than HD, namely Beta-amyloid for Alzheimer’s disease and Alpha-synuclein for Parkinson’s disease, could also be suppressed, thus confirming the idea of a common mechanism among a larger class of disorders involving fibrillar aggregates.

CONCLUSIONS

The wide variety of functional approaches used to study HD has brought a lot of information about the functional processes associated to the pathology. This information helps to dissect the disease into many of its facets, but still does not allow creating a general model of the disease. The symptoms of HD have conventionally been linked to cell death in the particularly vulnerable medium spiny neurons in the striatum and in cortical neurons, but other symptoms in peripheral tissues such as weight loss and skeletal-muscle wasting indicate that HD is not only a brain disease [37]. These evidences suggest that novel targets for therapeutic intervention and new biomarkers could be discovered by opening the research activities beyond the brain. They also indicate that the whole picture still needs to be assembled, probably through an integration of the results from various technologies including proteomics, interactomics, metabolomics and transcriptomics, in pathway models. Such a systems biology effort will help to understand the combination of all the processes involved across the body along time. This endeavour requires assembling teams with different expertises supported by state of the art technologies, like high-throughput whole-genome sequencing, induced pluripotent stem cell and user-friendly integration bioinformatics tools with flexible visualizations. The expectation is that such a combination will quickly benefit the HD patients, and that some findings will also be transposable to other neurodegenerative disorders.

Key Points

- The issue of causality: it is not possible to discriminate if the changes observed using proteomics or transcriptomics-based technologies in experimental models of HD are causative or epiphenomenal.
- Functional approaches based on genome-scale loss of function studies using either RNAi or mutagenesis in HD models provide a clear ‘cause to effect’ link.
- Integration of the information generated by the large number of functional approaches and models is complex but required to understand HD at the patient level and to transpose certain mechanisms to other neurodegenerative diseases.
References


