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Huntington's Disease: Current Status and Prospects

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Abstract

Huntington's disease (HD) is a hereditary neurodegenerative disorder caused by the mutant *HTT gene*. Its monogenetic nature offers unique advantages in HD basic and translational research. Recently, major advances in the revealing of its mechanisms have led to promising therapeutic strategies, which provide novel insights into other neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD) and ataxias. In this review, we will briefly discuss recent important progresses in HD research.

Introduction

Neurodegenerative diseases are a class of serious diseases that cause progressive neuronal death which gradually lead to dysfunction of nervous system until collapse. It is more common in middle-aged and older people, causing a huge social burden. The importance of research on neurodegenerative diseases is evident as the aging population of China increases. Currently there is no disease-progression modifying treatment for these diseases.

The most extensively studied neurodegenerative diseases are AD, PD, amyotrophic lateral sclerosis (ALS) and HD. HD is one of the most important monogenic diseases, which is caused by mutations in the *HTT gene*. This clear genetic etiology has two advantages: first, HD can be diagnosed early by sequencing *HTT gene*, providing possibility for timely treatment; second, a clear genetic driver makes it easy for scientists to establish disease models to simulate HD from genotypes to phenotypes, which facilitates unraveling the disease mechanisms. Therefore, regardless of the lower incidence of HD compared to AD and PD, the study of HD's mechanism and treatment is more likely to make a breakthrough. On the other hand, the clinical manifestations and molecular signaling pathways of HD are very similar to other neurodegenerative diseases, which are all related to protein misfolding and accumulation. Therefore, HD researches also provide important information for the study of the pathogenesis of other neurodegenerative diseases such as AD and PD.

The major clinical symptoms of HD are dance-like involuntary movements (gradual loss of moving capacity in the late stage), mental disorders and progressive cognitive impairment [1]. The average onset age of HD is 40 - 50 years old, but it is also occasionally seen in children and adolescents, the latter being called juvenile onset HD. The incidence of HD is about one in ten thousand around the world, regardless of gender and ethnicity. The average survival times of patients are 10 - 20 years. HD was first systematically described and named by doctor George Huntington in 1872 [2]. After nearly a hundred years of efforts to reveal the genetic causes of HD, scientists finally discovered its pathogenic gene HTT (also known as IT15) in 1993 [3]. The *HTT gene* is located on chromosome 4. It has CAG repeats in the exon 1 region, and the number of CAG repeats in the HTT from normal population is 6 to 35. When the CAG repeat length expands to more than 40, it will lead to HD accompanied by abnormal motor symptoms. When between 36~39, some patients will develop symptoms, some will not [1].

Since the discovery of the disease-causing gene HTT in 1993, scientists have made a series of important breakthroughs in the field of HD research. The following is a brief review of the progresses over the past 25 years from three aspects: disease models, disease mechanisms, and treatment strategies.

Disease Models

Currently, HD has neither curative treatment nor clear mechanism of disease occurrence and progress. Only a few symptom-improving drugs exist, and their effects will disappear due to resistance after a period of time. Therefore, the establishment of cell and animal models that accurately simulate disease phenotypes and pathologies is essential for the development of effective disease treatments.

Rodent models

The rodent models of HD are mainly divided into two categories. The first type mainly transgenically overexpresses the N-terminal fragment of mutant HTT (mHTT) containing polyQ sequence, including R6/2, R6/1 model [4] and N171-82Q model [5]. The second type of HD rodent model expresses full length mHTT. One way is to express the transgenic human mHTT by artificial chromosome, mainly including YAC128 (using yeast artificial chromosome) and BACHD (using bacterial artificial chromosome) [6,7]. Another way to express full-length mHTT is to replace exon 1 of the mouse *HTT gene* with that of the human *HTT gene* containing a long CAG repeats. This knock-in (KI) method is genetically the closest to HD patients. The commonly used KI models are Q140, Q150, Q175, etc., according to the number of CAG repeats [8-13].

Compared to other HD animal models, the HD rodent models have unique advantages: they have high genetic fidelity and consistent behavioral phenotypes with HD patients; in addition, various genetic and biochemical tools are available for experiments in rodent models. The rodent models have provided critical information for HD mechanisms and therapeutic studies. For example, the antisense

oligonucleotide (ASO) treatment of HD mentioned below is based on a key research using the rodent models, and further clinical success has been achieved.

Large animal models

In spite of its various advantages, HD rodent models have drawbacks. Rodent models show no clear evidence of selective death of striatal neurons, which is an important pathological feature in HD patients. In addition, the lack of higher-level structures such as sulcus makes the mouse brain insufficient for modeling brain diseases. Moreover, the size difference between mouse and human being affects the accuracy of pharmacokinetics and metabolic experiments.

To fill this gap, scientists have established a series of large animal HD models. By lentivirus injection of the exon 1 of mHTT, which carries 84 CAG repeats, to egg cells, Professor Xiaojiang Li and his collaborators established the HD rhesus monkey model in 2008 [14]. In addition, an HD sheep model has been constructed by microinjection of a full-length HTT cDNA carrying 73 CAG repeats into fertilized eggs [15-18].

Another promising model is the HD pig model. The pig has sulcus in the brain and is similar to human in body weight, phylogeny, and metabolism. Therefore, the pig models of brain diseases have unique advantages and related studies are easier to translate into clinical applications. The first HD pig model was constructed by transgenic overexpression of the N-terminal fragment of mHTT [19]. However, the gene expression was too toxic for the animal to survive, so a stable line could not be obtained. Since then, researchers have used the CRISPR/Cas9 gene editing technology to insert human mutant *HTT gene* into endogenous pig locus, and have established a knock-in pig model (KI) using somatic cell nuclear transfer technology. This model perfectly simulated the typical pathological features of selective death of medium spiny neurons in HD patients [20] and exhibited HD-like phenotypes. More importantly, these pathological features and behavioral abnormalities can be stably passed on to future generations [20].

Other animal models and cell models

In addition to the rodent models and large animal models described above, other HD models, such as the yeast model [21], the *C. elegans* model [22], the drosophila model [10], the zebrafish model [23], etc., were all established and used for HD mechanism and therapeutics research. These models also have specific advantages, such as high throughput, short lifecycle, and easy phenotypic screening.

Aside from animal models, the cell models are also very important. Cell models are easy to observe phenotypes such as cell death. In addition, human cell can better mimic human diseases. The human primary neuron HD model originates from human ESC or iPSC-derived medium spiny neurons [24,25]. The recently developed fibroblast direct trans-differentiation technique [26] has been utilized to better mimic the disease state by avoiding stem cells-inducing steps and retaining the senescence state of patient fibroblasts, which is very important for simulating aging-related neurological diseases such as HD.

In summary, there are various animal models and several cell models available for HD (the main models are summarized in (Table 1) that have good simulations of HD patients from genotypes to phenotypes. Each model has its own advantages and provides powerful tools for studying the pathogenesis and treatment of HD.

Model	Promotor and gene	copy	CAGs	Disease phenotype	Reference
Mice knock-in model (N-terminal fragment)					
R6/1	HTT promotor HTT exon1 fragment	1	116	Late onset, slight tremor and intermittent involuntary movements, seizures	4
R6/2	HTT promotor HTT exon1 fragment	3	144	Static tremor, chorea, rigid-involuntary movement	4
N171	Prp promotor N-terminal HTT fragment	1	82	Early onset of tremor, decreased motor function, abnormal gait, early death	5
Mice knock-in model (full length)					
HdhQ111	HTT exon1: mHtt chimera	1 or 2	111	Abnormal gait	16
CAG140	HTT exon1: mHtt chimera	1 or 2	140	Less activity, abnormal gait	14
HdhQ150	HTT exon1: mHtt chimera	1 or 2	150	Motive defect, abnormal gait	15
zQ175	Derived from CAG140 mice	1 or 2	~188	Motor and grip defects, cognitive defect, weight loss	11

Mice transgene model (full length)					
YAC128	HTT promotor; Full-length HTT	several	128	Hyperkinesia, progressive motor deficits, hypokinesia, striatum and cortex atrophy	8
BACHD	HTT promotor Full-length HTT	5	97	Progressive motor deficits, synaptic dysfunction; striatum and cortex atrophy, neuronal degeneration	9
Large animal model					
Rhesus	Ubiquitin promoter HTT exon1	One to several	84	Dystonia, chorea	17
Sheep (OVT73)	HTT promotor; Full-length HTT cDNA	One to several	73	No obvious phenotype	18-21
Tibet mini-pig	β-actin promotor; N-terminal mHTT fragment	One to several	105	Low survival rate; no obvious phenotype in surviving individuals	22
Pig	CMV promotor; CAG repeats knock-in pig *HTT gene*	1	150	Low weight, cutis laxa, early death, walking abnormality, abnormal breathing patterns and behaviors, striatal atrophy	23

Note: Genotype and phenotype information of major mice and large animal models. Copy number of *HTT gene* is 1 or 2 in some models, respectively corresponding to heterozygote and homozygote. Heterozygotes are commonly used as transgene model, so copy number is calculated according to heterozygote.

Table 1: Summary of major mice and large animal models of HD.

Pathogenesis

HD is a genetic disease caused by gain-of-function mutations of the *HTT gene*

HD is an autosomal monogenic dominant disease caused by mutations in the *HTT gene*. The underlying cause of single-gene-mutation diseases may either be loss of function or gain of function of the gene. The differentiation between these two is a prerequisite for further study of disease mechanisms and treatment strategies.

The experimental evidence obtained in the mouse genetic models indicates that HD is mainly a gain-of-function disease, and the main evidence lies in two findings. First, deletion (knockout) of the *HTT gene* did not cause phenotypes similar to HD. Complete knockdown of Htt in mice, despite embryonic lethality, did not cause neuronal death and was inconsistent with HD cell phenotypes [27-29]. Heterozygous knockout of mouse Htt or knockdown of Htt in adult mice over four months of age using gene editing techniques did not cause HD-related phenotypes [27,30,31]. On the other hand, transgenic mice expressing the variant *HTT gene*, such as the BACHD and YAC128 models, still showed HD-related phenotypes while retaining the expression of the original wild-type *HTT gene* [6,7]. In recent years, studies on conditional knockout of wild-type Htt (wtHtt) have shown that loss of wtHtt may also cause cytotoxicity by affecting selective autophagy, thus

involving in pathogenesis of the disease [32,33]. However, most of the evidence supports that HD is gain-of-function disease.

Soluble mHTT protein is likely the major cause of HD

The mutant HTT protein (mHTT) expressed by mutant *HTT gene* is neurotoxic, leading to HD [34]. Recent studies have found that RNAs transcribed by mutant *HTT genes* may produce proteins with other amino acid repeats (including polyAla, polySer, polyLeu, and polyCys) in addition to mHTT protein by Repeat-associated non-ATG (RAN)-translation of the repeat sequence. These proteins may also cause cytotoxicity and are involved in the disease [35]. In addition to proteins, RNAs transcribed by the mutant *HTT gene* may also be toxic due to mechanisms such as phase transitions mediated by CAG repeats, and are involved in the occurrence of diseases [7,21,36]. But this hypothesis still lacks reliable functional evidence. Despite the recent discovery that these new molecules may be involved in HD pathogenesis, most of the evidence still points to mHTT as the main cause of HD. For example, the mutant *HTT gene* expressed in the BACHD mouse model replaced nearly half of the CAG sequences in the CAG repeats with CAA, which in principle prevented the production of RAN-translation products and RNA transcripts containing overly long CAG repeats. This model still showed a significant HD-related phenotype, and the relative time of disease

onset was similar to that of HD patients, suggesting that mHTT protein may be the leading cause of disease [7].

Therefore, why mHTT produces neurotoxicity that ultimately leads to HD is a key question in disease mechanism research. mHTT differs from wtHTT only in the length of poly-glutamine repeats (polyQ) near the N-terminus. So the excessive polyQ may be the reason of mHTT-caused neurotoxicity, but the mechanism remains unknown.

Neurotoxicity caused by excessive polyQ was first attributed to changes in protein solubility. HTT has been found to form oligomers and insoluble protein aggregates in vitro and in cells [37], and excessive polyQ significantly increases the rate of aggregation of insoluble proteins [38]. This change in biophysical properties may cause neurotoxicity of mHTT. However, recent evidence suggests that soluble mHTT protein is the main cause of disease. First, soluble mHTT causes toxic cellular responses such as endoplasmic reticulum stress, mitochondrial autophagy, and oxidative stress [39-41]. Consistent with this, soluble mHTT, rather than mHTT aggregates, interacts with multiple transcription factors [42]. More direct evidence comes from research on cell death. HD rat neurons died in the absence of insoluble mHTT aggregates, and the time of death was significantly correlated with the level of soluble mHTT [43]. The study of human stem cell differentiated HD neuron models reached similar conclusion [25]. In summary, soluble mHTT may be the major protein that produces cytotoxicity leading to HD. Therefore, the structural basis of cytotoxicity caused by excessive polyQ in soluble mHTT is the key in elucidating the molecular mechanism of HD.

The "polymorphism" of the polyQ conformation may be the structure basis for its cytotoxicity

Two possible models for the above findings have been proposed. The first one is called the "linear lattice model": the polyQ chain consists of the same structural unit which may have intrinsic toxicity. The longer the polyQ chain is, the stronger the cytotoxicity is, and the severer the disease is. Briefly, polyQ has a linear arrangement of structural units with intrinsic toxicity [44]. The second model is the "emergent conformation model", that is, after the polyQ length reaching a certain threshold, a new conformational unit different from the "linearly arranged structural unit" appears. And this new conformational unit is the main cause of neurotoxicity. This idea does not negate the linear lattice model, but only speculates that in addition to the linear lattice conformation, the polyQ long chain also has a truly toxic "emergence conformation" and is therefore a mixture of multiple conformations [45].

The biggest controversy between the two models is whether the polyQ has many different conformations, namely, whether there is "polymorphism" in the conformation. Both models have some indirect evidence [46], but lack direct structural biology evidence. The structure of the mHTT protein is very difficult to resolve due to poor solubility and the instability of the polyQ conformation.

In order to overcome these difficulties, a recent study focused on a new perspective of this issue--protein degradation. On one hand, if the same protein in the same cell has different degradation rates, it means that the protein has different conformations; on the other hand, the degradation rate of mHTT is significantly negatively correlated with its neurotoxicity in HD neurons. Specifically, the slower the mHTT degradation is, the greater the toxicity is [47]. Therefore, a slower degradation conformation may be more toxic. Using a newly established method of protein degradation rate measurement based on click chemistry and homogeneous time-resolved fluorescence, CH-chase [48], the researchers found that the degradation rate of the mHTT conformation recognized by the polyQ antibody 3B5H10 was significantly lower than that of the mHTT conformation recognized by other polyQ antibodies, which directly demonstrated the existence of different polyQ types. Further mechanism studies found that the ubiquitination of lysine 63 in this conformation was almost absent in brain tissue and cells of HD patients. So it cannot be degraded through selective autophagy due to failure in recognition by the selective autophagy linker protein p62. The rate of degradation was slowed, resulting in higher toxicity. Therefore, the long polyQ conformation in mHTT is polymorphic, resulting in conformation dependent different degradation rates. The "toxic conformation" with slower degradation rate may lead to cytotoxicity and HD disease.

Downstream molecular mechanisms of mHTT-induced toxicity

mHTT causes cytotoxicity through its downstream molecular signaling pathway, thereby leading to the onset of HD. HTT is a huge protein with 3144 amino acids without known functional domain [49], on the basis of the wild type protein which has a polyQ number of 25. HTT has five regions enriched in HEAT repeat motifs, suggesting that HTT may function by interacting with many other proteins, similar to scaffold proteins [49]. mHTT may indirectly affect important processes in the cell due to its interaction to new proteins or loss of the original binding proteins. Currently, evidence exists that mHTT causes neurological abnormalities or regression at least through interfering with the production and transportation of brain-derived nerve growth factor (BDNF), mitochondrial function, calcium signaling, oxidative stress response, protein transportation, amino acid metabolism, apoptotic signaling pathways, cysteine synthesis, etc. [50]. Proteomics and RNAomics studies suggest that the factors contributing to these effects are likely to be the changes in the HTT protein interaction group in HD [51] and the resulting changes in the transcriptional expression profile (transcriptome) [10].

Some clinical manifestations of HD may be closely related to the downstream molecular mechanisms of mHTT-produced toxicity. For example, the age of onset of HD was found to be significantly negatively correlated with the polyQ length of mHTT (i.e., the number of CAG repeats in the mutant *HTT gene*), but the mechanism is unclear. Prof. William Yang's group and collaborators found that a "module" of a particular transcriptome in the HD striatum and cortex was different from that of wild type in a series of mouse models expressing *HTT gene*s containing different CAG repeats, and this difference was exacerbated by age. More interestingly, the rate at which some of the modules (such as the 13 functional blocks in the striatum) increased with age was positively correlated with the number of CAG repeats [10]. Therefore, the greater the number of CAG repeats is, the faster the transcriptome function module's changes are, and the earlier the onset is, thus explaining the above clinical manifestations.

Another clinical manifestation of HD and other neurodegenerative diseases is the increasing levels of disease proteins and symptoms over time. A recent study has revealed that mHTT can positively regulate its own level by increasing the expression of downstream kinase HIPK3 and the activity of downstream kinase MAPK11, thereby forming a positive feedback regulation of its own level, explaining the possible molecular mechanism of mHTT accumulation over time [52]. This may provide a possible explanation for the accumulation of mHTT over time and the progressive development of symptoms in HD.

Brain region specificity in HD

HD is mainly caused by the mHTT-induced cytotoxicity. mHTT is widely expressed in various types of cells including neurons in all the different brain regions, but neurodegeneration in HD mainly occurs in the striatum, in which the medium spiny neurons expressing dopamine type 2 receptor (D2) die the earliest [53,54]. Why this brain region specificity exists in HD is also an important issue of concern in the field. Elucidating the nature of this regional specificity helps to understand disease mechanisms at the cellular and neural pathway levels.

Two major mechanisms have been proposed to explain the brain region specificity in HD. One mechanism is cell autonomous, in which striatum neurons overexpress certain specific genes, amplifying the toxicity of mHTT or making these neurons more sensitive to mHTT toxicity, which ultimately makes these neurons easier to regression and die. For example, the striatum-enriched small G protein Rhes may increase striatum neuronal death by increasing the toxicity of mHTT via SUMOylation [55]. In addition, the striatum-enriched orphan G-protein coupled receptor GPR52 also stabilizes striatal mHTT, leading to specific death of striatal neurons [24]. Another possible mechanism is non-cell autonomous, in which striatum neurons receive signals transmitted by other types of cells and die specifically. The main evidence comes from genetics: In the BACHD mouse model, when the expression of mHTT was specifically shut down in the striatum, the HD-related phenotypes still existed with only a slight improvement. Whereas specifically shutting down the expression of mHTT in the cortex can almost completely rescue the relevant phenotypes related to HD [56]. In addition, some recent studies have also shown that the BDNF secretion and neural circuit projection of the cortex to the striatum may have important contributions to the brain specificity of HD [57,58].

In summary, important breakthroughs have been made in the genetic mechanism, biochemical mechanism, downstream molecular pathways and brain-specificity of HD since the discovery of its pathogenic gene HTT. Among them, the downstream molecules of mHTT and the mechanisms of pathogenesis at the cell/brain level are more relevant to the clinical manifestations of the disease. Treatments targeting these downstream pathways are easier, but they only treat the symptoms without addressing the root cause of the disease. mHTT itself is closer to the root cause of the disease. Although mHTT-targeting treatment is more difficult, it's more promising to cure the disease.

Treatment strategies for HD

Treatment for HD symptoms and downstream mechanisms of mHTT

In terms of patient care, physiotherapy and nutritional supplementation are important to improve the quality of life of HD patients. But no treatment can slow down the progression of the disease. A small number of drugs that improve symptoms have been used clinically with only temporary inhibitory effect on involuntary dance-like movements associating with HD. Some antidepressants are also used clinically to improve depressive symptoms caused by HD.

As the understanding of how mHTT causes neuronal dysfunction and death deepens, the number of rational therapeutic targets that can be attacked also gradually increases. For example, targeted inhibitor of histone deacetylase (HDAC) may improve various symptoms of the

disease by preventing aberrant transcription induced by mHTT; blockers that target the phosphodiesterase PDE10A may reduce dyskinesia and striatal atrophy in HD patients by regulating striatal synaptic function; direct or indirect supplementation of HD neurotrophic factor BDNF may protects neurons affected by HD [59]. These methods are currently in the process of development or clinical trials.

Treatments targeting mHTT level

The above treatments, although promising, have no direct effect on the underlying cause of HD, mHTT, and they can only temporarily relieve symptoms without slowing down the disease progression. Therefore, treatments directly reducing mHTT protein level may be more effective and fundamental.

This strategy has been extensively validated in disease models [60]. Induced expression of mHTT N-terminal fragment in a mouse model cause HD-associated phenotype, and cessation of expression thereafter can gradually attenuate the phenotype until disappearance [61]. In multiple HD mammalian models, knockdown of HTT using shRNA or siRNA can effectively rescue HD-associated phenotypes, and inhibition of HTT mRNA translation by ASO can also permanently improve disease phenotypes [62]. Genetic screening studies revealed a number of druggable targets that can effectively reduce HTT levels, and subsequent studies further confirmed that manipulating these genes by genetics or targeting them with small molecule drugs can effectively rescue HD-related phenotypes [24,25,46,48,52,60,63-69].

Various treatments for lowering mHTT levels have not yet been formally approved. Strategies in the research and development phase fall into three main categories: targeting HTT DNA, targeting HTT mRNA, and targeting HTT proteins. In recent years, gene editing technologies, especially CRISPR technology, have been developed to knock out the *HTT gene* and reduce the effect of mHTT protein. This idea has been tested and succeeds in mouse models [70]. Also, the potential off-target effects, safety, and clinical feasibility of CRISPR require further testing. Another recently developed technique for targeting HTT DNA uses a point mutation-inactivated zinc finger cleavage enzyme (ZFN) to bind to the HTT promoter region to achieve an effect of inhibiting *HTT gene* expression [71].

Two major methods have been applied to target HTT RNA: knockdown of HTT mRNA by RNA interference using siRNA or shRNA, and reduction of HTT mRNA level or inhibition of HTT translation by ASO [62]. In addition, recent work has found that kinase MAPK11 positively regulates the stability of HTT mRNA, so small molecule blockers of MAPK11 may be a new means of reducing HTT mRNA level [52].

Another promising strategy is to target mHTT protein, which is more likely to be achieved by small molecule drugs than targeting HTT mRNA, thereby solving the problem of drug delivery and reducing the cost. In order to obtain drug targets and small molecule drugs that can reduce mHTT levels more specifically, Dr. Boxun Lu's group has performed a number of genetic screenings and anti-screenings, and obtained a series of potential drug target genes that may specifically regulate mHTT levels, such as NUB1 and GPR52, etc. [24, 52, 68, 69]. These genes regulate mHTT levels through a variety of different molecular mechanisms, and small molecule drugs that target the proteins expressed by these genes may become candidates to cure HD. For example, a G protein-coupled receptor expressed by the GPR52 gene has high pharmaceutical potential. Small molecule

antagonists screened from compounds inhibiting GPR52 activity have been found to be effective in reducing mHTT levels and rescue HD-related phenotypes, opening a new avenue for disease treatment [69].

Prospects for Huntington's Disease Research

In summary, neurodegenerative diseases are a type of diseases that seriously affect the health of the population without any curative treatment. Huntington's disease, as one of the main monogenic diseases, has unique advantages in mechanism and therapeutics research. In recent years, scientists have made important breakthroughs in the study of HD, establishing HD disease models from patient cell differentiated neurons to mice and large animals, revealing the etiology and possible downstream mechanisms of HD. And the feasibility of reducing mHTT levels in the treatment of diseases has been clinically verified. Future work will focus on completing clinical trials that use ASO to reduce mHTT levels, exploring various small molecule drugs that reduce mHTT levels, and conducting clinical trials to verify these small molecule drugs.

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