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Review

Huntington disease: Can a zebrafish trail leave more than a ripple?

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ABSTRACT

Over the past decade, zebrafish has proved to be very useful in modeling neurodegenerative conditions. It poses a number of advantages and has been accepted as one of the best models for elucidating pathophysiological mechanisms of neurodegenerative diseases, including Huntington disease (HD). HD is a debilitating neurodegenerative genetic disorder that affects a person's ability to think, talk, and move. The pathophysiology of HD is not completely understood, which prevents the development of effective therapeutic approaches. Using zebrafish as a model organism, scientific advancements can be made in understanding the HD pathology/mechanisms with the hope of developing potential therapies in the near future.

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1. Introduction

Danio rerio, more commonly known as the zebrafish, was first described by Francis Hamilton in 1822, but it was in the early 1970s when phage geneticist, George Streisinger, who highlighted its value as a model organism. Due to the versatile and unique features, zebrafish provides a convenient and powerful platform for modeling human diseases (Table 1). An increased number of articles recently published attests to the fact that it is also gaining popularity in Huntington disease (HD) research. In this brief review, we have presented the recent progress made in modeling HD using zebrafish.

2. Huntington disease

Huntington disease (HD) is an autosomal dominant (chromosome 4) neurodegenerative disorder, associated with serious motor, cognitive and psychological problems (Table 2). Although the gene responsible for HD was identified in 1993, there is still no cure or effective treatment for this debilitating disease (HDCRG, 1993). There is a 50% probability for a child to inherit the fatal gene from the parents. All carriers of this gene are diagnosed with HD inevitably at some point of their life. A small number of cases (1–3%) are sporadic, meaning they occur even though there is no family history of HD (Myers et al., 1993).

The disease is caused by a mutation on either of an individual's two copies of a gene called Huntingtin (*HTT*) or interesting transcript 15 (*IT15*), which code for the protein Huntingtin (Htt). Htt is a large 350 kDa protein ubiquitously expressed in the brain. The HD gene product lacks sequence similarity to other proteins in the

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Table 1
The zebrafish has a number of characteristics and experimental advantages that makes it a fantastic species for modeling human disease.

Fertilization and development	The fertilization and development occur externally, thus allowing easy observation and manipulation of the embryos As the embryo is transparent, many morphological developments can be easily viewed using light microscope, without any surgery The optical transparency allows direct observation of internal organ development, suitable for high resolution imaging and time-lapse analysis The high number of embryo per clutch (100–200 embryos/weekly) not only provides good sample size, but also makes the process cost efficient Development is rapid, with all major organs developing within 36 h, and larvae display food-seeking and active avoidance behaviors within five days after fertilization
Embryo permeability	Zebrafish embryos are highly permeable Small molecules added directly to the fish water can easily diffuse into the embryo, simplifying drug dispensing and assay processing Large or lipophilic molecules can be easily injected into several hundred embryos
Evolutionary relationship to humans	70% of protein-coding human genes are related to genes found in the zebrafish 84% of these genes known to be associated with human disease have a zebrafish counterpart
Generation time and life span	Short generation time (2–4 months) Comparatively longer life span (2–4 years) than other traditional laboratory organisms like drosophila, *C. elegans* and mice
Forward and reverse genetics	Relatively easy to perform forward and reverse genetic screens for gene identification and to understand specific gene function Highly amenable to high-throughput screening
Behavioral phenotypes	Possess all major neurotransmitters, and their neuroendocrine system provides robust physiological responses to stress Tractable species for behavioral experiments Exhibit complex social interactions, being a shoaling fish Well-developed sensory organs, detect diverse environmental stimuli, and show well-defined behavioral responses to them Ideally suited to studies of social and cognitive behavior

current database (HDCRG, 1993). It is one of the nine neurological disorders caused by the expansion of a CAG trinucleotide repeat that encodes an extended polyglutamine tract within the respective disease proteins. Typically, more than 35 repeats of the CAG sequence leads to a chance of HD but more than 40 repeats certainly leads to HD (Goldberg et al., 1993). The primary pathology of HD involves gradual and selective death of medium spiny γ-aminobutyric acid (GABA)-utilizing neurons of the striatum, and neurons in the deeper layers of the cerebral cortex.

HD affects an estimated 7 per 100,000 people in the European countries (Walker, 2007). The disorder appears to be less prevalent in other populations such as African Americans and Japanese. In the United States alone, it is projected that about 30,000 people have HD with estimates of its prevalence about 1 in every 10,000 people. The worldwide prevalence of HD is 5–10 affected per 100,000 persons (Harper, 2005).

3. Zebrafish model of HD

As the first step toward exploring a potential role for HD gene in early vertebrate development, Karlovich et al. (1998) isolated the homolog of the HD cDNA in zebrafish. This cDNA was found to code a predicted protein product of 3121 amino acids with 70% identity to human htt. The first exon was predicted to encode four glutamines, followed by only one proline, which demonstrated that the polymorphic polyproline stretch found in mammalian HD sequences was absent in the zebrafish. A sequencing of approximately 900 bp upstream from the predicted start codon showed that it lacked a TATA box, CCAAT box and Sp1 binding sites. Western blot analysis revealed that the protein was expressed at a high level in late embryonic development and at moderate levels in the adult head (Karlovich et al., 1998).

The C-terminal Hsp70 (heat shock protein 70)-interacting protein (CHIP), being both a co-chaperone and ubiquitin ligase, plays an important role in protein quality control by linking molecular chaperones and the ubiquitin–proteasome system. To demonstrate the CHIP-mediated suppression of polyglutamine (polyQ) toxicity, zebrafish embryos were co-injected with plasmids expressing mutant polyQ proteins (Q71-GFPu and GFP-Q82-Htt) and empty vector, wild type (WT)-CHIP, or ΔTPR (tetratrico peptide repeat)-CHIP (Miller et al., 2005). It was observed that the embryos injected with mutant polyQ proteins and empty vector died at a high rate. Most surviving embryos were severely disturbed in their overall body pattern, showing developmental delay, and prominent patches of dead or dying cells. Co-expression of WT-CHIP rescued polyQ-mediated death at 24 h. Further, ΔTPR-CHIP-co-expressing embryos died at the same frequency as polyQ protein-expressing embryos co-injected with control vector and showed similar morphological disturbances among survivors. Co-expression of CHIP decreased toxicity of both a generic polyQ-containing fragment and a pathogenic Htt fragment in zebrafish embryos, suggesting the ability of CHIP to reduce aggregation and toxicity of mutant polyQ proteins (Miller et al., 2005). This novel finding suggested that the zebrafish model was predictive of at least one key biochemical mechanism implicated in pathogenesis of the polyQ neurodegenerative diseases, demonstrating that potential mechanistic insight into disease pathology could be gained by employing the zebrafish.

Table 2
Observable physical, cognitive and psychiatric impairments exhibited by the HD patients.

Movement	Cognition	Psychopathology
Slow eye movements	Lack of judgment	Sadness, apathy
Slow movement of body	Short term memory loss	Depression
Rigidity of muscle	Inability to concentrate	Anxiety, irritability
Jerking of the body	Impaired learning skills	Suicidal thoughts
Difficulty in production of speech	Mental confusion	Social withdrawal, irrational behavior
Swallowing disorder	Lack of organization skills	Insomnia or superfluous sleeping pattern
Problems with orientation	Speech problems	Denial due to intellectual deterioration
Fatigue		Uncontrolled aggression
Lack of coordination		Lack of inhibition
		Lack of sexual desire, inability of have sex or conflicting desires and needs
		Sleep disturbances

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