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A huntingtin–HAP1–PCM1 pathway in ciliogenesis

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Abstract

Huntington's disease (HD) is caused by expansion of a polyglutamine repeat in the N-terminal region of huntingtin (htt), a large protein that has been found to interact with a variety of proteins. It remains to be determined how the interactions of htt with other proteins are involved in the pathogenesis of HD. A recent publication by Keryer *et al.* demonstrates that htt regulates ciliogenesis by interacting with PCM1 through HAP1. This recent study shows that htt and HAP1 are essential for protein trafficking to the centrosome, as well as normal ciliogenesis, and that mutant htt causes abnormal ciliogenesis, providing a novel insight into the pathogenesis of HD.

Keywords

centrosome; ciliogenesis; HAP1; huntingtin; Huntington disease

Huntingtin (htt) is a large protein consisting of 3144 amino acids that carries a glutamine repeat in its N-terminal (amino-terminal) region, which is encoded by a CAG repeat in the htt gene on chromosome 4. Expansion of this repeat to more than 36 CAG/glutamine units causes Huntington's disease (HD), an autosomal dominant disorder that is characterized by abnormal movement (chorea), memory loss and moodiness. Htt is essential for cellular function and animal development, as its loss causes embryonic lethality in mice [1]. However, how the glutamine repeat expansion in htt leads to the selective death of neurons remains unclear. Despite this, it is known that this glutamine repeat expansion causes htt to abnormally interact with other proteins and to form aggregates or inclusions in the affected brain [2–4]. Therefore, the discovery of how mutant htt binds to other proteins and affects the function of its partners will be key to understanding the mechanism of HD and to developing a treatment for this tragic disease.

The selective neurodegeneration in HD raises the possibility that mutant htt affects the function of neuronal proteins via abnormal protein interactions. HAP1 is the first identified htt-binding partner and is a notable candidate for taking part in the selective neurodegeneration in HD, because Hap1, unlike htt that is ubiquitously expressed in all types of cells, is a neuronal protein enriched in the brain [5,6]. HAP1 has been characterized as an adaptor protein – the link between motor proteins and cargos that are transported along microtubules in neuronal cells [7]. Mutant htt binds HAP1 more tightly, preventing HAP1

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from functioning normally. This abnormal binding affects HAP1-dependent transport of various vesicles or receptors [8–10]. Moreover, a genetic modification of the HAP1 sequence that reduces mutant htt toxicity can delay the onset of HD symptoms in patients [11].

Intracellular trafficking is essential for maintaining the normal function of cilia, which are thin, tail-like projections from the cells and typically serve as sensory organelles. There are two types of cilia: motile cilia and nonmotile, or primary, cilia. Nearly all mammalian cells have a single nonmotile primary cilium, which coordinates a large number of cellular signaling pathways important for ciliary motility or cell division and differentiation [12]. Motile cilia are involved in the movement of cells or substances. As motile cilia constantly beat in a single direction, they can help a cell to move around or move substances such as fluid over or around the cell. Cilia move because of the interactions of a set of microtubules inside them, which together are called an ‘axoneme’. Proteins and other cargo are moved up and down the cilium by ‘molecular motors’ along microtubules [13,14]. Motile cilia are typically found in multiple copies on epithelial cells that line the lumenal ducts of various tissues. In the brain ventricles, motile cilia play a crucial role in circulating cerebrospinal fluid (CSF) inside the brain cavities. CSF contains ions, nutrients, neuroendocrine factors and neurotransmitters, and a key function of its flow is the clearance of brain catabolites to maintain normal brain homeostasis. Keryer *et al.* recently reported that htt and HAP1 are required for ciliogenesis in cells [15]. Importantly, mutant htt alters ciliogenesis and causes asynchronous beating of the cilia and abnormal CSF flow in HD mice [15]. These findings offer us a new insight into the HD pathology.

Summary of methods & results

Keryer *et al.* first identified that htt is localized at the centrosome in mouse striatal neuronal cells and that this localization depends on the HAP1-binding domain [15]. Furthermore, the centrosomal localization of htt is microtubule dependent, as depolymerization of microtubules depletes this localization. The colocalization of htt and HAP1 at the centrosome led to the observation that HAP1 and htt are also colocalized with PCM1 at the centrosome. To test the functional relevance of this colocalization, Keryer *et al.* suppressed htt expression via siRNA and found that downregulation of htt led to a marked dispersion of PCM1 from the perinuclear region and impaired primary cilia formation. To investigate whether htt mediates its effect on ciliogenesis through HAP1, they silenced HAP1 expression via siRNA. This silencing also led to the dispersion of PCM1 centrosomal staining. More interesting, depletion of HAP1 in cells dissociated htt from PCM1. Eliminating the HAP1-binding domain in htt prevented the colocalization of htt with PCM1. Taken together, these data convincingly show that the Htt–HAP1–PCM1 pathway regulates ciliogenesis.

It would be more important to know whether mutant htt with expanded polyglutamine (polyQ) can affect ciliogenesis. Keryer *et al.* then examined a cultured striatal neuronal cell line that expresses mutant htt [15]. In contrast to the hypotrophic cilia seen when htt expression was reduced, longer cilia or hypertrophic cilia were seen in the mutant cells in association with an increased accumulation of mutant htt and PCM1 at the centrosome.

More astrocytes also have cilia in HD knock-in mice, which express full-length mutant htt under the control of the endogenous mouse *htt* gene, than wild-type (WT) mice. Moreover, PCM1 aggregation and increased ependymal cilia length were seen in HD knock-in mice via immunocytochemistry staining and scanning electron microscopy. Using immunostaining and western blotting, the authors also found an increase in PCM1 immunoreactivity in human neostriatal specimens from HD patients (grade 2–4). All these findings suggest that mutant htt can increase abnormal ciliogenesis.

Since motile cilia play a crucial role in circulating CSF, the authors further examined CSF flow in HD knock-in mice. In WT mice, most of the cilia are oriented toward the CSF flow, whereas cilia in HD knock-in mice are randomly oriented. Furthermore, CSF flow in HD knock-in mice is disorganized at a reduced rate compared with the constant flow in a synchronous fashion in WT mice, suggesting there is altered CSF flow in HD mouse brains.

Discussion

Defects in cilia are associated with a range of human diseases, such as primary ciliary dyskinesia, hydrocephalus, polycystic liver and kidney disease, and some forms of retinal degeneration [16]. The underlying cause may be a dysfunctional molecular mechanism in the primary cilia structures in cells. The work carried out by Keryer *et al.* provides evidence for the potential ciliopathies in HD for the first time. Their findings will help to develop new strategies for treating this devastating disease.

Although HAP1 and htt have been reported to be involved in intracellular trafficking of membrane receptors and vesicles [8–10], the new findings of Keryer *et al.* provide another piece of important evidence for the roles of HAP1 and htt in intra cellular transport [15]. It should be pointed out that htt also interacts with other proteins, such as HIP1, which are involved in intra cellular trafficking [2,3]. However, HAP1 is a noticeable candidate for being involved in selective neurodegeneration in HD. This is because HAP1, unlike htt and other interacting proteins that are ubiquitously expressed, is enriched in neuronal cells in the brain [5,6]. Thus, its dysfunction caused by mutant htt is likely to contribute to neuronal dysfunction. Indeed, mutant htt with expanded polyQ binds HAP1 more tightly to affect its function in transport of membrane receptors and brain-derived neurotrophic factor [8–10]. Thus, although cilia are present in all mammalian cell types, mutant htt may selectively affect cilia function in neuronal cells via its abnormal binding to HAP1.

The role of HAP1 in ciliogenesis is also supported by recent findings that Ahi1, a protein that forms a stable complex with HAP1 [17], is critical for ciliogenesis, as lack of Ahi1 causes defective cilio-genesis [18,19]. The novel information provided by Keryer *et al.* also includes functional relevance of altered ciliogenesis in HD mice [15]. They found that CSF flow is altered in HD mice, suggesting that brain homeostasis may be affected by mutant htt. This finding could offer a new direction to develop therapeutics for HD.

Expert commentary & five-year view

The findings of Keryer *et al.* have provided new insights into the normal function of htt and the effect of mutant htt on ciliogenesis. Since ciliogenesis is critical for early development, it

would be interesting to know whether this effect of mutant htt occurs in early development, which might also contribute to late-onset neuro degeneration. If mutant htt only affects ciliogenesis in aged brains, an important issue is how this effect can be age dependent. Given the age-dependent formation of htt aggregates that are formed by N-terminal mutant htt fragments, it would be interesting to investigate whether N-terminal or full-length mutant htt has a greater impact on ciliogenesis.

Another interesting issue is the relative contribution of abnormal ciliogenesis to HD pathogenesis. This is because mutant htt can interact with a variety of proteins and mediates multiple pathological pathways, including transcriptional dysregulation, mitochondrial dysfunction and abnormal synaptic transmission [4,20]. Since only a fraction of htt and HAP1 are localized at the centrosome, whether or not abnormal ciliogenesis contributes to early HD pathology remains to be determined. Understanding this issue will help to develop an effective treatment for HD.

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- of considerable interest

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Key issues

- Huntingtin (htt) and HAP1 participate in microtubule-dependent transport in cells.
- Since HAP1 is a neuronal protein, its dysfunction is likely to contribute to selective neuropathology in Huntington's disease.
- Htt and HAP1 are colocalized with PCM1 at the centrosome.
- Loss of htt or HAP1 affects ciliogenesis.
- Mutant htt alters ciliogenesis by increasing the length of cilia.
- Mutant htt affects cerebrospinal fluid flow, which is carried out by cilia, and alters brain homeostasis.
- It remains to be investigated whether mutant htt affects ciliogenesis during early development.
- The contribution of abnormal ciliogenesis to age-dependent neuropathology in Huntington's disease remains to be determined.