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Review

Primary cilia and autophagic dysfunction in Huntington's disease

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Huntington's disease (HD) is an inherited, neurodegenerative disorder caused by a single-gene mutation: a CAG expansion in the huntingtin (HTT) gene that results in production of a mutated protein, mutant HTT, with a polyglutamine tail (polyQ-HTT). Although the molecular pathways of polyQ-HTT toxicity are not fully understood, because protein misfolding and aggregation are central features of HD, it has long been suspected that cellular housekeeping processes such as autophagy might be important to disease pathology. Indeed, multiple lines of research have identified abnormal autophagy in HD, characterized generally by increased autophagic induction and inefficient clearance of substrates. To date, the origin of autophagic dysfunction in HD remains unclear and the search for actors involved continues. To that end, recent studies have suggested a bidirectional relationship between autophagy and primary cilia, signaling organelles of most mammalian cells. Interestingly, primary cilia structure is defective in HD, suggesting a potential link between autophagic dysfunction, primary cilia and HD pathogenesis. In addition, because polyQ-HTT also accumulates in primary cilia, the possibility exists that primary cilia might play additional roles in HD: perhaps by disrupting signaling pathways or acting as a reservoir for secretion and propagation of toxic, misfolded polyQ-HTT fragments. Here, we review recent research suggesting potential links between autophagy, primary cilia and HD and speculate on possible pathogenic mechanisms and future directions for the field.

Facts

- Autophagy is increased, but inefficient, in HD
- Primary cilia dysfunction impairs autophagy
- Autophagic disruption impairs regulation of primary cilia biogenesis and growth
- PolyQ-HTT exhibits increased interaction with huntingtin-associated protein 1 (HAP1), resulting in PCM1 accumulation at the centrosome, aberrant ciliogenesis and altered primary cilia structure
- Multiple types of neuronal aggregates containing misfolded disease-associated proteins, such as α-synuclein, tau, ALS-associated proteins and polyglutamine proteins, have been shown to spread in a prion-like manner—mutant huntingtin can undergo cell-to-cell transmission *in vitro* and *in vivo*

Open Questions

- How does altered ciliary structure affect primary cilia function in HD?

- What is the link between elongated primary cilia morphology and aberrant corticostriatal signaling in HD?
- Can normalizing primary ciliogenesis reverse neurodegeneration in HD?
- Are primary cilia secretory organelles in neurons or astrocytes and do they contribute to propagation of misfolded polyQ-HTT in the brain?

Huntington's disease (HD) is an inherited, autosomal dominant neurodegenerative disease characterized by progressive motor dysfunction, cognitive decline and psychiatric disturbances. The prevalence in the Western hemisphere is 4–10 in 100 000 and the average age of onset is 40 years.[1,2] Brain atrophy in HD manifests first in the striatum, and later, in other brain regions including the substantia nigra, hippocampus, cerebellum and cerebral cortex.[3] Striatal medium spiny neurons are the most vulnerable, while interneurons are spared.[3] There is currently no cure or effective treatment to halt the progression of neurodegeneration in HD.

HD is caused by a CAG repeat expansion in a single gene, huntingtin (HTT), located on chromosome 4, which codes for a

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Abbreviations: ATG, autophagy-related; BDNF, brain-derived neurotrophic factor; CMA, chaperone-mediated autophagy; ER, endoplasmic reticulum; GLI, glioma-associated oncogene homolog transcription factor; HAP1, huntingtin-associated protein 1; HD, Huntington's disease; HDAC6, histone deacetylase 6; hESC, human embryonic stem cell; HSC70, heat-shock cognate protein 70; HTT/Htt, huntingtin; IFT, intraflagellar transport; LAMP2A, lysosome-associated membrane protein 2A; LC3, microtubule-associated protein 1A/1B-light chain 3; mTOR, mammalian target of rapamycin; OFD1, oral-facial-digital syndrome type 1; PCM1, pericentriolar material 1 protein; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1-alpha; polyQ, poly-glutamine; polyQ-HTT, mutant huntingtin with a poly-glutamine tail; Ptc, Patched-1; ROCK1rho-associated, coiled-coil-containing protein kinase 1; Shh, Sonic hedgehog; Smo, Smoothened; SSTR3, somatostatin receptor type 3; TrkB, tyrosine-related kinase B; UPS, ubiquitin-proteasome system; WT, wild type

large protein with an expanded poly glutamine (polyQ) tail. The presence of 40 or more CAG repeats causes HD, whereas 36–39 repeats cause HD in some cases (often with later onset).[1,4,5] Evidence suggests that both loss-of-function and gain-of-function mechanisms play a role in polyQ-HTT-mediated pathology. Embryonic lethality of complete Htt knockout in mice[6,7] and the pathological significance of loss of normal functions of HTT, such as transport of brain-derived neurotrophic factor (BDNF),[8,9] are suggestive of a loss-of-function mechanism. However, the dominant inheritance pattern of HD and the ability of full-length and truncated forms of polyQ-HTT to induce HD-like phenotypes in animal models suggest that a toxic gain-of-function mechanism also contributes to HD pathology.[10]

Although the normal physiological functions of wild-type (WT) HTT are not completely understood, it is essential for neurodevelopment and is believed to function as a scaffolding protein in multiple pathways including microtubule-dependent vesicular transport, neuronal gene transcription and synaptic transmission.[1,3,8] Important recent findings also describe a potential function as a scaffold for multiple autophagy-associated proteins in selective autophagy.[11] Consistent with these putative functions, HTT contains multiple leucine-rich HEAT repeats that are involved in protein–protein interactions.[12,13]

The exact nature of the polyQ-HTT toxic gain of function remains elusive, but several possibilities have been identified. It has been suggested that proteolytic cleavage of polyQ-HTT might play an early role in HD pathogenesis, giving rise to toxic N-terminal fragments.[2,14] Cleavage products generated by caspase-3, caspase-6, calpains and matrix metalloproteinase 10 have been shown to accumulate in the HD brain before neurodegeneration.[14–19] However, more recent evidence suggests full-length polyQ-HTT monomers are more abundant and more toxic than fragments.[20]

Aberrant interactions of polyQ-HTT and other proteins have been shown to disrupt microtubule-based transport and gene transcription. HTT has an important role in vesicular transport via interactions with motor protein cargo adaptors such as HTT-associated protein 1 (HAP1) and dynactin.[2,8,21] This complex is disrupted by polyQ-HTT, resulting in reduced transport, including vesicular trafficking of BDNF.[2,8] PolyQ-HTT also interferes with transcription factors and abnormally interacts with genomic DNA, disrupting transcription factor binding and DNA conformation.[22] One result of the transcriptional disruption is reduced transcription of BDNF.[23] In addition, repression of the transcription co-activator, peroxisome proliferator-activated receptor gamma co-activator-1-alpha (PGC-1α), results in reduced mitochondrial biogenesis,[24] and polyQ-HTT interaction with mitochondrial proteins results in impaired energy metabolism stemming from mitochondrial dysfunction and altered dynamics.[25,26] The PGC-1α target and regulator of the autophagy-lysosome pathway, transcription factor E-B, is also impaired in HD mice.[27]

Similar to other neurodegenerative diseases, protein misfolding and aggregation are a hallmark of HD neuropathology. However, the process of polyQ-HTT aggregation is complex and untangling the pathways and determining the pathological significance remains a challenge. Although it was initially

hypothesized that large aggregates containing polyQ-HTT were the cause of neuronal death in HD, more recent evidence suggests that soluble, misfolded monomers and oligomers are in fact toxic and aggregate formation is a protective mechanism.[28] PolyQ-HTT aggregates correlate poorly with HD severity and progression.[29,30] *In vitro* studies suggested that polyQ-HTT fragments undergo a conformational change, forming soluble toxic β-sheet monomers and/or oligomers.[31,32] Analysis of human HD brain and cultured neurons revealed that native polyQ-HTT occurs primarily as a soluble, full-length monomer,[20] and in human embryonic stem cells (hESCs), there was a quantitative relationship between neurodegeneration and soluble monomeric (but not oligomeric or aggregated) polyQ-HTT.[33]

Finally, polyQ-HTT impairs proteasomal activity and autophagy by interacting abnormally with proteins involved in these pathways.[34,35] Dysfunctional autophagy in HD is the focus of this review and will be discussed in more detail in the following sections. This pathological pathway of HD has been extensively investigated, but the functional mechanisms of action and cellular regulators of dysfunction have yet to be sufficiently described. Here we review recent data suggesting a potential link between autophagic dysfunction and primary cilia—often overlooked cellular signaling organelles that are novel regulators of autophagy—in HD and neurodegeneration. We conclude by speculating on other potential avenues of primary cilia involvement in the pathogenesis of HD.

Autophagic Dysfunction in HD

There is substantial evidence that autophagy, the cellular process of protein and organelle degradation by lysosomes, is dysfunctional in HD. Autophagy has a vital role throughout the nervous system and loss of this cellular function causes premature aging and neurodegeneration.[36,37] The autophagy-lysosome system compliments the ubiquitin-proteasome system (UPS) in the degradation of misfolded proteins and is upregulated when proteasome function is impaired. In HD, polyQ-HTT overwhelms UPS chaperones such as Hsp70 and DNAJB, decreasing their availability to other misfolded proteins and increasing the flux of proteins to the proteasome.[35] An increased cellular load of ubiquitinated proteins, the relatively low basal functionality of striatal proteasomes[38] and an overall age-dependent loss of UPS activity[39] all might contribute to the increased burden placed on the autophagy pathway in HD. Endoplasmic reticulum (ER) stress also induces autophagy in HD and it has also been shown that polyQ-HTT disrupts calcium homeostasis,[40] impairs ER-Golgi-lysosome trafficking[41,42] and inhibits the ER-associated degradation pathway.[43] Furthermore, because post-mitotic striatal neurons are unable to dilute accumulated misfolded proteins through cell division, ineffective autophagy might disrupt cellular proteostasis and exacerbate polyQ-HTT toxicity.[44]

Autophagy can be divided into three subtypes based on cargo recognition and mechanisms of lysosomal delivery—microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy. CMA and macroautophagy are the two primary pathways in mammalian cells (Figure 1a). CMA is a selective form of autophagy that acts upon protein substrates
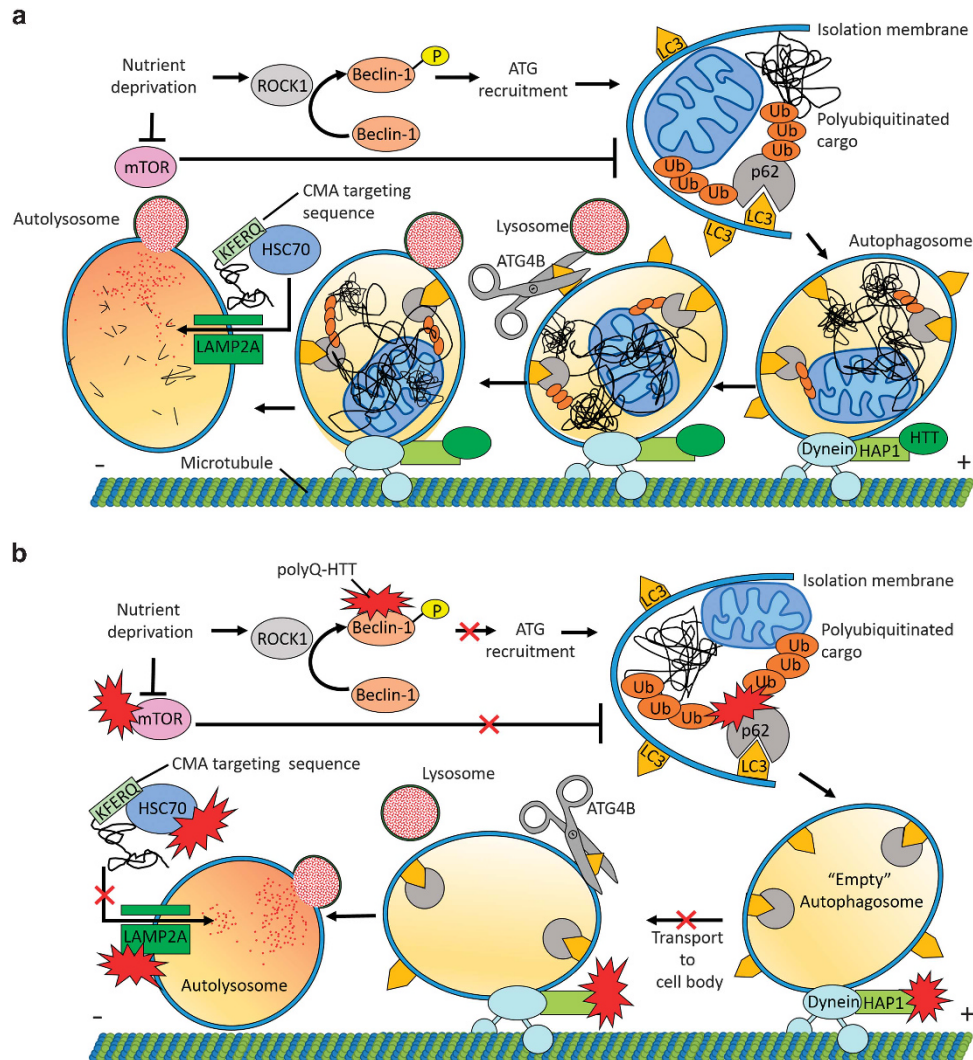
Figure 1 PolyQ-HTT impairs autophagy. (**a**) Nutrient deprivation induces macroautophagy, whereas activated mTOR acts as a major suppressor of this pathway. Upon nutrient deprivation, ROCK1 promotes autophagy by phosphorylating Beclin-1, a key regulator of autophagy. Beclin-1 orchestrates recruitment of additional ATG proteins required for autophagosome formation. The first stage of macroautophagy is formation of the double-membraned autophagosome. Small membrane structures called isolation membranes elongate and surround proteins or organelles destined for degradation. LC3 complexes associate with the isolation membrane early in autophagy. LC3 aids cargo sequestration through interaction with p62, a receptor essential for targeting polyubiquitinated cargo to the autophagosome. Autophagosomes colocalize with HTT and HAP1, enabling dynein-mediated retrograde transport toward the lysosome-enriched cell body. ATG4B cleaves LC3 from the outer autophagosome membrane, allowing fusion with lysosomes and cargo degradation by hydrolytic enzymes. Chaperone-mediated autophagy (CMA) acts upon protein substrates that contain pentapeptide amino-acid targeting sequences biochemically related to KFERQ. Chaperones, including HSC70, deliver misfolded proteins to lysosomes, where a membrane receptor complex containing lysosome-associated membrane protein 2 A (LAMP2A) recognizes and translocates cargo into the lysosomal lumen for degradation. (**b**) PolyQ-HTT impairs autophagy pathways through aberrant protein interactions. PolyQ-HTT sequesters mTOR, increasing autophagy induction. PolyQ-HTT sequesters Beclin-1, impairing autophagosome formation. PolyQ-HTT binds p62, disrupting LC3 interaction and causing cargo recognition defects with assembly of empty autophagosomes. PolyQ-HTT disrupts retrograde microtubule-based transport through interaction with HAP1 and dynein/dynactin. Autophagosome transport to the cell body is hindered, resulting in formation of fewer autolysosomes and decreasing overall autophagic degradation. CMA is also impaired in HD; polyQ-HTT sequesters the HSC70 chaperone that is important for transporting cargo to lysosomes. PolyQ-HTT also directly interacts with LAMP2A of the lysosomal membrane translocation complex, preventing the transport of cargo into lysosomes

that contain pentapeptide amino-acid targeting sequences biochemically related to KFERQ.[45] Chaperones, including heat-shock cognate protein 70 (HSC70), deliver misfolded proteins to lysosomes, where a membrane receptor complex containing lysosome-associated membrane protein 2A (LAMP2A) recognizes and translocates cargo into the lysosomal lumen for degradation.[46] Studies have shown that HTT is processed by CMA machinery. HTT contains putative CMA-targeting motifs, interacts with HSC70 and is delivered into the lysosome in a LAMP2A-dependent manner. In HD,

CMA function is diminished. PolyQ-HTT excessively interacts with HSC70 and LAMP2A, clogging this pathway[47] (Figure 1b).

Macroautophagy is a bulk clearance mechanism of proteins or organelles.[48,49] The three main stages of macroautophagy are autophagosome formation, maturation and fusion with lysosomes.[39] During the first stage, autophagosome formation, an isolation membrane forms to surround proteins or whole organelles.[49] Fifteen autophagy-related (ATG) proteins have key roles in formation of the autophagosome.[50,51]

After the autophagosomes are fully formed, they are transported along microtubules and fuse with lysosomes, which results in lysosomal degradation of the contents[49] (Figure 1a). Macroautophagy is abnormally induced in HD as evidenced by increased levels of autophagic vacuoles, the cargo adaptor p62 and processed autophagosome membrane marker microtubule-associated protein 1A/1B-light chain 3 (LC3)-II in HD patients and mouse models.[34,52] In addition, polyQ-HTT sequesters and inhibits mammalian target of rapamycin (mTOR), a negative regulator of macroautophagy[53] (Figure 1b).

Post-translational modifications of proteins can regulate their autophagic clearance and several such modifications have been shown to contribute to clearance or stabilization of HTT. For example, polyQ-HTT exhibits reduced serine (S13 and S16) phosphorylation, a modification that regulates the balance between ubiquitination and SUMOylation of adjacent lysine residues (K6, K9 and K15).[54,55] Inhibitor of κB kinase-mediated phosphorylation results in increased polyubiquitination and enhanced clearance of WT-HTT. By contrast, decreased phosphorylation of polyQ-HTT leads to SUMOylation, which in turn stabilizes polyQ-HTT and increases neurotoxicity.[55,56] Acetylation is another modification regulating polyQ-HTT clearance. Lysine-444 acetylation, mediated by the acetyltransferase activity of CREB-binding protein and occurring primarily in polyQ-HTT, is an important regulatory mechanism that targets polyQ-HTT to autophagosomes for degradation.[57] Myristoylation, another acylation modification, promotes protein–membrane interaction and is relevant to autophagy regulation. After caspase-mediated cleavage of HTT, an N-terminal glycine (G553) undergoes myristoylation.[58] Myristoylated $HTT_{553-586}$ fragments may influence autophagosome formation by sensing and inducing membrane curvature at the ER.[49] The ER membrane is the source of the isolation membrane,[59] an early structure required for engulfment of cytoplasmic materials.[60] Myristoylation has been shown to be significantly less prevalent in polyQ-HTT compared with WT,[58] and thus, further examination of this post-translational modification in the context of HD is required.

Despite induction of autophagy, there is inefficient polyQ-HTT clearance in HD. This stems from abnormal autophagic function arising from defects at several key points of the pathway. Normally, upon nutrient deprivation rho-associated, coil-containing protein kinase 1 (ROCK1) promotes autophagy by phosphorylating Beclin-1, a key regulator of autophagy.[61] PolyQ-HTT sequesters and inhibits Beclin-1, impairing autophagosome formation and polyQ-HTT clearance.[62] In addition, polyQ-HTT disrupts retrograde transport of autophagosomes to the lysosome-rich cell body, resulting in fewer fusion events and defective degradation.[63] There are also pronounced cargo recognition defects possibly resulting from aberrant polyQ-HTT–p62 interaction.[34] The p62 autophagic adaptor protein has a key role in selective macroautophagy, binding to polyubiquitin chains of targeted cargo and LC3 membrane proteins of the forming autophagosome.[64] Interaction with polyQ-HTT decreases p62 availability to other substrates. This results in the production of relatively empty autophagosomes, thereby compromising macroautophagy and further contributing to neurotoxicity in HD[34] (Figure 1b).

Results of recent studies in animal models of HD have suggested that HTT plays an important role in autophagy. For example, expression of full-length HTT lacking its polyQ (ΔQ-HTT) in a knock-in mouse model of HD increases autophagy markers and longevity.[65] *In vitro* expression of ΔQ-HTT also increases autophagosome synthesis and ATG5-dependent clearance of HTT aggregates.[65] In addition, a new study reported that WT-HTT serves as an important scaffold protein in multiple types of selective macroautophagy (not including starvation-induced autophagy) in *Drosophila* and mammalian cells (including mouse embryonic fibroblasts and striatal cells).[66] WT-HTT is able to bind simultaneously two important ATG proteins, p62 and unc-51-like autophagy activating kinase (ULK1).[66]

In sum, studies to date indicate abnormal autophagy function in HD and suggest that HTT has a functional role in autophagy. Upregulation of autophagy has been shown to enhance polyQ-HTT clearance[53] and drugs targeting autophagy—such as rapamycin/CCI-779, lithium, trehalose and rilmenidine—continue to be of interest as potential therapeutic agents for HD.[49] Given the somewhat contradictory findings of polyQ-HTT-mediated autophagy induction being detrimental and autophagy enhancer therapy being beneficial, the role of autophagy in HD appears to be even more complex than previously thought. A key piece of this puzzle might be the primary cilium, a novel regulatory organelle of autophagy.

Primary Cilia and Autophagy

Structure, function and biogenesis of primary cilia. Primary cilia are single, non-motile signaling organelles found on the surface of most mammalian cells (Figure 2). They are required for Sonic hedgehog (Shh) signal transduction and have essential roles in Wnt, platelet-derived growth factor and transforming growth factor–β signaling pathways.[67–70] Present in neurons, astrocytes and progenitors, these structures have an important role in neurodevelopment—acting in neuronal homeostasis, differentiation and survival.[71] Neurological defects associated with so-called ciliopathies, multi-system genetic disorders stemming from primary ciliary dysfunction, underscore the critical role of primary cilia in the nervous system. Primary cilia also have important functions in the adult nervous system, including neural stem cell regulation, neuronal signaling and regeneration.[72] Interestingly, primary cilia dysfunction has recently been implicated in late-onset neurodegenerative disorders such as Alzheimer's disease and HD.[73,74]

Primary cilia are covered in a specialized receptor-dense membrane and protrude into the extracellular environment like cellular antennae.[75] To facilitate their specialized signaling functions, primary cilia are compartmentalized from the rest of the cell. A septin GTPase structured ring embedded within the plasma membrane acts as a diffusion barrier[76] and a gate-like transition zone at the ciliary base regulates protein transport from the cytoplasm into the primary cilium.[77] A unique bidirectional intraflagellar transport (IFT) system is utilized for trafficking of signaling and structural components along a microtubule-based axoneme.[78] IFT employs kinesin-2 motors for anterograde transport (from ciliary base to tip) and
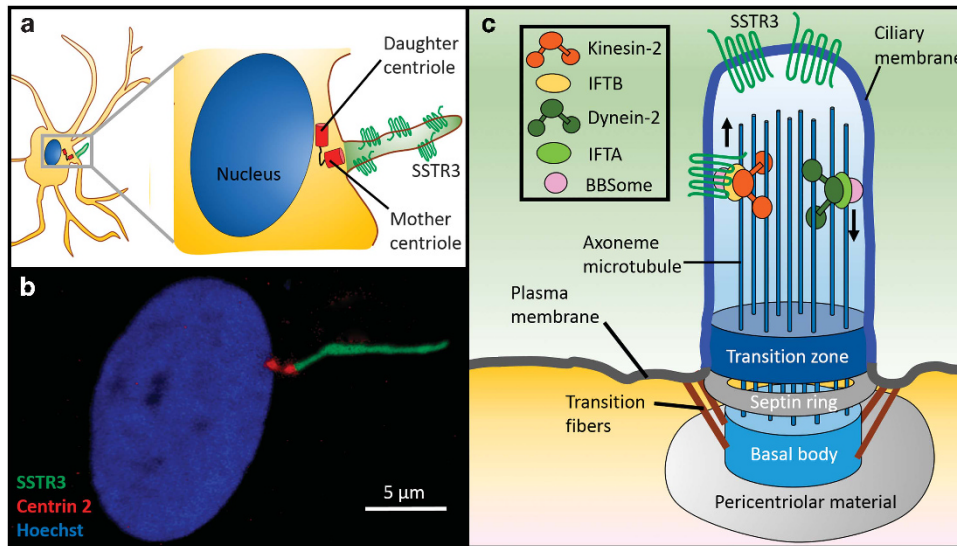
Figure 2 The primary cilium is a specialized signaling organelle. (**a** and **b**) Rat spinal astrocytes expressing dsRed-Centrin 2 (centriole marker) and Somatostatin receptor type III-GFP (primary cilia marker) were fixed and stained with Hoechst dye (nucleus marker). (**c**) The primary cilium is a specialized non-motile signaling organelle that is compartmentalized from the rest of the cell by unique structures, including an ordered membrane-bound septin ring structure and a transition zone at the base of the cilium. The microtubule-based axoneme nucleates out from the basal body after being anchored to the plasma membrane by transition fibers. Signal transduction is mediated by the receptor dense ciliary membrane and a specialized intraflagellar transport system (IFT) along the axoneme. The primary ciliary membrane is enriched in membrane receptors that include SSTR3. Cargo is transported into the primary cilium via the Bardet-Biedl Syndrome (BBSome) complex (which also coordinates cargo attachment to microtubule motors), IFT complex A (for dynein-2-mediated retrograde transport) and IFT complex B (for kinesin-2-mediated transport to the ciliary tip)

specialized cytoplasmic dynein-2 motors for retrograde transport (tip to base). IFT proteins link motor proteins and cargo, creating multi-protein IFT complexes that travel along the axoneme[79] (Figure 2).

Primary cilia formation involves axonemal extension from the basal body, a structure originating from the mother centriole in post-mitotic cells.[80] Following basal body anchoring to the plasma membrane, vesicles carrying material needed for ciliary growth dock at the ciliary base. Materials, such as ciliary membrane receptors, are incorporated into IFT particles for transport into the cilium,[81] and elongation is mediated by bidirectional IFT.[82] Bardet-Biedl Syndrome proteins play a key role in vesicular transport of signaling receptors to primary cilia, in addition to their role in regulation of IFT assembly and turnaround.[79] Primary cilia are formed in quiescent cells and their disassembly is required for cell cycle entry and progression.[83] Aurora A kinase, localized at the basal body, activates the tubulin deacetylase histone deacetylase 6 and promotes deacetylation of *a*-tubulin, ciliary resorption and axoneme disassembly.[84] Subsequent liberation of the centriole, required for reutilization in mitotic spindle bodies, regulates cell cycle re-entry.[85]

Primary cilia are important cellular environmental sensors, signaling back from the extracellular space information such as nutrient availability.[86,87] Primary cilia communicate external signals from growth factors and nutrients by regulating the activity of mTORC1,[88] a protein complex directly linked to the regulation of autophagy,[89] which is inhibited by nutrient stress. In cultured cells, primary cilia biogenesis and macroautophagy are both triggered upon growth arrest induced by nutrient withdrawal.[90,91] In addition to this temporal relationship, these processes also share similar sites of origin. Primary cilia have been observed as membrane-derived

extensions for well over 100 years and new data now describe the plasma membrane as a source for early autophagosome membranes.[92] These findings hint at a functional relationship between these pathways, and recent results from the Cuervo and Zhong groups elegantly describe an intricate crosstalk between primary cilia and autophagy.[50]

Primary cilia dysfunction impairs autophagy. The Cuervo lab recently reported that primary cilia are required for induction of autophagy, as evidenced by compromised autophagosome biogenesis in serum-starved cells deficient in IFT20 or IFT88.[93] These proteins modulate protein trafficking between the Golgi complex and primary cilia and are required for primary cilia assembly and growth. In these experiments, autophagy was induced through serum withdrawal, a stimulus that promotes both autophagy and ciliogenesis. The investigators demonstrated that reduced ciliogenesis limited upregulation of autophagy following nutrient deficiency. Further experiments showed that autophagy induction is dependent on Shh signaling,[93] a pathway mediated through the primary cilium, and that autophagic dysfunction can be attributed to impaired ciliary signaling (Figure 3). Through activation of Shh signaling, the researchers induced autophagy in serum-supplemented cells to levels observed upon serum removal. Various treatments and conditions increased autophagy, including treatment with purmorpamine, an agonist of the Shh signal transducer membrane protein Smoothened (Smo); knockout of Patched-1 (Ptc), a membrane receptor protein that represses Smo in the absence of Shh; and overexpression of glioma-associated oncogene homolog transcription factor (GLI), the most downstream element within the Shh pathway. Signaling proteins exit the cilium near the basal body, where regulatory
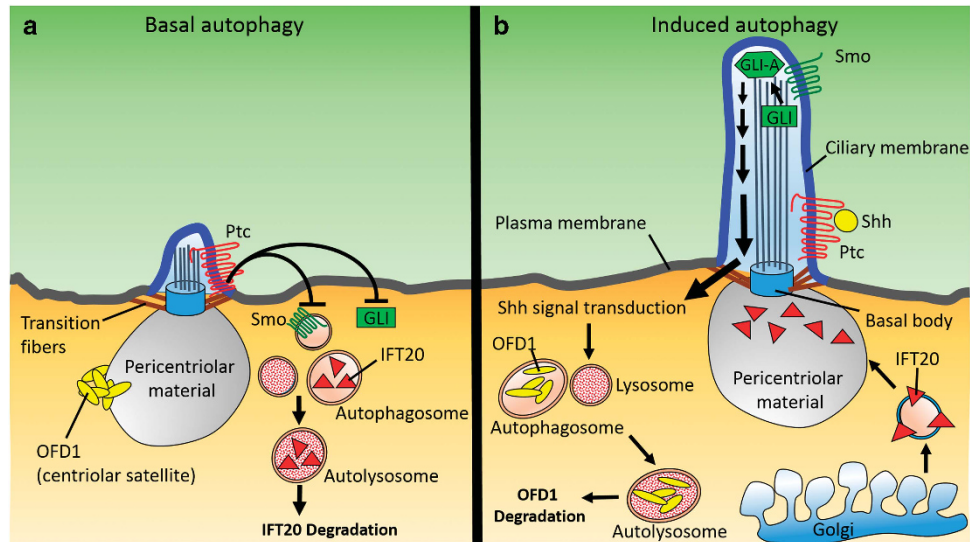
Figure 3 The functional relationship between primary cilia and autophagy. **(a)** In the absence of Sonic Hedgehog (Shh), the Patched (Ptc) receptor restricts Smoothened (Smo) entry into the primary cilium, which results in processing of the transcriptional effector GLI into its repressor form or targeting for degradation. Autophagy is not induced, OFD1 at centriolar satellites is not degraded and ciliogenesis is impaired. Basal autophagy degrades IFT20 when nutrient levels are normal. This reduces trafficking of IFT components to the pericentriolar material, thus impairing the ciliary transport required for growth of primary cilia. **(b)** Primary ciliogenesis and autophagy are induced during starvation or periods of low-nutrient availability. Autophagy induction is dependent on Shh signaling through the primary cilium. Shh signal transduction is mediated by Smo, which undergoes localization to the primary cilium following Shh binding to Ptc. Smo accumulation within primary cilia promotes GLI protein processing into its transcriptional activator form (GLI-A), enabling downstream signal transduction. Shh-mediated signal transduction promotes the autophagic degradation of OFD1 at centriolar satellites, which is required for ciliogenesis

ATG proteins required for autophagosome formation are located. One such example is ATG16L, a protein that mediates the activation of membrane-bound LC3[94] and has an essential role in autophagy through modulation of nascent autophagosomal membrane elongation.[95] Upon serum withdrawal, Shh signaling promotes ATG16L recruitment to the ciliary base via IFT20-enriched vesicles,[93] demonstrating that Shh signaling and IFT collaboratively promote autophagosome formation and provide an important point of regulation for macroautophagy. Thus, aberrant primary cilia function in the form of altered Shh signaling can hinder cellular homeostasis through misregulation of autophagy. In the context of HD, this may also augment ciliary dysfunction by altering the structural integrity of primary cilia.

Compromised autophagy alters primary cilia growth and structure. Interestingly, autophagy itself has a key role in regulating primary cilia biogenesis and growth—suggesting that the relationship between primary cilia and autophagy is bidirectional. Disruption of either basal or induced autophagy, as seen in HD, alters ciliary regulatory factor availability and causes imbalanced growth of primary cilia. The Zhong lab showed that induced autophagy promotes primary cilia biogenesis through the degradation of oral-facial-digital syndrome type 1 (OFD1) protein at centriolar satellites[96]— the nonmembranous, electron-dense granules that cluster around centrosomes (Figure 3b). OFD1 also localizes to the centrioles, where it has been shown to be essential for primary ciliogenesis,[97] yet the centriolar satellite pool of protein exhibited significantly faster turnover due to induced autophagy. OFD1, a negative regulator of ciliogenesis, is spared during basal autophagy.[96] The Cuervo lab

demonstrated that basal autophagy regulates primary cilia growth: under normal nutrient conditions, IFT20 is degraded, limiting the ciliary trafficking of components required for lengthening of primary cilia[93] (Figure 3a). Thus, disruption of either basal or induced autophagy has the potential to affect primary cilia growth by altering availability of IFT20 and OFD1. Increased autophagy induction results in degradation of OFD1 at the centriolar satellites, removing the block on ciliary growth and allowing continued lengthening of primary cilia. On the other hand, blockage of basal autophagy increases the amount of IFT20, also resulting in enhanced primary cilia growth. Interestingly, elongated ciliary morphology is evident in HD mice and human HD brains, in which autophagic dysfunction has been reported.[74]

Primary Cilia and HD Pathogenesis
Primary cilia morphology is altered in HD. The association between HTT, microtubules and motor proteins is critical for trafficking within the cell. HTT binds directly to dynein[98] and interacts indirectly with dynactin through HAP1,[99] a neuronal-binding partner of HTT that participates in intracellular trafficking.[100,101] Microtubule-based trafficking is essential for many cellular processes, including primary ciliogenesis. The formation of primary cilia depends on the proper localization of regulatory factors and building components to the pericentriolar material, a matrix of proteins surrounding the centrosome that serves as a binding platform for the microtubule nucleation required for axoneme assembly.[102] Proper primary cilia biogenesis is dependent on the centrosomal trafficking of pericentriolar material 1 protein (PCM1), a centriolar satellite protein essential for microtubule organization[103] and recruitment of proteins to the centrosome.[104]
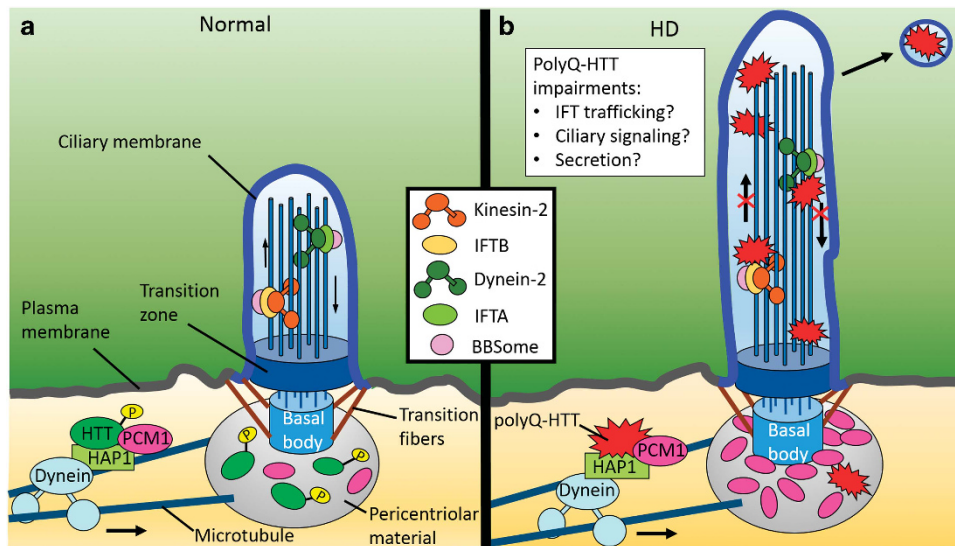
Figure 4 Abnormal ciliogenesis and hypertrophic cilia are observed in Huntington's disease. (**a**) HTT and HAP1 regulate primary cilia formation through the transport of PCM1 to the centrosome. (**b**) PolyQ-HTT exhibits increased binding to HAP1, causing accumulation of PCM1 at the centrosome, increased ciliogenesis and lengthening of the primary cilium. Increased ciliogenesis may perturb neuronal homeostasis and affect neuron survival. Altered polyQ-HTT–HAP1 interaction may also result in aberrant transport of membrane receptors and potential ciliary signaling dysfunction. PolyQ-HTT is also hypophosphorylated within its N17 domain, an area important for intracellular targeting, and exhibits increased localization within the primary cilium. Accumulated polyQ-HTT may interfere with IFT machinery, altering microtubule-based transport along the axoneme and impairing primary ciliary signaling. In light of recent evidence of vesicular secretion from neuronal primary cilia, these organelles may act as a conduit for the intercellular transfer of polyQ-HTT

The Saudou group demonstrated that HTT, in conjunction with HAP1, mediates the transport of PCM1 between the cytoplasm and pericentriolar material. Their data elucidate a role for HTT in ciliogenesis through the HAP1-PCM1 pathway[74] (Figure 4a). Significantly, they found that polyQ-HTT exhibits increased interaction with HAP1, thereby causing PCM1 accumulation at the centrosome, increased ciliogenesis and primary cilia elongation[74] (Figure 4b). The resulting hypermorphic primary cilia may be of particular significance in HD, as altered function caused by changes in ciliary structure may affect autophagic regulation. Primary cilia downregulate mTOR signaling[88] and polyQ-HTT sequesters and inhibits mTOR.[53] Because mTOR is a negative regulator of autophagy, the combinatorial effects of elongated primary cilia and polyQ-HTT may explain the increased autophagy in HD.

Primary cilia-mediated signaling is dysfunctional in HD. Changes in primary cilia-mediated signaling may contribute to HD by affecting overall ciliary function, as many signaling pathways relevant to HD operate through primary cilia (Figure 4b). Various pathway impairments are linked to HD pathogenesis, including decreased BDNF signaling at corticostriatal terminals. The clinical manifestations of striatal atrophy have long been attributed to reduced levels of BDNF,[105] a secreted neurotrophin vital for neuronal development and synaptic plasticity. Deficits in BDNF-mediated downstream tyrosine-related kinase B (TrkB) signaling have also been reported.[106–109] TrkB receptor localization and activation are primary cilia-dependent processes and primary cilia loss impedes activation of TrkB.[110] Conversely, upregulation of p75^{NTR} signaling has been shown to disrupt TrkB signaling.[106] Because p75^{NTR} also localizes to primary

cilia[111] and is the only other known receptor for BDNF, the effect of changes in ciliary-mediated signal transduction in HD merits further examination.

PolyQ-HTT accumulates within primary cilia. PolyQ-HTT may also have a more direct impact on primary cilia structure and function, as recent data have demonstrated that polyQ-HTT accumulates within primary cilia. Transport of HTT between the basal body and primary cilium is mediated by phosphorylation (S13 and S16) within the amino-terminal alpha-helical N17 domain (N17). PolyQ-HTT exhibits lower N17 phosphorylation than WT-HTT, and thus, polyQ-HTT accumulates within the primary cilia stalk[112,113] (Figure 4b). With HTT acting as a scaffolding protein connecting cargo to kinesin or dynactin for microtubule-based transport, polyQ-HTT may convey toxicity by directly interfering with IFT components localized to the primary cilium. In addition to promoting localization to primary cilia, N17 phosphorylation induces clearance of polyQ-HTT through the autophagic pathway,[55] again demonstrating the intricate relationship between primary cilia and autophagy in HD pathogenesis.

Primary Cilia: Secretory Organelles?

Another avenue by which primary cilia could participate in HD pathogenesis is by secretion of polyQ-HTT from neurons (Figure 4b). As outlined in the next section, increasing evidence suggests that progression of late-onset neurodegenerative diseases such as HD is driven by transmission and propagation of toxic forms of disease-associated proteins via a mechanism akin to that of prion diseases. Such protein secretion could further overload and overwhelm the protective cellular maintenance systems of vulnerable neurons.

Although the secretory capabilities of primary cilia in humans remain uncertain, several lines of evidence suggest that cilia might act as secretory organelles.

First, IFT proteins, key components of the ciliary structure, have been shown to play a role in exocytosis and extracellular signaling in non-ciliated cells.[114,115] T lymphocytes express IFT proteins, including IFT20, and kinesin-2, the IFT-dependent motor. These proteins associate with the centrosome and Golgi.[114] Given that transport protein machinery characteristic of and critical to ciliary structure and function is important for protein secretion in cells that do not develop cilia suggests the possibility that this machinery might play a similar protein secretory role in primary cilia.

Second, studies of simple organism model systems have identified release of extracellular vesicles from ciliary structures. EM analysis showed that cilia of *Chlamydomonas*, a unicellular green alga, secrete bioactive vesicles containing a proteolytic enzyme.[116] This finding is of potential relevance to human primary cilia because green algae have traditionally served as a reliable model of mammalian cilia—other ciliary components first identified in algae and later found to be conserved in mammals include the IFT proteins.[116,117] In addition, ciliated sensory neurons in *Caenorhabditis elegans* were recently shown to release extracellular vesicles.[118]

Finally, early studies in mammalian cells, although far from conclusive, have yielded indicators of protein secretion from primary cilia. For example, a study of neuroepithelial cells found that the distribution of prominin-1, a stem cell marker, on primary cilia was suggestive of budding of small membrane vesicles from the tips of differentiating cells.[119] In addition, the protein contents of purified human urinary exosome-like vesicles suggested that the structures possibly originated from primary cilia.[120] To date, evidence of secretion of proteins from primary cilia in humans is at best preliminary, but because of observations in simpler organisms and the potential importance of such a mechanism, further exploration is warranted.

Primary Cilia: Propagation of Unfolded Proteins?

Prion theory. Increasing evidence supports a model of neurodegenerative disease in which pathological progression is driven by prion-like protein seeding and propagation. This prion theory of neurodegeneration postulates that fibrillary protein seeds are taken up from adjacent or connected cells in the brain and induce aggregation of normally structured proteins, leading to spreading of characteristic misfolded proteins.[121] This process is analogous to classical prionopathies in which PrP[SC], the misfolded form of the normal prion protein PrP[C], propagates through neuronal networks by inducing pathological conformational changes of native prion protein. Most neurodegenerative diseases are characterized by dysfunction in discrete brain regions in the early stages and more widespread dysfunction in later stages, suggestive of a spreading pathology.[121,122] For example, in HD, pathophysiological progression is quite predictable, beginning in the striatum and later progressing to the cortex.[123] Furthermore, spreading of protein aggregates has been shown to mirror the overall clinical pathophysiology of neurodegeneration.[121]

In recent years, multiple studies have investigated the prion-like capabilities of pathological proteins and protein aggregates associated with various neurodegenerative diseases.[124] Exosomal secretion of small fractions of amyloid-β peptides has been observed.[125] Internalization of aggregates containing α-synuclein, tau, ALS-associated proteins and polyglutamine proteins has also been shown in cultured cells.[121] Specifically, uptake of α-synuclein has been observed in cultured human dopaminergic neuronal cells by a mechanism that involved endocytosis.[126] In addition, cultured HEK and C17.2 cells have been shown to take up tau aggregates and transfer aggregates between cells that are able to interact with native tau directly and convert the native protein to fibrillar forms.[127,128] Furthermore, seeding of cell cultures with aggregated forms of ALS-associated proteins SOD1 and TDP-43 resulted in misfolding of WT proteins[129–131] and cell-to-cell transmission of misfolded WT SOD1 has been observed.[132] Finally, polyQ aggregates have been shown to be internalized by COS7, HEK293 and neuro2A cell lines *in vitro* and internalized aggregates colocalized with cytosolic quality control components such as ubiquitin and proteasome subunits; however, cell-to-cell transmission of aggregates between intact cells was limited and relatively inefficient.[133] By contrast, expression of polyQ-HTT fragments in CAD neuronal cells and primary cerebellar granule neurons *in vitro* did result in spontaneous and efficient transfer to neighboring cells that did not require release from dying cells.[134] In this instance, transfer required cell-to-cell contact and aggregates were found in tunneling nanotubes, which were increased by polyQ-HTT expression.[134] Perhaps providing the most compelling evidence to date of propagation of polyQ-HTT *in vitro*, a new study has reported that when hESC-derived neurons were introduced into brain slices from HD disease model R6/2 mice, the hESC-derived neurons developed HTT aggregates and exhibited signs of toxicity.[135] Similarly, when R6/2 cortical brain slices were co-cultured with striatal brain slices from WT mice, polyQ-HTT aggregates developed in the WT striatal neurons.[135]

Evidence of the propagation ability of neuronal aggregates has also been found *in vivo* in animal models and human patients. For example, multiple studies of animal models of neurodegeneration have reported strong evidence of cell-to-cell transmission of α-synuclein,[126,136] tau[137–140] and amyloid-β.[141] Moreover, it was recently shown that lentiviral transduction of polyQ-HTT exon 1 into cortical neurons of WT mice led to the development of polyQ-HTT aggregates in striatal medium spiny neurons located in regions innervated by the transduced cortical neurons[135,142] and polyQ-HTT aggregates in *Drosophila* were found to effect prion-like conversion of soluble WT-HTT in the cytoplasm of phagocytic glia.[143] Finally, transplant studies in PD and HD patients have confirmed propagation in humans. Engrafted fetal mesencephalic dopaminergic neurons developed ubiquitin- and α-synuclein-positive Lewy bodies when transplanted in PD patient brains.[121,123,144–147] Similarly, polyQ-HTT aggregates were found in grafted tissue in HD patient brains, although the aggregates were extracellularly located and not within engrafted neurons.[148]

Mechanism of polyQ-HTT cell-to-cell transmission: primary cilia involvement? Although the ability of toxic proteins such as polyQ-HTT to propagate and spread in the brain is becoming well-established, the mechanism of such cell-to-cell transmission remains a mystery. To date, the study by Costanzo and colleagues is the only one, to our knowledge, to suggest a mechanism of cell-to-cell transmission of polyQ-HTT—namely, tunneling nanotubes.[134] In light of the clues suggesting protein secretion from primary cilia, we speculate that primary cilia might be important, as yet unexplored mediators of prion-like spreading of polyQ-HTT (and potentially other toxic proteins) in the brain.

Remarks and Future Outlook

As discussed in this review, there are multiple avenues through which primary cilia could contribute to HD pathogenesis. It is well documented that autophagy is both increased and inefficient in HD, resulting in wasted energy expenditure without removal of toxic species or recycling of macromolecules. This autophagic increase causes degradation of OFD1 at centriolar satellites, promoting ciliogenesis and extending primary cilia length. Studies also show that autophagy induction depends on primary cilia signaling. With these points in mind, we speculate that elongated primary cilia, enriched in ciliary membrane receptors and signaling components, contribute to HD by exacerbating autophagy induction. Abnormal structure may also alter primary cilia function and affect pathways impaired in HD, such as striatal BDNF signaling. In sum, we propose that primary cilia and autophagic dysfunction have multifaceted and interdependent roles in HD pathogenesis, many of which require further investigation and characterization.

Autophagy and primary cilia. It should be noted that in the experiments investigating interrelationship between primary cilia and autophagy, serum starvation was used to induce autophagy. Although this method is commonly used to induce autophagy in non-neuronal cells, previous *in vivo* studies show that starvation does not induce autophagy in the brains of mice.[149] Therefore, further investigation of primary cilia-autophagy interaction in a system more relevant to primary neuronal cells merits consideration.

Primary cilia in HD. Data from the Saudou group demonstrate that polyQ-HTT affects primary ciliogenesis, altering primary cilia structure and function.[74] Based on this finding, we postulate that decreased BDNF-TRkB signaling in HD stems from these structural changes. However, it is possible that signaling through primary cilia is not adversely impacted by increased overall length. There are other potential points along this signaling pathway that may be susceptible to polyQ-HTT. One such area may involve trafficking of TRkB receptors. A recent study shows that HAP1 stabilizes and prevents the degradation of TRkB receptors,[150] and in the context of HD, increased polyQ-HTT–HAP1 interaction may interfere with this important function. Establishing these connections will be an important avenue of investigation.

Conflict of Interest

The authors declare no conflict of interest.

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