The Roles of Cytoskeleton in Huntington's Disease

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Abstract: This article indicates partly roles of cytoskeleton in Huntington's Disease. First, mutant huntingtin may change the vesicle transport due to the fact that phosphorylation of huntingtin as a switch to regulate the anterograde/retrograde transport in neurons. Secondly, dysfunction of cellular morphology regulated by huntingtin, actinin and growth factor may be related in the occurrence of the Huntington's Disease. Finally, the alterations of Tau in total level, imbalance of isoforms produced by alternative splicing or by post-translational modifications imply that Huntington's Disease and Alzheimer's Disease may have similar occurrence mechanism. According to specific description of these, this article hopes to provide new treatments for Huntington's Disease or new research orientations for diseases with similar characteristic of Huntington's Disease.

1 INTRODUCTION

With the continuous improvement of medical level and health environment, the average life expectancy of the population is gradually increasing and some diseases associated with the aging of the population are also gradually highlighted, especially in neurodegenerative diseases such as Alzheimer's disease and Huntington's disease, which cause huge economic burden to the society and family.

Huntington's Disease is a rare genetic autosomal-dominant neurodegenerative disease that results from expansion of a CAG trinucleotide repeat (>35) in the HTT(Huntingtin) gene on the short (p) arm of chromosome 4 at position 16.3 and first involves basal ganglia (caudate nucleus and putamen) (Taran et al. 2020). In neuropathology, Huntington's Disease is characterized by neuron death, primarily a progressive atrophy of the basal ganglia produced by medium-sized spiny neurons of the striatum, and the presence of spherical inclusions due to aggregation of mutant Huntingtin (Htt) in the neuronal nucleus and cytoplasm (Marta et al. 2020). The clinical manifestations of this disease are movement disorders, cognitive decline and a range of somatic symptoms. Progressive worsening comes with a bedridden state and patients finally die in 20 years after the onset of symptoms (Anne-Catherine et al. 2019). Huntington's disease affects approximately 1 in 10,000 people worldwide, and the average age of onset is between 40 and 50 years (An et al. 2018) .

Although etiology of Huntington's Disease is clear, there is no radical treatment for it currently and the mechanism of its occurrence and development is unequivocal. In recent years, through the further understanding of the cytoskeleton, more and more studies have proposed that the cytoskeleton plays an important role in the pathogenesis of Huntington's disease.

This review is designed to integrate the remarkable recent advances that have led to new insights into the possible pathogenesis of Huntington's disease from the perspective of cytoskeleton.

2 THE CYTOSKELETON IN HUNTINGTON'S DISEASE

2.1 Huntingtin, Kinesins and Dyneins

Dynein is a minus end-directed microtubule motor protein, while kinesin is a plus end-directed microtubule motor protein. The property of motor protein that carry vesicles along MTs determines the correct intracellular transport of membranous organelles and cargoes. In neurons, kinesins are responsible for anterograde transprot of vesicles from

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cell centre to the end of neurites. On the contrary, dyneins are responsible for the retrograde transport of vesicles and organelles back towards the cell centre. Dynactin is a dynein activator that binds to both dynein and MT to form the complex which plays a critical role in intracellular transport, such as vesicular transport from the endoplasmic reticulum to the Golgi and lysosomal motility. Besides, Juliane et al found that proper localization of huntingtin in the cell depends on functional dynein/dynactin complex (Caviston et al. 2007).

Huntingtin, a protein with 3,144 amino acids, is present in all cells especially neurons and has a large number of huntingtin partner proteins (Taran et al. 2020) involved in vesicales in neurons and endocytic vesicles through directly binding to intermediate chain of dyneins and to huntingtin-associated protein-1(HAP1). HAP1 interacts with the subunit p150Glued of dynactin and the heavy chain of kinesin-1 by its coiled-coil domain. What is more, the subunit p150Glued of dynactin can also interact with kinesin-1. The fact that mutant huntingtin can cause disturbance in axonal transport also indirectly demonstrates the important role of Huntington proteins in vesicle transport along microtubules. The function of huntingtin to facilitate anterograde and retrograde transport along MT in neurons is regulated by phosphorylation of huntingtin at serine 421 by Akt upon IGF-1 stimulation. When huntingtin S421 is phosphorylated, it recruits kinesin-1 to vesicles and MTs to facilitate anterograde transport. In contrast, when huntingtin S421 is not phosphorylated, huntingtin directly combine with intermediate chain of dynein to facilitate dynein/dynactin-mediated retrograde transport of vesicle along the axon like Figure 1 shows. Furthermore, phosphorylation of huntingtin does not affect basic characters of MT such as nucleation, dynamics or stability (Colin et al. 2008).

Although phosphorylation of huntingtin S421 indicates no differences in binding between huntingtin and HAP1 or between HAP1 and p150Glued of dynactin or between HAP1 and kinesin-1, the signal marked the interaction of p150glued of dynactin and kinesin-1 increase. It shows that the phosphorylation of huntingtin S421 leads to the stable interaction between dynactin and kinesin-1 (Colin et al. 2008).

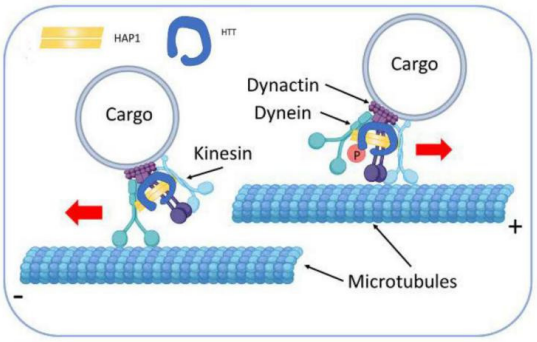

Figure 1. Participation of Huntingtin in vesicle transport along microtubule (Taran et al. 2020).

Huntingtin as a scaffold for the dynein-dynactin complex and its phosphorylation determines the direction of vesicle transport along the microtubules.

2.2 Huntingtin and Actin

Huntingtin is essential for cellular adhesion and for actin cytoskeleton in response to stimulation of growth factor, like platelet derived growth factor (PDGF), to change the normal morphology. The deletion of huntingtin results in reduced adhesion and altered morphology. Moreover, mutant huntingtin in Huntington's Disease inhibits the regulation of growth factor stimulation in morphology changes and increases numbers of vinculin-positive focal adhesion. Immunoreactivity indicates huntingtin is localized to actin stress fibers, vinculin-positive adhesion contacts and membrane ruffles in fibroblasts. Other studies further to show that first 14 amino acids of a purified fragment of huntingtin have a direct interaction with actin in vitro. Besides, huntingtin co-localizes with α-actinin and regulates its localization at membranes to impact on maintenance of adhesion and cellular morphologic changes (Tousley et al. 2019). The Figure 2 shows the co-localizations between huntingtin with α-actinin-1 in primary human fibroblasts (a and b).

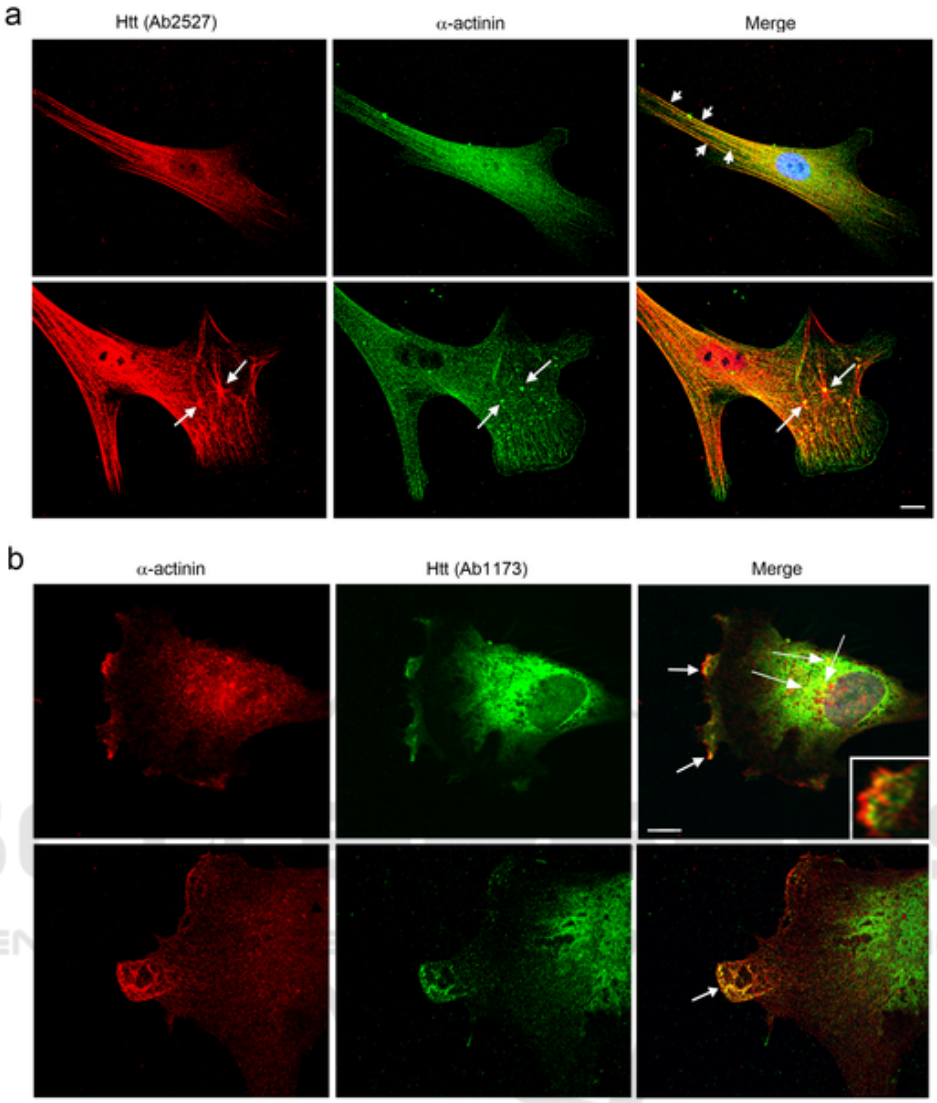

Figure 2: Co-localizations between huntingtin with α-actinin-1 in primary human fibroblasts (a and b) by double-label immunofluorescence (Tousley et al. 2019).

Red shows huntingtin detected with Ab2527, green shows α-actinin detected by a monoclonal antibody and yellow show them co-localize at stress fibers in serum-starved cells(top, short arrows). (b) Green shows huntingtin detected with Ab1173, red shows α-actinin detected by a monoclonal antibody and yellow shows them co-localize in lamellipodia at ruffled membranes(top panel, arrows and inset) and in protrusions at the leading edge of lamellipodia(bottom panel, arrow). 60x oil objective. Scale bars = 10μm.

α-actinins are actin binding proteins including different isoforms, like α-actinin-1 and α-actinin-2 (Tousley et al. 2019). Huntingtin can bind to α-actinin-1, 2 and 4. The function of α-actinin is to bundle and crosslink actin filaments in both contractile and non-contractile cells and link actin filaments to integrins in focal complexes and focal adhesions that persists during hierarchical assembly. Moreover, Actin and α-actinin-2 are concentrated in the dendritic spines of neurons and play a role in regulating the morphology of the spine and stabilizing postsynaptic membrane proteins (Tousley et al. 2019).

α-actinin-1 and huntingtin co-localized to stress fibers, membrane and ruffles and lamellar protrusions in fibroblasts through double-label immunofluorescence. Proximity ligation assays indicate that α-actinin-1 have a close interaction with huntingtin in human fibroblasts and neurons (Tousley

et al. 2019). Adelaide et al. found that huntingtin is responsible for regulation of α-actinin-1 proper localization on the membrane and combination of growth factor with actin polymerization at new sites of adhesion (Taran et al. 2020).

α-actinin-2 interacts with 399-969 amino acids region of huntingtin. However, full interaction between α-actinin-2 and huntingtin demands additional amino acids N-terminal to huntingtin residue 399. Highly dynamic α-actinin-2 is concentrated in dendritic spines of neurons in brain, where it regulates morphology and maturation of dendritic spines and the transport of the AMPA subtype of glutamate receptors to post-synaptic. Huntingtin is also essential for development of excitatory synapses in the cortical-striatal pathways in brain. Hence, the interaction between huntingtin and α-actinin-2 may induce maturation and function of excitatory synapses on neurons (Taran et al. 2020).

On one hand, IP 3-kinase in respond to growth factor stimulation activates Akt and produces PI (3,4,5) P3 and PI (3,4) P2. Activated Akt can phosphorylate huntingtin at serines 419 and 421 to interfere the combination between huntingtin and α-actinin-2 or facilitate their dissociation. On the other hand, α-actinins can bind to both PI (4,5) P2 and PI (3,4,5) P3. Huntingtin binds to PI (4,5) P2 with a low affinity while binds to PI (3,4) P2 and PI (3,4,5) P3 with a high affinity. As the result of the fact that PI (4,5) P2 is more abundant in the membrane than PI (3,4,5) P3, α-actinins bind to PI (4,5) P2 with lack of huntingtin. With the interaction of huntingtin, α-actinins bind to PI (3,4,5) P3 with a high affinity at highly specialized regions. These may be pathways for huntingtin interacting with actin and actinin to impact the cellular morphology, induced adhesion and neuronal maturation and they may alter in Huntington's Disease. Nevertheless, it still requires more relevant experiments and studies to prove (Taran et al. 2020).

It is worth noting that α-actinin-2 and dynein have the same region S421 of interaction on huntingtin and their competitive binding to huntingtin perhaps play a critical role in regulating the transport of vesicle from MTs to actin filaments (Tousley et al. 2019).

2.3 Alternation of Tau in Huntington's Disease

Recently, with further studies of Huntington's Disease, increasing evidences of multiple alterations of Tau have been found in brains of Huntington's Disease patients, which implies that abnormal alterations of Tau is likely to pathogenic for contributing to the process of Huntington's Disease.

Tau, a microtubule-associated protein, is encode by the MAPT gene that is located in the long(q) arm of chromosome 17 at position 21.31 and contains 16 exons. Multiple Tau isoforms is generated by alternative splicing. For instance, the exclusion of exon 10 results in 3R isoform of Tau while inclusion of exon 10 results in 4R isoform. The difference between 3R and 4R is in the C-terminal region of Tau, where exon 10 encodes a 31 amino acid sequence and provides one of the four probable tubulin-binding repeats. The proportion of Tau isoforms as well as post-translational modifications such as phosphorylation and acetylation influence the affinity of Tau for microtubules (Marta et al. 2020).

The MAPT gene is mainly expressed in neurons of the central nervous system, which is related to its function of maintaining neuronal polarity by regulating microtubule assembly and stability. In general, Tau is almost exclusively located in the axon of healthy neurons. The N-terminal region of Tau binds to plasma membrane components and participates in the formation of microtubule bundles as a spacer between microtubule bundles while the C-terminal region binds to microtubules to regulate their dynamic assembly. Besides, Tau is involved in the transport of mRNA and proteins along axons in intracellular, neurite extension and synaptic plasticity (Marta et al. 2020).

The alterations of Tau are mainly reflected in increased total levels, imbalance of isoforms produced by alternative splicing or by post-translational modifications and the presence of Tau nuclear rods (TNRs) or Tau-positive nuclear indentations (TNIs) (Marta et al. 2020).

Marked by Tau-5 antibody, Tau showed a high increase in the cortex of Huntington's Disease patients while no changes were found in the striatum. Moreover, elevated Tau total mRNA levels in the putamen of Huntington's Disease patients and attenuate motor abnormalities by Tau knock-down in an HD mouse model also demonstrate that excess of Tau contributes to the process of Huntington's Disease (Marta et al. 2020).

In Huntington's Disease patients, another prominent manifestation of alteration of Tau is an increase in the ratio 4R-Tau/3R-Tau isoforms, which is regulated by alternative splicing of exon 10. It shows an increase of the level of 4R-Tau protein in the cortex while in the striatum, an increase of the level of 4R-Tau is accompanied by a decrease of the level of 3R-Tau. Alternative splicing of exon 10 is mainly regulated by the family of the serine- and

arginine-rich (SR) proteins, especially SRSF6. Post-translational modifications of SR proteins, like phosphorylation of serine and threonine residues, regulate their activity and localization. In general, phosphorylation of SR proteins is benefit to its translocation from the cytoplasm to the nucleus. In Huntington's Disease, increased levels of phosphorylation of SRSF6 in the striatum and cortex, as well as sequestration by mutant Huntingtin inclusions result in a decrease of SRSF6 activity. In addition, SRSF6 regulates alternative splicing of MAP-2, which also alters in Huntington's Disease (Marta et al. 2020).

Marking by AT-8 antibody, it found that an increase of phosphorylation of Tau at Ser396, 404, 199 and Thr205 epitopes in the putamen of Huntington's Disease patients. GSK-3 is one of the main kinases to phosphorylate Tau. Further studies found that the level and activity of GSK-3 decreased and the phosphorylation of GSK-3β Ser9, inactive form of the kinase, increased in Huntington's Disease. What's more, a decrease of phosphatases (PP1, PP2A and PP2B) implicated in dephosphorylation of Tau is detected in the R6/2 mouse model. All of these implicate that the reason of Tau hyperphosphorylation in Huntington's Disease is the deficiency of dephosphorylation of Tau (Marta et al. 2020).

Finally, some research has found the presence of TNIs, known as TNRs, in the striatum and cortex of Huntington's Disease patients by antibodies that recognize 4R-Tau isoforms, 3R-Tau isoforms, total Tau or Tau oligomers. The ordered filamentous ultrastructure of TNIs/TNRs fills neuronal invaginations of nuclear envelope and partially or totally span the neuronal nuclear space (Marta et al. 2020).

3 CONCLUSIONS

All in all, this review describes the roles of cytoskeleton in Huntington's Disease from three aspects. When huntingtin S421 is phosphorylated, it recruits kinesin-1 to vesicles and MTs to facilitate anterograde transport. In contrast, when huntingtin S421 is not phosphorylated, huntingtin directly combine with intermediate chain of dynein to facilitate dynein/dynactin-mediated retrograde transport of vesicle along the axon. Huntingtin interacts with actin and actinin to impact the cellular morphology, induced adhesion and neuronal maturation. Moreover, the competitive binding of α-actinin-2 and dynein to huntingtin S421 may be the switch on vesicle transport from MT to actin. As a result, mutant huntingtin in Huntington's Disease may changes its function above. In addition, the alterations of Tau like total level, alternative splicing and post-translational modification suggest that Tau may be an independent factor that results in Huntington's Disease, or together with Huntingtin leads to the occurrence and development of the disease.

This article through to partly roles of cytoskeleton in Huntington's Disease, on the one hand, hopes to provide new targets for clinical treatment and method. On the other hand, such as Alzheimer's and Huntington's part neurodegenerative diseases are characterized by accumulation of protein misfolding, which may have a certain similarity in pathogenesis, this article may provide help for researches on these diseases with similar characteristics.

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