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Special Focus: Molecular and Cellular Events Controlling Neuronal and Brain Function and Dysfunction

Huntingtin associated protein 1 and its functions

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Huntington disease (HD) is caused by a polyglutamine expansion in the protein huntingtin (Htt). Several studies suggest that Htt and huntingtin associated protein 1 (HAP1) participate in intracellular trafficking and that polyglutamine expansion affects vesicular transport. Understanding the function of HAP1 and its related proteins could help elucidate the pathogenesis of HD. The present review focuses on HAP1, which has proved to be involved in intracellular trafficking. Unlike huntingtin, which is expressed ubiquitously throughout the brain and body, HAP1 is enriched in neurons, suggesting that its dysfunction could contribute to the selective neuropathology in HD. We discuss recent evidence for the involvement of HAP1 and its binding proteins in potential functions.

Introduction

Huntington disease (HD) is an inherited neurodegenerative disease caused by mutant Huntingtin (Htt) with cytosine-adenine-guanine (CAG) trinucleotide repeats in exon 1 which codes for polyglutamine (polyQ) expansion.[1] HD is characterized by cognitive decline, chorea, dementia and other psychiatric symptoms. Although the disease affects a number of brain regions such as the cortex, thalamus and subthalamic nuclei, the neuropathological hallmark of HD is the severe atrophy of the striatum.[2,3] The normal Htt protein with 6 to 34 polyQ tract does not cause the disease whereas disease symptoms can be observed when polyQ extension is greater than 40 in the N-terminal fragment of Htt. Htt is ubiquitously expressed in the brain and peripheral organs.[2] The polyQ region contributes to the modification of the three-dimensional structure of the whole protein and difference in the physiological reaction with other related proteins. This is why the number of ployQ repeat plays a key role in the disease pathogenesis.[4]

Hungtingtin-associated protein 1 (HAP1) was the first Htt interacting proteins to be identified in yeast two-hybrid screens.[5] HAP1 binds more tightly to Htt with an expanded glutamine repeat than to wild type Htt, and the binding is enhanced by lengthening the glutamine repeat.[6] Unlike Htt which is expressed ubiquitously, HAP1 is expressed predominantly in the central nervous system (CNS), particularly in the basal forebrain, cerebral cortex, cerebellum, the accessory olfactory bulb and the pedunculopontine nuclei, and highly expressed in the olfactory bulb, the hypothalamus, and the supraoptic nucleus.[5,7-9] Rat HAP1 consists of two isoforms (HAP1-A, 75 Kd and HAP1-B, 85 Kd) which have different C-terminal sequences (amino acids 579–599 in HAP1-A and amino acids 579–629 in HAP1-B). HAP1-A has a unique sequence of 21 amino acids, whereas HAP1-B has a different sequence of 51 amino acids.[5,10,11] The expression ratios of rat HAP1-A to HAP1-B are different in various regions. In the olfactory bulb and spinal cord, the level of HAP1-A is lower than that of HAP1-B.[11] In the striatum and other regions their levels are almost the same.[12] Human HAP1 is detected as only one major form (75 Kd) which shares a great similarity with HAP1-A mainly in the hippocampus and caudate, while the levels are lower in the cerebral cortex and cerebellum, and no expression is found in the thalamus and white matter.[6,8,13]

In investigating the function of two HAP1 isoforms, it was found that the common region of HAP1-A and HAP1-B binds to other molecules which constitute the cytoplasmic inclusions; however the C-terminus of HAP1-B takes the role for inhibition of the formation of inclusions whereas the unique C-terminal region of HAP1-A seems to be critical for inclusion formation. Both isoforms can aggregate in different proportions or self-associate in vivo, where HAP1-A accelerates the formation of inclusions and HAP1-B suppresses this formation simultaneously. Whether there are inclusions in the cell body depends on the proportion of the two isoforms.[11] The dynamic association between the isoforms regulate the variable size of the inclusions in the body.[11] The expression level of HAP1-B is normally higher than that of HAP1-A in most brain regions. This could explain in part why the majority of native HAP1 in the brain is cytosolic and diffusely distributed in the neurons.[11]

Like Htt, HAP1 is a cytoplasmic protein with neither conserved transmembrane domains nor nuclear localization signals,[14] and associates with microtubules and many types of membraneous organelles, such as mitochondria, endoplasmic reticulum, tubulovesicles, endosomal and lysosomal organelles.[15] In adult mouse brain neurons, HAP1 is highly enriched in large dense organelles, large

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Table 1 **HAP1-interacting proteins**

Name	Function	Region in HAP1 for binding	Refs
Huntingtin	Scaffold protein	Amino acids 278–370	12
$p150^{Glued}$	Microtubule-dependent transporter	Amino acids 278–445	12, 26
KLC	Vesicular trafficking		24
$GABA_A$ receptors	Membrane receptor	Amino acids 220–520	47
TrkA	Nerve growth factor receptor		25
Hrs	Vesicular trafficking	Amino acids 246–425	23
EGFR NeuroD(ND)	Neuronal transcription factor	Amino acids 247–446	64
$InsP_3R1$	Membrane receptor	Amino acids 273–599 in HAP1A	70
14-3-3 protein	Multifunctional regulatory protein		
Duo	GDP-GTP exchange factor	Animo acids 1–313	79
AR	Androgen receptor		91
TBP	Transcription factor		93
AHI1	Signal transduction, RNA processing, transcriptional regulation, cytoskeleton assembly, vesicle trafficking and cell division		98

endosomes (multivesicular bodies) and moderately locate in small vesicles, tubulovesicular structures, plasma membrane, coated and budding vesicles and microtubules.[13] The localization of HAP1 and Htt is similar which suggests that HAP1 and Htt have the role in intracellular transport.[15]

As HAP1 is expressed more abundantly in the hypothalamus, which is well documented to regulate feeding behavior, the postnatal HAP1 knockout mice show suckling defects that ultimately leads to malnutrition, dehydration and premature death.[16-19] Nipple-searching behavior and attachment to the nipple, in most mammals, is believed to be mediated primarily by olfactory and tactile cues.[20] Absence of olfactory bulbs or surgical lesions in the olfactory system in newborns lead to a reduction in nipple attachment efficiency, and consequent early postnatal lethality due to starvation.[21] All HAP1 knock out pups exhibited a normal rooting reflex in response to manual stimulation of their mouth region, indicating normal tactile sensation and motor control. Moreover, HAP1 knock out pups' mothers do nest, crouch over their pups in a typical nursing manner, and collect them when they are scattered, further indicating that olfaction is not affected in HAP1 knock out mice.[16]

Htt act as a scaffold protein which enables the packaging of various proteins for transport along microtubules. As the binding protein of Htt, HAP1 is as one of the components of cargo-motor molecules and participates in intracellular trafficking.[14,22-25] The common region of both HAP1 isoforms contains three predicted coiled-coil domains,[23,26] which may be responsible for binding with the interacting proteins, such as Htt (amino acids 171–230).[27] In this review, we attempt to discuss all HAP1 interacting proteins discovered so far to explain the functions of HAP1 (Table 1). Based on the functions of these interacting proteins and the direct evidence revealed in the literature, HAP1 is likely involved in the vesicular transport, gene transcription regulation, membrane receptor trafficking and other functions such as calcium release and protein aggregation.

HAP1 Regulates Vesicular Transport by Interacting with Accessory Molecular Motor Proteins and the Signaling Molecules

Substantial evidence suggests that HAP1 plays important roles in the vesicular transport within neurons and axons. Li and Li have made an excellent review on this topic.[14] Here in this review, we focus its roles in vesicular transport by elaborating different interacting molecules.

$p150^{Glued}$. $p150^{Glued}$ is the largest member of all the dynactin subunits. Dynactin is a multisubunit protein complex that binds to dynein which is the microtubule motor that participates in retrograde transport in cells.[26] Dynactin binds dynein directly and allows the motor vehicle to travel over long distances.[28] The N-terminal fragments of $p150^{Glued}$ contain a conserved CAP-Gly (cytoskeleton-associated protein, glycine-rich) motif which plays a very important role in dynactin binding to microtubules.[29-31] This motif also contributes to microtubule minus-end anchoring at interphase centrosomes and mitotic spindle poles.[32-34] In addition to microtubules, the $p150^{Glued}$ CAP-Gly domain binds proteins such as EB1 and CLIP-170, both of which are themselves microtubule-binding proteins.[28] The middle region of $p150^{Glued}$ is responsible for interacting with microtubule-based motors.[28]

HAP1 binds to $p150^{Glued}$ and induces the microtubule-dependent retrograde transport of membranous organelles. HAP1 may influence the transport of various proteins that bind to $p150^{Glued}$.[12,26] From the colocalization experiment of HAP1 and $p150^{Glued}$ in transfected cells, the cytoplasmic inclusions were found to be colocalized with HAP1. Thus the cytoplasmic inclusions could be transported along microtubules with HAP1 and $p150^{Glued}$.[12] It is reported that the common region of HAP1 also binds to Htt and $p150^{Glued}$ and acts as a scaffold linking Htt to dynactin complex.[12,35]

Kinesin light chain (KLC). Kinesins are the largest superfamily of microtubule-dependent motors for anterograde transport with 45 members in mice and human and they are the most abundant motors in many cell types. Conventional kinesin, kinesin I, was originally discovered in the context of vesicle transport in axons. It

is a tetramer consisting of a kinesin heavy chain (KHC, 110–120 Kd) dimer and two kinesin light chains (KLC, 60–70 Kd).[36,37] The N-terminal globular motor domain of KHC contains a microtubule-binding sequence and an ATP-binding sequence. The C-terminus has a unique sequence and is linked with the N-terminal coiled-coil domain of KLC.[38-41] The C-terminus of KLC consists of six tetratrico peptide repeat domains, which are involved in protein-protein interactions and are proposed to link KLC to receptor proteins on vesicular cargoes.[42,43] This diversity of domains is thought to regulate motor activity and binding to different cargoes.[44] HAP1 was found to interact with KLC that drives anterograde transport along microtubules in neuronal processes and HAP1 gene deletion suppressed kinesin-dependent transport of amyloid precursor protein vesicles. HAP1-A preferentially binds KLC as compared with HAP1-B.[24] These findings demonstrate that HAP1 plays an accessory role not only in retrograde transport but also anterograde transport along microtubules.

14-3-3 protein. The 14-3-3 family contains well conserved and ubiquitously expressed regulatory proteins. 14-3-3 proteins are multifunctional regulators, and they bind a large number of proteins, including cytoskeletal and trafficking proteins and are involved in the regulation of many crucial cellular processes, such as signal transduction and protein trafficking.[45,46] Using the yeast two-hybrid system, HAP1 was found to interact with 14-3-3 proteins. The overexpressed 14-3-3 decreases the trafficking of HAP1-A to the neuronal processes and neurite tips and inhibits the function of HAP1-A in promoting neurite outgrowth.[47]

Duo. Duo was identified by using the yeast two-hybrid system as one of HAP1-binding proteins, which is a membrane cytoskeletal protein[48] and belongs to RhoGEF superfamily.[49,50] Guanine exchange factors (GEF) stimulate Rho and Rac signal transduction by switching them from the inactive (GDP-bound) to the active (GTP-bound) form. These molecules are often involved in organizing the cytoskeleton and act as axon guidance molecules.[51] Duo contains at least four or five spectrin-like repeats which enable it to bind to actin, one GEF domain,[48,52] peptidylglycine α-amidating monooxygenase (PAM) binding region, and HAP1 binding region. The cytoplasmic domain of PAM, which binds Duo is believed to be involved in the biogenesis of secretory granules and has a sorting signal for internalization from the cell surface.[53] Duo is a rac1-specific binding protein, which regulates cytoskeleton (actin) organization, endocytosis, exocytosis and free radical production. Thus HAP1 is proposed to play a role in vesicle trafficking and cytoskeletal functions, and takes part in a ras-related signaling pathway.[50]

Hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs). Hrs, a mammalian homologue of yeast vacuolar protein sorting protein Vps27p, contains a phosphatidylinositol 3-phosphate-binding FYVE domain and the association of Hrs with early endosomes was well established.[54-57] Hrs binds Vps23, recruiting ubiquitinated membrane proteins to form protein complexes called endosomal sorting complex required for transport (ESCRT) which transports endocytic membrane proteins to multiple vesicular bodies.[58] HAP1 also interacts with Hrs, which plays a role in the regulation of vesicular trafficking and signal transduction,[59,60] and regulates endocytic trafficking through early endosome.[23] The association of HAP1 with Hrs is mediated via a coiled-coil interaction between the central coiled-coil domains of both proteins. HAP1 co-localizes with Hrs on early endosomes.[23] The increased expression of mutant Htt causes abnormal interactions of HAP1 with Hrs, which results in aberrant endocytic trafficking.[23,61]

Abelson helper integration site 1 (AHI1). Mouse AHI1 was initially identified as a common helper provirus integration site for murine leukemias and lymphomas.[62] The protein encoded by the human AHI1 gene (AHI1 or Jouberin) contains 7 WD40 repeats, an SH3 domain, potential SH3 binding sites, and an N-terminal coiled-coiled domain.[63] WD40 domains are present in proteins that are involved in a variety of functions, including signal transduction, RNA processing, transcriptional regulation, cytoskeleton assembly, vesicle trafficking and cell division.[64] SH3 domains are a common feature of signalling molecules involved in numerous pathways.[65] Using immunoprecipitation and mass spectrometry, mouse Ahi1 was found bound tightly to HAP1 and formed a stable protein complex in the brain. Ahi1 and HAP1 form a stable protein complex in vivo. HAP1 and Ahi1 stabilize each other and are important for maintaining the level of tyrosine kinase receptor B (TrkB) and BDNF signalling, which is critical for neuronal differentiation and cerebellar development.[66]

How HAP1 exactly regulates the vesicular transport is not fully understood. HAP1 clearly interacts with molecular motor accessory proteins which may directly holds cargos forward and backward. It may also likely interact with signaling molecules providing scaffold to interact with GTP-GDP exchangers which are necessary for vesicular fusion, fission and trafficking. HAP1 can interact with proteins such as 14-3-3 and Hrs which are known to have motives which directly interacts with membrane receptor proteins and regulates the membrane receptor trafficking.

HAP1 may Regulates Gene Transcription by Interacting with Transcription Factors

NeuroD (ND). ND is a basic helix-loop-helix transcription factor important for regulation of neuronal development and survival in vertebrates.[67-69] Disruption of ND causes massive cell death in subsets of differentiating and mature neurons.[67-69] ND is also involved in the development and survival of pancreatic β cells and in the transcriptional activation of the insulin gene. Mutations in ND cause diabetes in mice and humans.[67] By a yeast two-hybridization screen, ND was found to interact with Htt, HAP1 and mixed lineage kinase 2 (MLK2).[67] MLK2 is a protein kinase that phosphorylates MKK4/7 and consequently activates the JNK signaling pathway.[23,70-72] MLK2 is also enriched in neurons and associated with Htt.[72] Htt interacts with ND via HAP1, and Htt and HAP1 facilitate MLK2 phosphorylation which stimulates the activity of ND. This mechanism implicates Htt and HAP1 may play a role in transcriptional regulation by ND. This assumption requires further investigation.

TATA-binding protein (TBP). Like HD and SBMA, Spinocerebellar ataxia 17 (SCA17) is also caused by expansions in the polyglutamine (polyQ) repeats in TBP. TBP is a general transcription factor that functions in initiation by all three nuclear RNA polymerase.[73] Nuclear Htt aggregates typically contain proteasome subunits, chaperones and ubiquitin. Aggregates also contain transcription factors, including TBP.[74] Using unbiased two-hybrid screens, TBP is found to interact with HAP1. The binding mapping shows that HAP1 has two regions (amino acids 157 and 261, amino acids

473 and 582) both bind the conserved C-terminal TBP domain.[74] When HAP1 or TBP were expressed independently in COS-7, 293, or Neuro-2a cells, all TBP localizes to the nucleus and all HAP1 assembles into cytoplasmic stigmoid-like bodies (STLBs). When co-expressed, a portion of the TBP was assembled into the HAP1 positive STLBs while the remainder was localized to the nucleus. The HAP1 and TBP binding is not polyQ-length-dependent manner, but removal of the TBP Q repeat can reduce the proportion of TBP that is assembled into STLBs, whereas expansion of the Q (repeat) had no significant affect on TBP subcellular localization.[74] However, whether HAP1 is involved in the expression of the genes regulated by TBP is yet to be determined.

HAP1 Regulates Recycling of Membrane Receptors and is Involved in the Signal Transduction

Recent evidences suggest that HAP1 may regulate the turnover and stabilization of membrane receptors on the cell surface to maintain neuronal responses to neurotransmitters and neurotrophic factors. HAP1 increases the levels of cell surface receptors by inhibition of lysosomal degradation pathway and enhances endocytic recycling pathways. Thus HAP1 may maintain neuronal transmission and neurotrophic functions on developing neurons by regulating receptor reccycling and degradation.

γ-aminobutyric acid type A receptors ($GABA_A$ receptors). γ-aminobutyric acid type A receptors ($GABA_A$ receptors) regulate neuronal excitability by the level of stability on the cell surface.[75,76] Most benzodiazepine-sensitive $GABA_A$ receptors are constructed from α, β and γ2 subunits.[75,76] HAP1 binds the $GABA_AR$ β subunit specifically.[77] Synaptic $GABA_A$ receptors are undergoing clathrin-dependent endocytosis.[78-80] Accordingly, the internalized $GABA_A$ receptor can be either recycled to the cell membrane surface or targeted for lysosomal degradation.[14] During the cell signaling pathway of $GABA_AR$, HAP1 inhibits the receptor lysosomal degradation and at the same time facilitates the receptor recycling back to the cell membrane. In this way, over expressed HAP1 increases $GABA_AR$ cell surface number, therefore increase the neuronal excitability.[77] Suppression of HAP1 by siRNA decreases the level and activity of $GABA_A$ receptors in the hypothalamus. Food intake and body weight of mice was also reduced by the HAP1 siRNA and in HAP1 knockout mice.[16,18] The inhibition of hypothalamic HAP1 expression elevated insulin circulation which in turn crucially decreases hypothalamic activity and feeding activity.[18] These findings suggest that HAP1 might function as a mediator for regulating the activity of hypothalamic $GABA_A$ receptors in control of the feeding behavior.

TrkA. Neurotrophins mainly activate two kinds of cell surface nerve growth factor receptors, the high affinity of tyrosine receptor kinase (Trk) family which includes three members, TrkA, TrkB and TrkC and the low affinity p75 neurotrophin receptor (p75NTR) which is a member of the tumor necrosis factor (TNF) receptor superfamily. In nerve terminals, endocytosis and trafficking of nerve growth factor receptors are essential for synaptic transmission and plasticity. The dynactin $p150^{Glued}$ or kinesin microtubule-dependent transporters participate in receptor internalization at nerve terminals.[25,81] HAP1-A is phoshorylated on the C-terminal site. The phosphorylated HAP1-A binds less dynactin $p150^{Glued}$ and KLC than non-phosphorylated HAP1-A. Mutant Htt can affect kinesin- or dynactin-associated transport[35,82,83] and inhibit neurite outgrowth. HAP1 maintains the normal level of membrane TrkA by preventing the degradation of internalized TrkA. HAP1 deficiency can reduce the level of TrkA and neurite outgrowth.[25] HAP1 also increases the level of TrkB on cell surface by interacting with Ahi1 and regulates the development of cerebellum by maintaining BDNF/trkB signaling.[65]

HAP1 may regulate the turnover of epidermal growth factor receptor (EGFR) which is highly expressed in the developing brain and important for neuronal survival[84,85] and proliferation.[86] Overexpression of HAP1 prevents the trafficking of internalized EGFR from early endosomes to lysosomes, and in turn suppresses ligand-induced degradation of internalized EGFR.[61] Inhibition of HAP1 expression decreases EGRF signaling and cell viability, whereas overexpression HAP1 enhances this signaling activity and inhibits mutant Htt mediated cytotoxicity.[61]

The type 1 inositol (1,4,5)-triphosphate receptor ($InsP_3R1$). The type 1 inositol (1,4,5)-trisphosphate receptor ($InsP_3R1$) is another membrane receptor that also binds to HAP1.[87] $InsP_3R1$ is an intracellular Ca^{2+} release channel which is very important in the neuronal Ca^{2+} signal pathway.[88] Htt could directly interact with the $InsP_3R1$ C-terminus and the binding of Htt to the $InsP_3R1$ C-terminus is dependent on both the presence of HAP1 and the polyQ expansion. Mutant Htt can bind to the $InsP_3R1$ C-terminus either directly or indirectly through HAP1.[89] But the interesting finding is that the functional effects of mutant Htt on $InsP_3R1$-mediated Ca^{2+} release are attenuated in medium spiny striatal neurons (MSN) of HAP1 knockout mice when compared with wild-type mice MSN. Thus, HAP1 potentiates functional effects of mutant Htt on $InsP_3R1$ function in vivo. As already known, increases in neuronal Ca^{2+} represent early events in the pathogenesis of HD.[87,90]

Androgen receptor (AR). Human AR gene has been reported to have a CAG-repeat motif near its 5'-terminus, as Htt, being translated to AR protein with a polyQ sequence near the N-terminus.[91] Another distinct polyQ-neurodegenerative disease, spinal and bulbar muscular atrophy (SBMA) [Kennedy disease or Kennedy-Alter-Sung syndrome (KAS)], is elicits by polyQ AR.[92,93] Like Htt, HAP1 interacts with AR in an AR-polyQ-length-dependent manner in HEp-2 cells cotransfected with HAP1 and/or normal ARQ25, SBMA-mutant ARQ65 or deletion-mutant AR cDNAs, and forms prominent cytoplasmic aggregations sequestering AR. HAP1 has a higher binding affinity with ARQ65 than ARQ25. The overexpressed HAP1 can rescue the SBMA-mutant-ARQ65-induced apoptosis.[93]

HAP1 may Play a Role in the Generation of Inclusion Bodies

A number of reports show that the overexpression of HAP1 in vitro results in the formation of cytoplasmic inclusions,[74,93] suggesting that HAP1 directs assembly of similar cytoplasmic inclusions in neuronal and non-neuronal cell types. In the physiological condition, HAP1 is also found to associate with in large inclusion bodies.[11,15] One of these types of aggregates called Stigmoid Bodies (SBs). The SBs are structures found in the cytoplasm of various types of neurons in the central and peripheral nervous system.[94-96] They are distinct, spherical-to-ovoidal and non-membrane-bound neuronal cytoplasmic inclusions (~0.5–3 μm in diameter) with a granulo-fuzzy texture and moderate-to-low electron density,[97] and are found abundantly in the preoptic, hypothalamic and limbic

forebrain regions of the rat.[98] Although the subcellular functions of SBs have not yet been understood, it is important to know that SBs contain HAP1.[11,15] SBs containing HAP1 also are found to contain the unknown human placental antigen complex X-P2 (hPAX-P2) and apolipoprotein E receptor, SorLA/LR11 and sortilin (two members of the vacuolar protein sorting 10 (VPS10) domain-containing family).[94-96] HAP1 is a core component of the SBs and important for fetal and early postnatal neural development, particularly in the hypothalamic or limbic networks and HAP1/SBs has been assumed to play a protective role against neurodegeneration in HD.[96,99] Whether HAP1 plays any role in the pathological protein aggregation such as in Alzheimer disease, Parkinson disease and Huntington disease is not clear.

Concluding Remarks

In conclusion, HAP1 plays critical role in the trafficking of intracellular organelles and membrane proteins by interacting with a number of proteins. HAP1 acts as an accessary molecule for microtubule associated molecular motors carrying related cargos towards both plus and minors ends of microtubules, maintaining normal cellular functions such as calcium homeostasis, neurite growth, neurotrophic functions, neuronal differentiation and synaptic transmission and plasticity. The polyQ expansion mutations on the N-termini of several proteins such as Htt, AR and TBP may alter the interaction property of HAP1 with these proteins, leading to dysfunctions of intracellular cargo trafficking and neurodegeneration. HAP1 may also participate in the regulation of gene expression by interacting with transcription factors. Further characterization of HAP1 functions will provide precise molecular targets for the treatment of neurodegenerative diseases resulted from these dysfunctions of protein-protein interactions.

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