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Mini Review

ESCRT, autophagy, and frontotemporal dementia

*Jin-A Lee & Fen-Biao Gao**

Gladstone Institute of Neurological Disease and Department of Neurology, University of California, San Francisco, CA, USA

Many age-dependent neurodegenerative diseases are associated with the accumulation of abnormally folded proteins within neurons. One of the major proteolytic pathways in the cell is the autophagy pathway, which targets cytoplasmic contents and organelles to the lysosomes for bulk degradation under various physiological and stressful conditions. Although the importance of autophagy in cellular physiology is well appreciated, its precise roles in neurodegeneration remain largely unclear. Recent studies indicate that components of the endosomal sorting complex required for transport (ESCRT) are important in the autophagy pathway. Reduced activity of some ESCRT subunits leads to the accumulation of autophagosomes and failure to clear intracellular protein aggregates. Interestingly, rare mutations in CHMP2B, an ESCRT-III subunit, are associated with frontotemporal dementia linked to chromosome 3 (FTD3). Mutant CHMP2B proteins seem to disrupt the fusion of autophagosomes and lysosomes in cell culture models. These findings suggest a potential mechanism for the pathogenesis of FTD3 and possibly other neurodegenerative diseases as well. [BMB reports 2008; 41(12): 827-832]

INTRODUCTION

To understand the various neurodegenerative diseases, it is helpful to keep in mind that unlike many other cell types, neurons in the adult brain extend many fine branches, both dendritic and axonal (1). Dendrites participate actively in neuronal function, both as an apparatus for processing neuronal signals and as major sites of synaptic plasticity. The abnormal growth and degeneration of dendrites are closely associated with many neurodegenerative diseases. During normal aging, for example, the number of hippocampal neurons gradually decreases, and the number and length of terminal dendritic branches of remaining neurons increase progressively, probably to compensate for the loss of other neurons (2-4).

In Alzheimer's disease (AD) patients, however, some surviving neurons fail to increase the growth of terminal dendrites (3, 4), suggesting an AD-associated defect in dendritic growth in adult brains. Thus the age-related dementia seen in AD may be due, at least in part, to the failure of neurons to adapt morphologically and functionally to the changing environment (5). Degeneration of dendritic branches in diseased brains has also been well documented (e.g. 6-10) and has been confirmed in some animal models of neurodegenerative diseases (e.g. 11, 12). The exceedingly large surface area of all dendritic fine branches and, in many neurons, their long distance from the cell body, pose challenges in maintaining the structural integrity of neuronal processes during normal aging and pathological conditions.

Endosomal sorting complex required for transport (ESCRT)

One of the important pathways involved in dendritic maintenance is the endosomal-lysosomal pathway, which plays a major role in the homeostatic regulation of transmembrane proteins, such as many receptor-signaling molecules in neurons and many other cell types. Endocytic cargos in early endosomes are either returned to the cell surface or trafficked to lysosomes for degradation. A critical step in the latter process is the formation of multivesicular bodies (MVBs)-late endosomal compartments formed through the inward invagination and budding of vesicles into the lumen of endosomes. MVBs can fuse with lysosomes and deliver transmembrane proteins on the intraluminal vesicles (as well as lipids) for degradation.

The molecular machinery that controls MVB formation has been intensively studied. The first group of proteins essential for MVB formation was identified in *Saccharomyces cereveisiae*. Genetic studies isolated class E vacuolar protein sorting (*vps*) mutants in which large endosomal structures with stacked membranes accumulated and normal MVB formation was disrupted (13). Subsequent molecular analysis revealed that the biogenesis of MVBs was controlled by three multimeric protein complexes, called the endosomal sorting complex required for transport (ESCRT)-I, -II, and -III (14-16). ESCRT-I and ESCRT-II are thought to be soluble heteromeric complexes, while ESCRT-III subunits are soluble monomers until they are recruited to endosomal membranes and form an insoluble lattice.

ESCRT-I contains Vps23, Vps28, Vps37, and Mvb12 (14, 17) and is recruited to the endosomal membrane by Vps27 (18, 19). Vps27 is localized to endosomes and can bind to the endosomal lipid phosphatidylinositol 3-phasphate and interact

*Corresponding author. Tel: 415-734-2514; Fax: 415-355-0824;
E-mail: fgao@gladstone.ucsf.edu

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with ESCRT-I via Vps23. Vps27 also binds to ubiquitin, which serves as a signal to target cargos into the MVB pathway. ESCRT-I bound to early endosomes then recruits ESCRT-II, which contains Vps22, Vps25, and Vps36. ESCRT-I and ESCRT-II appear to work together to cluster and target multiple ubiquitinated cargos into the intralumenal vesicles of MVBs (20). Endosomal ESCRT-II further recruits ESCRT-III components from the cytosol. There are at least six ESCRT-III subunits in yeast and ten in humans (16, 21). One, Vps20, interacts with ESCRT-II and forms a subcomplex with Snf7 before interacting with other subunits at a later stage of ESCRT-III assembly. ESCRT-III components in mammals are also known as charged MVB proteins or chromatin-modifying proteins (CHMPs), which are classified into six subfamilies corresponding to each ESCRT subunit in yeast (22).

Another critical step in MVB biogenesis is the dissociation of ESCRT-III, which is essential for additional rounds of invagination and cargo sorting into MVBs. This step is catalyzed by Vps4, an AAA type ATPase. The microtubule-interacting and transport domain of Vps4 interacts directly with the conserved motifs in the carboxyl termini of the ESCRT-III subunits Did2 and Vps2 as well as related mammalian proteins (23, 24). The vital role of Vps4 is demonstrated by the effects of its loss of function on ESCRT accumulation and mistargeting of MVB cargos (16).

ESCRTs play diverse roles in many biological processes. In addition to their well-established functions in MVB biogenesis, ESCRTs are also involved in retrovirus budding. For instance, HIV-1 Gag proteins interact with Tsg101, an ESCRT-I subunit, and AIP1, a protein that also interacts with components of ESCRT-III (25, 26). This interaction is essential for HIV-1 release from host cells, and related ESCRT-III subunits show a differential effect in this process (27). Interestingly, cytokinesis in animal cells also requires ESCRT-I (28), indicating that ESCRTs play a broad role in cellular processes that require membrane fission and are topologically similar to the invagination and budding of intralumenal vesicles of MVBs.

One study suggested that ESCRT-II was also involved in *bicoid* RNA localization in *Drosophila* oocytes, but this function seems to be independent of its role in the endosomal pathway (29). Components of ESCRT-II and ESCRT-III are also implicated in nuclear functions but this process remains poorly understood (30, 31). Developmental studies indicate that the ESCRT-II subunit Vps25 controls epithelial cell proliferation by regulating Notch trafficking and activity in *Drosophila* and functions as tumor suppressor in a non-cell-autonomous manner (32, 33). Mutations in Tsg101 have a similar effect (32-34). In the nervous system, reduced activity of Shrub, the fly homologue of the yeast ESCRT-III subunit Snf7, leads to increased branching of both dendrites and axons, suggesting that the MVB pathway is essential in neuronal morphogenesis (35). Loss of mSnf7-2 activity causes dendritic retraction and eventual neuronal cell death in mature cortical neurons, indicating a critical role for ESCRT-III in dendritic maintenance and neuronal survival (36).

ESCRT and autophagy

Autophagy (referring to macroautophagy) is an evolutionarily conserved, highly regulated cellular process for bulk degradation of proteins and organelles (37). Cytosolic contents are sequestered by a double-membrane whose origin is unknown, and the resulting vacuoles, called autophagosomes, are delivered to the lysosomes for degradation (Fig. 1). It is unclear how autophagosomes mature and fuse with lysosomes. Some studies suggest that autophagosomes can also fuse with early and late endosomes (38-41). These observations suggest that newly formed autophagosomes fuse with some vesicles from early endosomes and MVBs to form amphisomes. Whether this fusion step is essential for autophagosome maturation is unclear, and the molecular mechanisms have not yet been fully elucidated.

A few recent reports indicate that ESCRT-deficient cells exhibit defects in the autophagy pathway. In rodent cortical neurons, loss of mSnf7-2 activity, an essential ESCRT-III component, leads to the accumulation of autophagosomes as labeled by microtubule associated protein 1 light chain 3-GFP (LC3-GFP) (36). LC3 is the mammalian homolog of the yeast autophagy protein Atg8, and its phosphatidylethanolamine (PE)-modified version (LC3-II) specifically binds to autophagic membranes. Therefore, it is used widely as a molecular marker of autophagosomes, although with some potential caveats (42). Since LC3-GFP puncta induced by a deficiency of mSnf7-2 can be suppressed by the autophagy inhibitor 3-methyladenine (36), these may well indicate autophagosome formation. Another way to examine autophagic activity is to measure the ratio of endogenous LC3-II to LC3-I (42). Indeed, in rodent cortical neurons deficient in mSnf7-2, there is an increase in the LC3-II level and the ratio of LC3-II to LC3-I (36). Moreover, electron microscopy analysis demonstrates a massive accumulation of autophagosomes and multilamellar bodies that resemble those reported by others (36). Since the percentage of LAMP1-positive autophagic compartments is decreased in ESCRT-III-deficient neurons, it seems that ESCRT-III dysfunction leads to abnormal fusion between autophagosomes and endosomal compartments or lysosomes (43).

Similar conclusions were reached independently by another study demonstrating the essential roles of several ESCRT components, such as the ESCRT-I subunit Tsg101, the ESCRT-II subunit Vps22, and ESCRT-III subunit Vps24, in autophagic degradation in HeLa cells (44). Although loss of basal autophagy activity in *atg5* knockout mice causes neurodegeneration (45, 46), these findings suggest that excess accumulation of autophagosomes is detrimental to neuronal survival. However, more studies are needed to dissect the exact functions of ESCRTs in the autophagy pathway at a more detailed molecular level.

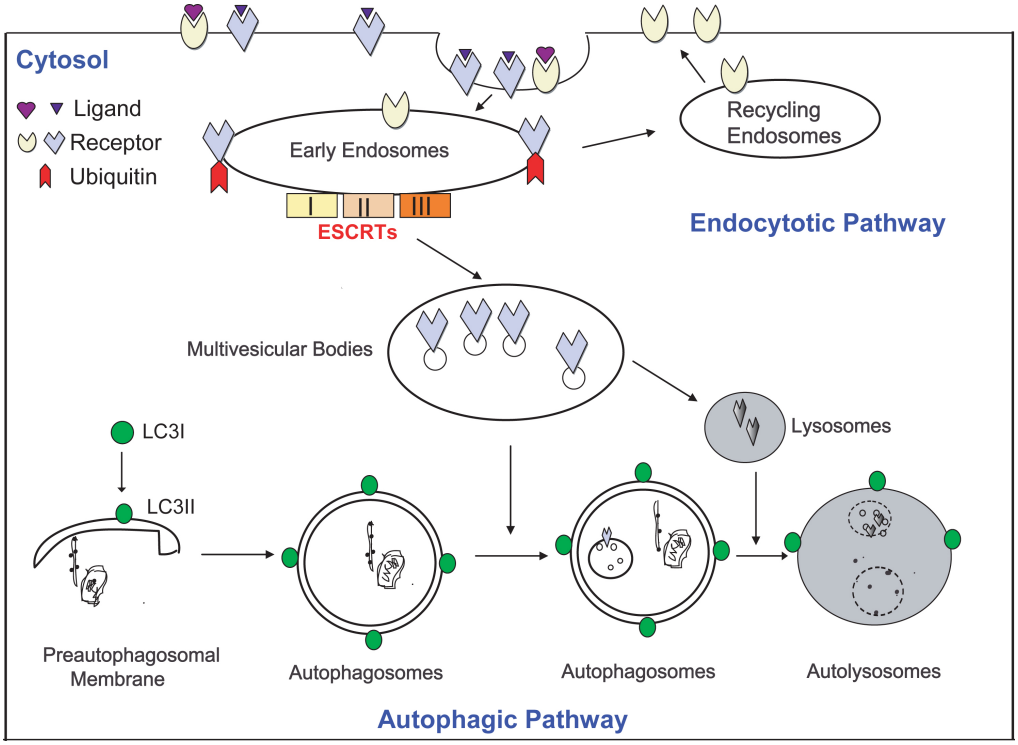

Fig. 1. ESCRTs in the endosomal-lysosomal and autophagy pathways. Transmembrane proteins or receptors are internalized and transported into early endosomes for further cargo sorting. Some cargo proteins can be recycled back to the plasma membrane; others with ubiquitin tags can be sorted into intralumenal vesicles of MVBs by ESCRT-I, -II, and -III protein complexes. Cargos are trafficked into lysosomes for degradation. Cytosolic proteins or organelles can be degraded by the autophagy pathway. Once autophagy is activated, autophagosomes with double membranes or multimembranes are formed. During their formation, LC3-II (modified from LC3-I) is specifically localized to autophagosome membranes. Cytosolic components or cellular organelles are engulfed by autophagosomes that are fused with MVBs and lysosomes for actual degradation. Recent findings show that depletion of ESCRT proteins causes accumulation of autophagosomes, indicating an important role for ESCRT in the autophagy pathway.

ESCRT and frontotemporal dementia

Frontotemporal lobar degeneration (FTLD) is a progressive neurodegenerative condition associated with focal atrophy of the frontal and/or temporal lobes. Frontotemporal dementia (FTD), a major clinical syndrome of FTLD, is now regarded as the most common form of senile dementia in individuals below 65 years of age. The most prominent clinical and neuropsychological features of FTD are changes in personality and behavior, such as poor insight, loss of personal awareness and social awareness, lack of empathy, aggressiveness, inappropriate social behaviors, dietary changes, and the development of a new personality (47).

In some FTD brains, tau neurofibrillary tangles are apparent in some neurons. However, many FTD patients show tau-negative histopathology; therefore, other mutations are also involved in the pathogenesis of FTD. Indeed, in addition to VCP (48), progranulin (49, 50), and TDP-43 (51, 52), a point mutation within *CHMP2B* was identified in 11 affected members of a Danish family but not in unaffected family members or in control populations; this was thought to be responsible for FTD linked to chromosome 3 (FTD3) (53). The presence of this mutation was confirmed by another group (54) and also found in a new branch of the Danish family with several affected members (55).

Human CHMP2B is a protein with 213 amino acids containing a coiled-coil domain near the N-terminus. There is no other known domain near the C-terminus. Its yeast ortholog, Vps2, is a component of ESCRT-III. This point mutation (G to C transition) results in abnormal mRNA splicing and production of mutant CHMP2B proteins lacking the C-terminal 36 amino acids ($CHMP2B^{intron5}$) or with an abnormal 29-amino acid C-terminus ($CHMP2B^{\Delta 10}$) (53). The aberrant mRNAs were much less abundant than the wild-type mRNAs in FTD patient brains (53). A second C-terminal truncation mutation (Q165X) was found in another FTD patient (56), further supporting the notion that CHMP2B mutations are responsible for some FTD cases. However, a different C-terminal truncation mutation (R186X) has been found in non-affected members of an FTD family (57). Thus, the carriers of the R186X mutation have not yet developed symptoms, or not all truncation mutations are pathogenic in vivo. Since mutations in CHMP2B are very rare, identification of additional familial disease-causing mutations

in CHMP2B will provide further human genetic evidence for the importance of CHMP2B and ESCRT-III in FTD.

The neurotoxicity of different mutant CHMP2B proteins has been examined in cell culture models. Ectopic overexpression of $CHMP2B^{intron5}$ in undifferentiated PC12 cells led to the accumulation of vesicular structures (53). A similar phenotype was observed when other CHMP2B C-terminal truncating mutations were expressed in SK-N-SH cells (56). Studies in cultured mature cortical neurons suggested that $CHMP2B^{intron5}$ caused dendritic retraction, autophagosome accumulation, and eventual neuronal cell loss (36). As with the loss of mSnf7-2, the effects of $CHMP2B^{intron5}$ on autophagy were documented with LC3-GFP, Western blot, electron microscopy analysis, and 3-MA inhibition studies (36). $CHMP2B^{intron5}$ appears to block the maturation of autophagosomes in mature cortical neurons (43). Similarly, expression of mutant CHMP2B proteins in HeLa cells led to the accumulation of protein aggregates containing ubiquitin and p62 (44). Although $CHMP2B^{intron5}$ is highly toxic in cultured mature cortical neurons, neither $CHMP2B^{\Delta 10}$ nor CHMP2B containing a missense mutation (D148Y) found in an FTD patient, causes neurotoxicity in this assay (36), suggesting that not all mutant CHMP2B proteins are pathogenic. The finding that knockdown of CHMP2B by siRNA did not cause neuronal cell loss (36) further suggests that simple loss of CHMP2B activity is not responsible for the neurodegeneration seen in FTD3. Biochemical analysis demonstrates that $CHMP2B^{intron5}$ associates with mSnf7-2 more avidly than $CHMP2B^{WT}$. Moreover, a dominant-negative form of the AAA-type ATPase SKD1, the mammalian homolog of the yeast protein Vps4, causes a neuronal phenotype very similar to that caused by loss of mSnf7-2 or ectopic expression of $CHMP2B^{intron5}$ (36). Indeed, structural analysis revealed that the C-terminus of CHMP2B directly interacts with the microtubule-interacting and transport (MIT) domain of Vps4, an AAA type ATPase responsible for ESCRT-III disassembly (58, 59). These findings in neuronal and non-neuronal cells suggest a mechanistic model for CHMP2B neurotoxicity in which dysfunctional ESCRT-III fails to dissociate, leading to abnormal accumulation of autophagosomes and eventual neurodegeneration.

Conclusions and future perspectives

Both human genetics analysis and cell culture studies strongly suggest that some CHMP2B C-terminal truncating mutant proteins are pathogenic in FTD3. Mutant CHMP2B causes neurodegeneration, likely through the failure of dysfunctional ESCRT-III to dissociate properly. Considering the inherent limitations of neuronal cell culture studies, *in vivo* animal models will be useful to further dissect the genetic pathways underlying the pathogenesis of FTD3. Although CHMP2B mutations are very rare among FTD patients, their involvement in the MVB and autophagy pathways may have important implications for FTD and other neurodegenerative diseases in general. Further understanding of CHMP2B neurotoxicity will shed light on the molecular pathogenesis of this horrific disease.

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