Autophagy 9:5, 801–802; May 2013; © 2013 Landes Bioscience

TECPR2

A new autophagy link for neurodegeneration

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Autophagy dysfunction has been implicated in a group of progressive neurodegenerative diseases, and has been reported to play a major role in the pathogenesis of these disorders. We have recently reported a recessive mutation in *TECPR2,* an autophagy-implicated WD repeat-containing protein, in five individuals with a novel form of monogenic hereditary spastic paraparesis (HSP). We found that diseased skin fibroblasts had a decreased accumulation of the autophagy-initiation protein MAP1LC3B/LC3B, and an attenuated delivery of both LC3B and the cargo-recruiting protein SQSTM1/p62 to the lysosome where they are subject to degradation. The discovered *TECPR2* mutation reveals for the first time a role for aberrant autophagy in a major class of Mendelian neurodegenerative diseases, and suggests mechanisms by which impaired autophagy may impinge on a broader scope of neurodegeneration.

Keywords: hereditary spastic paraparesis, autophagy, endosomal trafficking, exome sequencing, MAP1LC3, skin fibroblasts

Submitted: 02/04/13

Revised: 02/11/13

Accepted: 02/11/13

http://dx.doi.org/10.4161/auto.23961

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Punctum to: Oz-Levi D, Ben-Zeev B, Ruzzo EK, Hitomi Y, Gelman A, Pelak K, et al. Mutation in TECPR2 reveals a role for autophagy in hereditary spastic paraparesis. Am J Hum Genet 2012; 91:1065–72; PMID:23176824; http://dx.doi.org/10.1016/j.ajhg.2012.09.015

Autophagy is a major intracellular mechanism for degradation of compromised proteins and organelles in the lysosome, and is essential to maintain cellular homeostasis and survival. Autophagy impairment has been implicated in the pathogenesis of several neurodegenerative and muscle diseases, such as Huntington, Alzheimer and Parkinson diseases, spinocerebellar ataxias (SCAs), and amyotrophic lateral sclerosis. In these diseases, the buildup of protein aggregates leads to a decline in proteasome activity, making nerve cells more dependent on autophagy for degradation, and often resulting in upregulated autophagy.

Neurons are highly vulnerable to impairment of autolysosomal clearance and must continually traffic autophagy-related compartments long distances back to the cell body where substrate clearance by lysosomes is most active. Constitutive autophagy is highly active in motor neurons and has a protective role by preventing the accumulation of cytosolic unsequestered cargo and potentially toxic proteins that lead to disruption of the transport mechanisms in axons. Even a minor inhibition in lysosomal proteolytic activity disrupts the transport of autophagic vacuoles and causes them to selectively accumulate, creating an axonal "traffic jam." Thus, autophagy failure, depending on where the defect is along the pathway, can specifically trigger neuronal cell death and bring about a neurodegenerative phenotype. These mechanisms of decreased autophagosome formation or autophagosomal aggregation and their contribution to neurodegeneration are yet to be further investigated.

Hereditary spastic paraparesis (or paraplegia) is a heterogeneous group of neurodegenerative disorders characterized by axonal degeneration of the corticospinal or pyramidal motor and sensory tracts that control the lower extremities, resulting in progressive spasticity and paralysis of the lower limbs. We recently identified a novel form of recessive HSP (SPG49) in Jewish Bukharians, caused by a single base deletion resulting in a premature stop codon in the gene *TECPR2*, leading to full degradation of the protein.

TECPR2 is an uncharacterized protein that belongs to the tectonin β-propeller

repeat-containing protein family, containing WD (tryptophan-aspartic acid repeat) and TECPR domains, both mammalian-specific, and implicated in protein-protein interactions involved in diseases. TECPR2 shows some similarity to two other human proteins: TECPR1, implicated in selective recruitment of bacteria into the autophagosome, and HPS5, underlying a specific type of Hermansky-Pudlak syndrome involving dysfunction of lysosome-related organelles. This is generally consistent with a role for TECPR2 in the autophagic pathway. More specifically, a recent proteomic analysis of the autophagy interaction network has demonstrated a positive autophagy regulation by TECPR2 via interaction with the six human Atg8 orthologs, including the *MAP1LC3* (LC3) subfamily.

We thus investigated how the mutated TECPR2 protein affects autophagy, potentially resulting in the observed phenotype, by examining the amount and cellular disposition of two endogenous autophagy-related proteins. The first is SQSTM1, a polyubiquitin-binding adaptor protein for autophagy. The second is the ubiquitin-like autophagy-initiation protein MAP1LC3B (LC3B), which upon autophagy initiation transits from the cytosolic form (LC3B-I) to the autophagosome-associated, phosphatidylethanolamine-conjugated form (LC3B-II). Autophagy was induced by nutrient depletion in skin fibroblasts from a patient and from an unrelated healthy control, either in the presence or absence of bafilomycin A_1, which inhibits protein degradation in lysosomes. We found that while starvation alone leads to a slightly decreased amount of both proteins in control cells, the addition of bafilomycin A_1 leads to their considerable increase. In comparison, the patient sample showed an across-the-board diminution of such augmentation of SQSTM1 and LC3B-II levels under all conditions. siRNA-mediated knockdown of *TECPR2* in HeLa cells revealed similar results indicating a major reduction of the bafilomycin A_1-induced LC3B-II, but a much less pronounced effect on SQSTM1. The combined results suggest that the mutated TECPR2 in the spastic paraparesis patients brings about a decreased accumulation of lipidated LC3B and reduces the delivery of LC3B-II and SQSTM1 to the lysosome. This indicates that the autophagy pathway is impaired, but not completely eliminated, as we observe a lower impact on SQSTM1 protein levels. Of note, SQSTM1 is selectively recruited into autophagosomes and therefore even partial autophagic activity may be sufficient for its lysosome delivery.

The core neuropathology of HSP is distal degeneration of the lateral corticospinal tract. Many biochemical mechanisms contribute to axonal integrity within the tract, including mitochondrial function, endoplasmic reticulum shaping, endosomal trafficking, and microtubule stability, affecting both anterograde and retrograde axonal transport. The importance of these allied intracellular membrane transport pathways supports the implication of an autophagic defect in the cellular mechanism of HSP.

One of the rare autosomal dominant forms of HSP (SPG8) is caused by mutations in *KIAA0196*, encoding the protein also referred to as strumpellin. This protein is involved in the fission of endosomes and interacts directly with VCP, a member of the AAA-ATPase family that acts through SQSTM1, with multiple cellular functions including vesicular trafficking and degradation of proteins by the ubiquitin-proteasome system. Missense mutations in *VCP* have been recently identified as causing a late onset form of autosomal dominant complicated HSP.

A form of recessive, early onset complicated HSP (SPG20, Troyer syndrome), with considerable phenotypic similarity to SPG49 that we are studying, with a homozygous frameshift mutation in the gene *SPG20,* leads to degradation of the protein also known as spartin. SPG20 is another member of the AAA-ATPase family, and is implicated in regulating endosomal trafficking and mitochondria function. Taken together, the accumulated data show a strong tie between HSP and intracellular trafficking, and highlight our own findings as a novel implication of yet another such mechanism, that is, autophagy dysfunction, in hereditary spastic paraparesis in particular, and in neurodegeneration in general, thus broadening the scope of autophagy-associated neurodegenerative diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.