FEATURED NEW INVESTIGATOR

Evidence for autophagic gridlock in aging and neurodegeneration

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Autophagy is essential to neuronal homeostasis, and its impairment is implicated in the development of neurodegenerative pathology. However, the underlying mechanisms and consequences of this phenomenon remain a matter of conjecture. We show that misexpression of human tau in *Drosophila* induces accumulation of autophagic intermediates with a preponderance of large vacuoles, which we term giant autophagic bodies (GABs), which are reminiscent of dysfunctional autophagic entities. Lowering basal autophagy reduces GABs, whereas increasing autophagy decreases mature autolysosomes. Induction of autophagy is also associated with rescue of the tauopathy phenotype, suggesting that formation of GABs may be a compensatory mechanism rather than a trigger of neurodegeneration. Last, we show that the peculiar Biondi bodies observed in the choroid epithelium of both elderly and Alzheimer's disease human brains express immunoreactive markers similar to those of GABs. Collectively, these data indicate that autophagic gridlock contributes to the development of pathology in aging and neurodegeneration. (Translational Research 2014;164:1–12)

Abbreviations: AD = Alzheimer's disease; GAB = giant autophagic body; GFP = green fluorescent protein; LAMP-2 = lysosomal-associated membrane protein 2; PBS = phosphate-buffered saline

Pathologic evidence of autophagic dysfunction has been reported in a number of neurodegenerative disorders, including Alzheimer's disease (AD),[1] Parkinson's disease,[2,3] Huntington's disease,[4] and amyotrophic lateral sclerosis.[5] AD brains show extensive involvement of the autophagic machinery in diseased neurons, with abundant, immature autophagic vacuoles, which are otherwise scarce in normal brains.[1,6]

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AT A GLANCE COMMENTARY

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Background

Autophagy is essential to neuronal homeostasis, and its impairment is implicated in the development of neurodegenerative pathology. Using a *Drosophila* model of human tauopathy, we characterize a vacuolelike pathology that we term giant autophagic bodies (GABs), which are reminiscent of dysfunctional autophagic entities and are associated with a gridlock in autophagic flux.

Translational Significance

Although most of the hallmarks of neurodegenerative pathology have been reported in neurons and glia, only a few have been described in the choroid plexus. We propose that GABs are the invertebrate counterparts of the peculiar pathology referred to as Biondi bodies observed in the choroid epithelium of elderly and Alzheimer's disease brains.

Models of neurodegeneration in cell culture, rodents, and flies, among other systems, have shed light on the link between autophagy and neurodegeneration. Although much of this work has focused on the therapeutic potential of autophagy in clearing protein aggregates and ameliorating neurodegeneration,[7-11] there is a relative paucity of mechanistic work *in vivo* focusing on the means by which the autophagic machinery itself is impacted in neurodegeneration.[5] Defects in autophagosome maturation have been found as a consequence of disease-causing mutations in a number of genes, including *charged multivesicular body protein 2b (Chmp2b)*,[12,13] *valosin containing protein (Vcp)*,[14] *presenilin 1*[15] and *ATPase type 13A2 (ATP13a2)*.[16] A similar defect in maturation has not been reported in a tauopathy model nor in aging outside the context of a specific disease protein.

A peculiar feature observed in brains of elderly individuals and those with AD is the presence of Biondi bodies in the choroid epithelium.[17] Although these bodies were first described more than 7 decades ago,[18] their etiology remains unknown. Their strong association with aging pathology[17] is strengthened with the demonstration that they are abundant in a case with multiple mitochondrial DNA deletions.[19] Ultrastructural analysis reveals they are composed of filaments, which stain with thioflavin S, that surround a lipid core and are confined exclusively to the intracellular compartment.[20] Lipofuscin granules, which are residues of lysosomal digestion, are also present.[17] Given the high turnover of cerebrospinal fluid and its recycling by the choroid plexus, we posited that these bodies are a manifestation of a catabolic function such as autophagy. The role of autophagy in the choroid epithelium is poorly understood. We examine the relationship between Biondi bodies and the autophagic machinery in human brain. We then use a *Drosophila* model of human tauopathy to generate similar structures, which we term giant autophagic bodies (GABs). We use a variety of optimized genetic tools in combination with high-resolution microscopy to characterize them *in vivo* and to unravel their association with autophagic flux.

MATERIALS AND METHODS

***Drosophila* stocks and genotypes.** Stocks were maintained in Jazz-Mix medium (Fisher Scientific, Pittsburgh, Pa). All crosses were carried out at 25°C. The *gl*-Tau flies were engineered previously by our lab.[21] The green fluorescent protein (GFP)-tagged *gl*-Tau flies were generated by inserting GFP complementary DNA at the 5′ end of the human tau complementary DNA in the pEx-*gl* expression vector. Microinjection was carried out by BestGene (Chino Hills, Calif); stocks were backcrossed a minimum of 8 generations. The following stocks were obtained from the Bloomington Stock Center: UAS-ATG5.GFP, UAS-LC3.GFP, UAS-Rab5^{S43N}, UAS-Rab7^{T22N}, and UAS-Rab14^{S49N}, and UAS-mCherry.eGFP.ATG8.[22,23] The ATG1$^{\Delta 3D}$ allele was the kind of gift of Dr Thomas Neufeld (University of Minnesota); this is an imprecise excision allele that has a deletion upstream of ATG1 extending into the translation start site.[24]

Immunohistochemistry of choroid epithelium cells. The glomus was dissected out of autopsy cases divided into young (20, 31, 32, 41, and 46 years) and elderly (64, 65, 66, 70, 74, 77, and 90 years) groups. Biondi bodies increased with aging and followed a distribution similar to that described by Wen et al.[17] The tissue was fixed in 10% formaldehyde and embedded in paraffin. Seven-micrometer tissue sections were deparaffinized in 4 changes of xylene for 5 minutes each and then rehydrated through a series of graded alcohols with a final rinse in distilled water. Endogenous peroxides were quenched by soaking sections in 2 changes of 3% H_2O_2 in methanol for 5 minutes each. No antigen retrieval was needed for tau. Citrate (pH 6.0) and heat retrieval were needed for ATG5, and heat retrieval was needed for LC3, lysosomal-associated membrane protein 2 (LAMP-2), and WDFY-3. The slides were washed and placed in the blocking solution (Abcam, Cambridge, Mass), and incubated in the primary antibody at specified concentrations: tau, 1:800

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