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Lysosome size, motility and stress response regulated by fronto-temporal dementia modifier TMEM106B

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ABSTRACT

Fronto-temporal lobar degeneration with TDP-43 (FTLD-TDP) is a fatal neurodegeneration. *TMEM106B* variants are linked to FTLD-TDP risk, and TMEM106B is lysosomal. Here, we focus on neuronal TMEM106B, and demonstrate co-localization and traffic with lysosomal LAMP-1. pH-sensitive reporters demonstrate that the TMEM106B C-terminus is lumenal. The TMEM106B N-terminus interacts with endosomal adaptors and other TMEM106 proteins. *TMEM106B* knockdown reduces neuronal lysosomal number and diameter by STED microscopy, and overexpression enlarges LAMP-positive structures. Reduction of TMEM106B increases axonally transported lysosomes, while TMEM106B elevation inhibits transport and yields large lysosomes in the soma. TMEM106B overexpression alters lysosomal stress signaling, causing a translocation of the mTOR-sensitive transcription factor, TFEB, to neuronal nuclei. TMEM106B loss-of-function delays TFEB translocation after Torin-1-induced stress. Enlarged TMEM106B-overexpressing lysosomes maintain organelle integrity longer after lysosomal photodamage than do control lysosomes, while small TMEM106B-knockdown lysosomes are more sensitive to illumination. Thus, neuronal TMEM106B plays a central role in regulating lysosomal size, motility and responsiveness to stress, highlighting the possible role of lysosomal biology in FTLD-TDP.

Introduction

FTLD is amongst the most prevalent neurodegenerative dementias after Alzheimer's. Symptomatology includes pronounced behavioral changes with altered motivation, personality and/or language function, with pathology in the frontal and temporal lobes (Mackenzie et al., 2010). The largest subset of FTLD exhibits neuronal TDP-43 aggregates. Common inherited causes of FTLD-TDP are repeat expansions in *C9ORF72*, and loss-of-function in the granulin (*GRN*) gene encoding progranulin (PGRN) protein. PGRN is internalized and concentrated in neuronal lysosomes after binding to Sortilin receptors (Hu et al., 2010). In one family, homozygous *GRN* mutation causes a lysosomal storage disorder (Smith et al., 2012). Thus, understanding of FTLD-TDP is intimately connected with the function of the neuronal lysosome.

A genome-wide association study of FTLD-TDP risk demonstrated linkage with polymorphisms near the *TMEM106B* locus (Van Deerlin et al., 2010). Risk association of *TMEM106B* variants was greatest for *GRN* mutation carriers, in which there was a decrease in the frequency of homozygous carriers of the rs1990622 minor allele (Finch et al., 2011; Van Deerlin et al., 2010). The most strongly associated SNP, rs1990622, is tightly linked to a missense T185S variant in TMEM106B (rs1042949), suggesting a protective effect of the p.185T form. This association has been replicated and extended to age of FTLD onset (Cruchaga et al., 2011), to clinical cohorts (van der Zee et al., 2011) and to cognitive change in ALS cohorts (Vass et al., 2011). There is evidence that this allele protects against hippocampal sclerosis pathology in those with Alzheimer's disease (Rutherford et al., 2012).

TMEM106B variation alters PGRN level in human serum (Cruchaga et al., 2011; Finch et al., 2011), but the cell biological basis of this effect is unclear. *TMEM106B* encodes a 274 aa transmembrane protein, and recent publications indicate that TMEM106B is predominantly localized at the endo-lysosomal membrane where it might interact indirectly with PGRN (Brady et al., 2013; Chen-Plotkin et al., 2012; Lang et al., 2012). However, TMEM106B's role in neurons and in lysosomal biology remains poorly defined. A report that appeared online while this manuscript was in preparation (Schwenk et al., 2014), describes TMEM106B interaction with MAP6 and a selective regulation on dendritic retrograde transport.

Recently, there have been advances in understanding lysosomal regulation. The transcription factor EB (TFEB) controls autophagy and lysosomal biogenesis by regulating expression of the Coordinated

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Lysosomal Expression and Regulation (CLEAR) gene network (Sardiello et al., 2009; Settembre et al., 2013). TFEB colocalizes with the mechanistic target of rapamycin complex 1 (mTORC1) on the lysosome. When nutrients are present, phosphorylation of TFEB by mTORC1 inhibits TFEB. Conversely, inhibition of mTORC1 or starvation or lysosomal disruption activates TFEB by promoting dephosphorylation and nuclear translocation (Settembre et al., 2012). TFEB acts to sense lysosome state at the lysosome and as an effector of lysosomal function when translocated to the nucleus. Lysosome-to-nucleus signaling allows organelle self-regulation (Settembre et al., 2012).

We sought to determine whether TMEM106B is present in the neuronal lysosome and whether it has a key role in determining organelle size, motility and stability. We report that depletion of neuronal TMEM106B reduces lysosome size and responsiveness to stress, but increases motility. These findings expand our understanding of lysosomal regulation and suggest that these pathways contribute to FTLD-TDP pathophysiology.

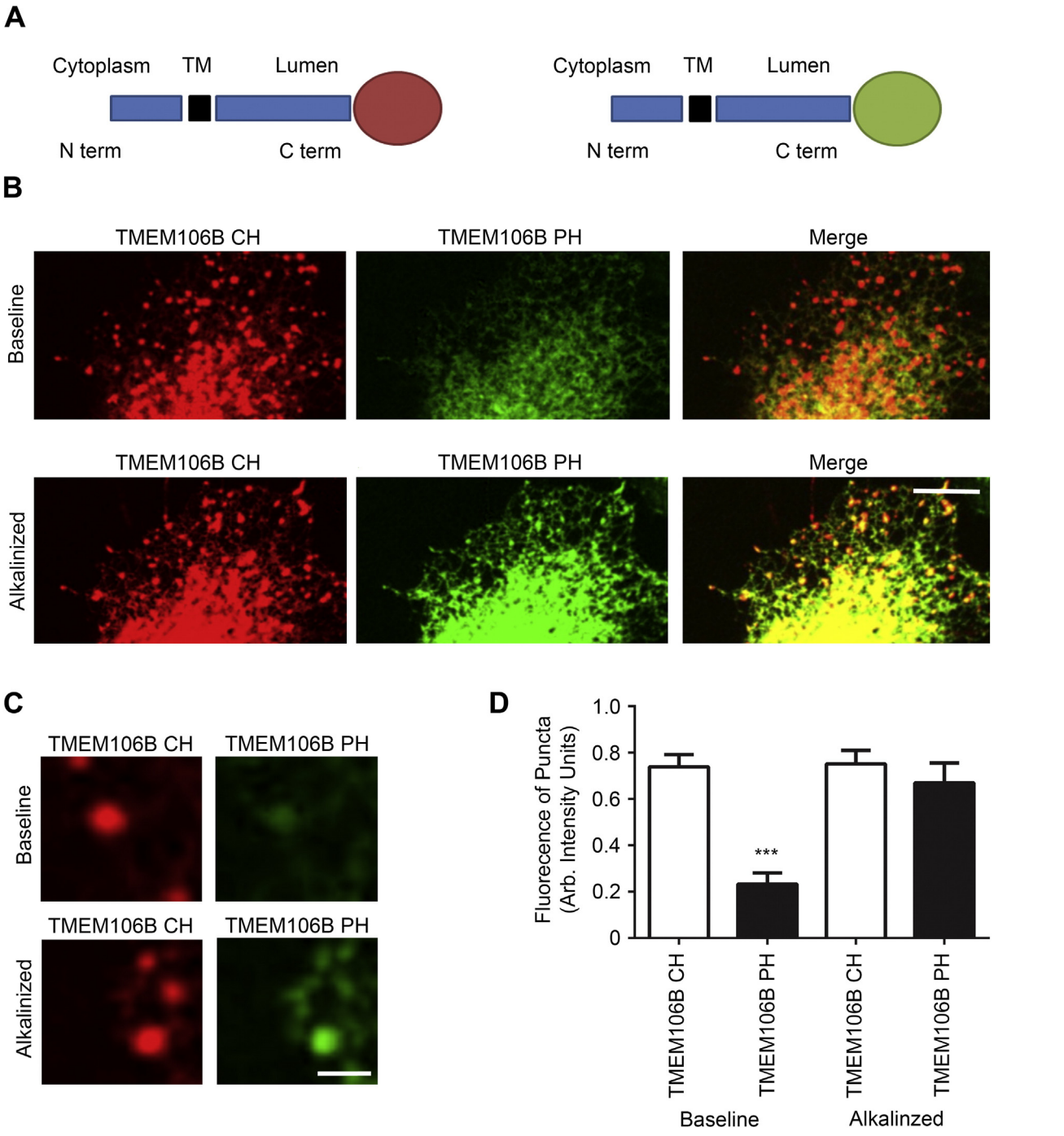

Fig. 1. Topology of TMEM106B. (A) Schematic of fluorescent fusion protein constructs of TMEM106B with Cherry (red) or pHluorin (green) tag and deduced membrane orientation. TM, transmembrane. (B, C) COS-7 cells were transfected with expression vectors for TMEM106B–Cherry (red) and TMEM106B–pHluorin (green) and then imaged in time lapse without fixation 48 h later. Prior to addition of 500 nM bafilomycin A1 and 0.075% (w/v) sodium bicarbonate, the red fluorescence is intense and the green signal is quenched. Twenty minutes after alkalinization of the lysosomal lumen, green fluorescence is increased. Panel C shows a higher magnification of individual puncta from B. Scale bars, 15 μm in B and 2 μm in C. (D) The fluorescence intensity in the Cherry and pHluorin channels from experiments as in B and C was measured for regions generated from a threshold mask in the red channel. Data are mean ± SEM from n = 7 independent experiments. One-way ANOVA with post-hoc pairwise testing relative to vector control, **, $P \leq 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)