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TFG: a novel regulator of ULK1-dependent autophagy

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ABSTRACT

TRK-fused gene (TFG) is a protein implicated in multiple neurodegenerative diseases and oncogenesis. We have recently shown that, under starvation conditions, TFG contributes to spatial control of autophagy by facilitating Unc-51 like autophagy activating kinase 1 (ULK1)-microtubule-associated protein 1 light chain 3 gamma (MAP1LC3C) interaction to modulate omegasome and autophagosome formation. Defective TFG-mediated autophagy could thus be postulated as a possible contributor to ontogenesis or progression of TFG-related diseases.

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TRK-fused gene (TFG) is an endoplasmic reticulum (ER)-to-Golgi resident protein playing a crucial role in intracellular protein trafficking. In particular, TFG localizes to the ER exit sites (ERES) adjacent to the ER–Golgi intermediate compartment, known as the ERGIC, and allows clustering of coat protein complex II (COPII) vesicles between the ER and the ERGIC, helping secretion of cargo proteins.[1]

TFG was first identified as a fusion partner of the nerve growth factor receptor *(NTRK1)* as the result of a chromosomal arrangement that produces the papillary thyroid *TRK-T3* oncogene. Analogously, translocations between the *TFG* and anaplastic lymphoma kinase genes generate a fusion oncoprotein in some lung tumors.[2] Besides cancer, the importance of TFG is also emphasized by the identification of several pathogenic mutations that impair its activity in membrane trafficking and are linked to different neurological disorders, including hereditary motor and sensory neuropathy with proximal dominant involvement (HMSN-P), Charcot–Marie–Tooth disease and recessive hereditary spastic paraplegia.[3] Although the current model proposes that the main TFG function is regulating the integrity of the ER–Golgi interface, novel molecular insights are emerging, offering new hints for understanding the pathophysiology of *TFG*-related diseases. Our work further extends this by showing a link between TFG and autophagy and providing mechanistic details about this interplay and its control.[4] By using a combination of biochemical, molecular, high-resolution and live-cell imaging approaches, we found that *TFG* depletion affects the Unc-51 like autophagy activating kinase 1 (ULK1) steady-state levels and localization during autophagy, leading to the improper formation of both omegasomes and autophagosomes. ULK1 is essential for starvation-induced autophagy and integrates signals from upstream sensors, transducing them to the downstream autophagy pathway. In details, we found that TFG-dependent regulation of both ULK1 distribution andautophagy progression acts via a canonical LC3-interacting region (LIR) motif that mediates TFG interaction with microtubule-associated protein 1 light chain 3 gamma (MAP1LC3C, known as LC3C). Strikingly, while in control conditions and upon autophagy induction, there is an increase in ULK1 *puncta* that co-localize with both ER and autophagy-related 9 (ATG9), when *TFG* is depleted the percentage of ULK1 *puncta* juxtaposed to the ERGIC structures significantly increase. Our findings demonstrate a case for additional layer of complexity to the relationship between the ULK1 complex and ERES/ERGIC compartment, identifying TFG as a crucial player. Supporting this, wild-type TFG, but not an LC3C binding-deficient mutant ($TFG^{mutLIR3}$), rescued the effects on ULK1 canonical protein levels, *puncta* formation and intracellular localization. Intriguingly, by analyzing fibroblasts from a patient carrying the *R106C TFG* variant (previously associated with a complicated hereditary spastic paraplegia phenotype), we observed a defect in both ULK1 *puncta* formation and autophagy, together with the massive presence of "abnormal structures" in the cytoplasm.[4] At a mechanistic level, it remains unknown how the *R106C TFG* mutation causes this defect. One possibility is that the conformational change in TFG ring complexes could impair its ability to regulate ULK1-LC3C interaction, which is a prerequisite for proper autophagy progression. Besides autophagy, some other cellular alterations have been identified in the presence of the *R106C TFG* variant (Figure 1).[4–6]

An increasing number of studies suggest that one specific form of macroautophagy, aggrephagy (elimination of accumulated and aggregated proteins), has a particular relevance in neuropathies.[7,8] In a previous work, by analyzing biopsies from patients with a heterozygous p.P285L mutation in *TFG*, which is known to cause HMSN-P, accumulation of aggregates of TFG itself, TAR DNA-binding protein

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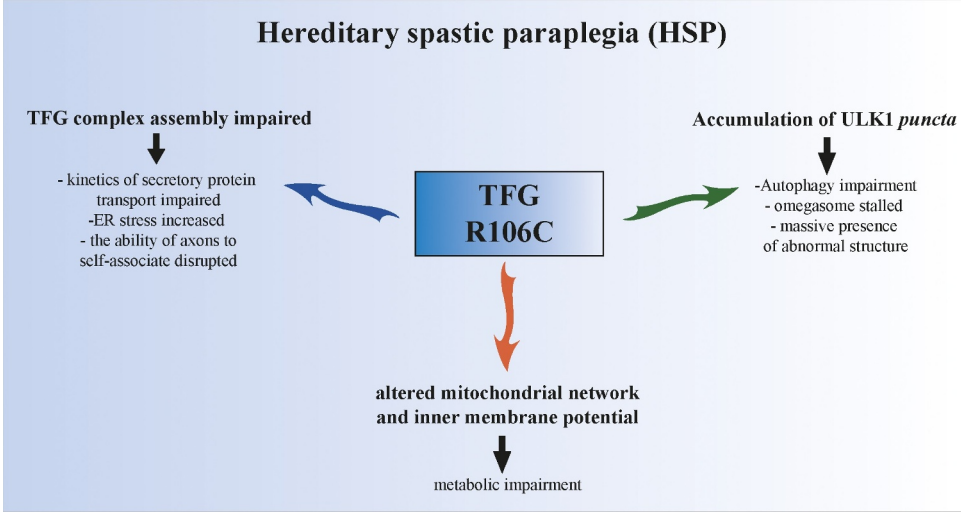

Figure 1. Cellular alterations imposed by TFG R106C. Biochemical characterizations of the mutant protein demonstrated a defect in (i) its ability to self-assemble into an oligomeric complex, and consequently specific defects in secretion from the endoplasmic reticulum (ER) and axon fasciculation; (ii) Unc-51 like autophagy activating kinase 1 (ULK1) canonical turnover and consequently defects in autophagy flux; (iii) mitochondria network and consequently defects in metabolism.

43 (TDP-43), and sequestosome 1 (SQSTM1, best known as p62) is found.[9] Consistent with this, by transmission electron microscopy analysis upon *TFG* depletion and autophagy induction, we have observed the presence of "abnormal structures", containing multiple smaller compartments, multilamellar structures and single-membrane vesicles including electron-dense intraluminal material. It remains completely unknown whether *TFG* mutations could also contribute to defective aggrephagy. Previous studies showed that LC3C promotes aggrephagy and it is involved in the degradation of disease-related protein aggregates also in a neural context.[7,8] Based on these data, it is reasonable to speculate that the TFG-LC3C axis may also be involved in the regulation of autophagic degradation of protein aggregates in a specific cellular context, such as neurons.

In addition to *TFG*, mutations in a number of other genes involved in the autophagy–lysosome pathway are responsible for inherited neurodegenerative disorders, but the degree and manner by which autophagy contributes to the manifestations of these different neurological disorders remain to be established.[10] A process as essential as autophagy for cell homeostasis is indubitably more vital in post-mitotic cells as neurons. The role of TFG in autophagy could thus be central in a novel pathogenic mechanism for *TFG*-associated neuropathies, and our results supports the idea that targeting autophagy may represent a potential approach to counteract, or at least delay, the onset of those specific illnesses. We are just at the beginning of a long journey and most of the current autophagy-based therapies use drugs, such as rapamycin or chloroquine, which are far from being specific. Although further studies are required to fully elucidate autophagy-related TFG role, especially in the nervous system, our report provides new insights on the relevance of autophagy functionality in the pathophysiology of *TFG*-related disease. Unraveling the exact molecular principles could be of great help in developing more specific compounds exclusively target autophagy-related proteins.

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Disclosure of interest

We declare no competing interests and no potential conflicts of interest.

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