Deviant lysosomal K+ fluxes and Parkinson's. A calci-centric point of view.

Martina Gregori, Stephen R. Bolsover and Sandip Patel*

Department of Cell and Developmental Biology,
University College London, Gower Street, London WC1E 6BT

*correspondence to patel.s@ucl.ac.uk

TMEM175 is a lysosomal K^+ channel linked to Parkinson's but little is known on how it is regulated. Wie et al characterised pathogenic variants in TMEM175, correlated channel activity with Parkinson's risk and revealed a novel kinase-independent role for AKT in gating.

The lysosomal lumen is a concentrate of H^+ together with Ca^{2+}, Na^+ and other ions such as Fe^{2+} and Zn^{2+} (1). Pumps, ion channels and transporters peppered throughout the lysosomal membrane support currents that set the lysosomal membrane potential and regulate cytosolic Ca^{2+} levels (1). Failures in this biological circuit underpin lysosomal morphology defects, impaired autophagy and deregulated vesicle trafficking, and have been associated with several diseases (1).

TMEM175 is a non-canonical K^+ channel located in late endosomes and lysosomes (2). Previous work had shown that in TMEM175-deficient cells, lysosomes are depolarised, thus implicating the channel in the regulation of the membrane potential (2). In addition, depletion of TMEM175 led to reduced lysosomal acidification during starvation, compromised lysosomal enzymatic activity, abnormal lysosomal-autophagosome fusion and clearance, as well as impaired mitochondrial respiratory capacity (2,3). TMEM175 is important because genome-wide association studies have linked variants in TMEM175 to risk for developing Parkinson's (4). Wie et al (5) show that similar to TMEM175 knock-out, knock-in cell models harbouring the pM393T variant linked to increased Parkinson's risk accumulate high levels of pathogenic alpha-synuclein (3,5), a hallmark of Parkinson's. Wie et al also provide complementary *in vivo* data showing that the lack of TMEM175 results in the selective loss of nigral dopaminergic neurons and, consequently, poorer motor coordination performances in mice (5). In accord, patients carrying the M393T variant trended towards more rapid cognitive and motor decline in two independent cohorts (5).

At the biophysical level, Wie et al. (5) patch clamped lysosomes dissected from mouse neurons and could readily record TMEM175-dependent K^+ currents but only under nutrient replete conditions. They deduced that activity was due to the presence of growth factors and identified AKT as the protein responsible for relaying the signals from the plasma membrane to TMEM175. This was achieved by the use of both molecular and pharmacological strategies, which showed that the absence of AKT correlated with a lack of substantial K^+ currents. AKT is a ubiquitously expressed Ser/Thr kinase that is activated by a variety of growth factors in phosphoinositide 3-kinase-dependent manner. As such, the binding of AKT to TMEM175 at a consensus site located near the cytosolic end of the channel pore was presumed to underpin kinase activation, with the subsequent phosphorylation and opening of the K^+ channel. This presumption was, however, debunked because kinase-dead AKT was still able to activate TMEM175. Multiple evidence was provided indicating that it was a conformational change in AKT between a closed and an active state that was sufficient to switch on TMEM175.

Wie et al. (5) went on to analyse channel activity of TMEM175 variants associated with Parkinson's. The activity of TMEM175 M393T was reduced consistent with a loss-of-function model underlying increased risk of Parkinson's. However, similar results were obtained with a second variant (Q65P) that *decreases* the risk of Parkinson's. Experiments performed under starvation conditions however showed that whereas activity of wild type TMEM175 and the

M393T variant was lost after 1h (consistent with AKT inactivation), currents through Q65P mutant were not. Results from Q65P knock-in mice also revealed that the channel was more resistant to run down upon nutrient depletion. Thus, the Q65P variant manifests as a gain-of-function in TMEM175 but only in the starved state. In accord, neurons from the mutant mice had much less stress-induced damage upon nutrient limitation than the wild-type counterparts, suggesting that the Q65P is a neuroprotective gain-of-function variant. Exactly how this relates to AKT regulation remains unclear. One possibility is that the Q65P mutation affects TMEM175:AKT dissociation.

So how does TMEM175 loss-of-function underpin the observed phenotypes? Wie et al. (5) suggest that TMEM175 might conduct counterions during lysosomal acidification. Thus, changes in the lysosomal pH could account for the drop in enzymatic activity and subsequent, autophagic anomalies in cells lacking the channel (2,3,5). However, TMEM175-deficient cells only showed an increase in luminal pH upon starvation i.e. when even the normal channel is inactive. This suggests that pH changes are independent of channel activity (2,3,5). AKT is thought to modulate V-ATPase assembly and activity under nutrient depleted conditions (6). Might TMEM175 function as a scaffold to recruit active AKT to the lysosome during starvation, with its absence leading to an impairment of acidification? Perhaps more relevant are TMEM175-dependent changes in membrane potential (2). Depolarisation of the lysosome upon TMEM175 deficiency would reduce the driving force for Ca^{2+} efflux through, for example, TPC2 (7) but would enhance the activity of voltage-gated ion channels, such as $Ca_v2.1$ (reportedly lysosomal; (8)) and TPC1. $Ca_v2.1$ in neurons has been to shown to trigger lysosomal fusion with endosomes and autophagosomes (8). Could Ca^{2+}, therefore, play a role in the acceleration of the autophagic fusion step observed in TMEM175 compromised cells? TPC1-mediated Ca^{2+} release in stressed cardiomyocytes has been suggested to trigger cytosolic Ca^{2+} oscillations, leading to mPTP opening, a key step in cell death (9). Interestingly, mitochondrial Ca^{2+} overload and opening of mPTP, has also been associated with Parkinson's (10). Lysosomal Ca^{2+} release, therefore, may be a key effector of dopaminergic neuronal loss in TMEM175-dependent Parkinson's.

Acknowledgments: Work in our lab is funded by the BBSRC, Parkinson's UK and the Wellcome Trust.

1. Xu H, Ren D. Lysosomal physiology. Annu Rev Physiol [Internet]. 2015 Feb 1 [cited 2021 Apr 24];77:57–80. Available from: https://www-annualreviews-org.libproxy.ucl.ac.uk/doi/abs/10.1146/annurev-physiol-021014-071649
2. Cang C, Aranda K, Seo YJ, Gasnier B, Ren D. TMEM175 Is an Organelle K+ Channel Regulating Lysosomal Function. Cell. 2015 Aug 27;162(5):1101–12.
3. Jinn S, Drolet RE, Cramer PE, Wong AHK, Toolan DM, Gretzula CA, et al. TMEM175 deficiency impairs lysosomal and mitochondrial function and increases α-synuclein aggregation. Proc Natl Acad Sci U S A [Internet]. 2017 [cited 2021 Apr 24];114(9):2389–94. Available from: www.pnas.org/cgi/doi/10.1073/pnas.1616332114
4. Krohn L, Öztürk TN, Vanderperre B, Ouled Amar Bencheikh B, Ruskey JA, Laurent SB, et al. Genetic, Structural, and Functional Evidence Link TMEM175 to Synucleinopathies. Ann Neurol [Internet]. 2020 Jan 18 [cited 2021 Apr 24];87(1):139–53. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ana.25629
5. Wie J, Liu Z, Song H, Tropea TF, Yang L, Wang H, et al. A growth-factor-activated lysosomal K+ channel regulates Parkinson's pathology. Nature [Internet]. 2021 Mar 18 [cited 2021 Apr 24];591(7850):431–7. Available from: https://pubmed.ncbi.nlm.nih.gov/33505021/
6. Collins MP, Stransky LA, Forgac M. AKT Ser/Thr kinase increases V-ATPase-dependent lysosomal acidification in response to amino acid starvation in mammalian cells. J Biol Chem [Internet]. 2020 Jul 10 [cited 2021 Apr 24];295(28):9433–44. Available from: http://www.jbc.org/article/S0021925817489642/fulltext
7. Hockey LN, Kilpatrick BS, Eden ER, Lin-Moshier Y, Cristina Brailoiu G, Brailoiu E, et

al. Dysregulation of lysosomal morphology by pathogenic LRRK2 is corrected by TPC2 inhibition. J Cell Sci. 2015;128(2):232–8.
8. Tian X, Gala U, Zhang Y, Shang W, Nagarkar Jaiswal S, di Ronza A, et al. A Voltage-Gated Calcium Channel Regulates Lysosomal Fusion with Endosomes and Autophagosomes and Is Required for Neuronal Homeostasis. PLoS Biol. 2015 Mar 26;13(3).
9. Davidson SM, Foote K, Kunuthur S, Gosain R, Tan N, Tyser R, et al. Inhibition of NAADP signalling on reperfusion protects the heart by preventing lethal calcium oscillations via two-pore channel 1 and opening of the mitochondrial permeability transition pore. Cardiovasc Res. 2015;108(3):357–66.
10. Zampese E, Surmeier DJ. Calcium, Bioenergetics, and Parkinson's Disease. Cells. 2020;9(9).

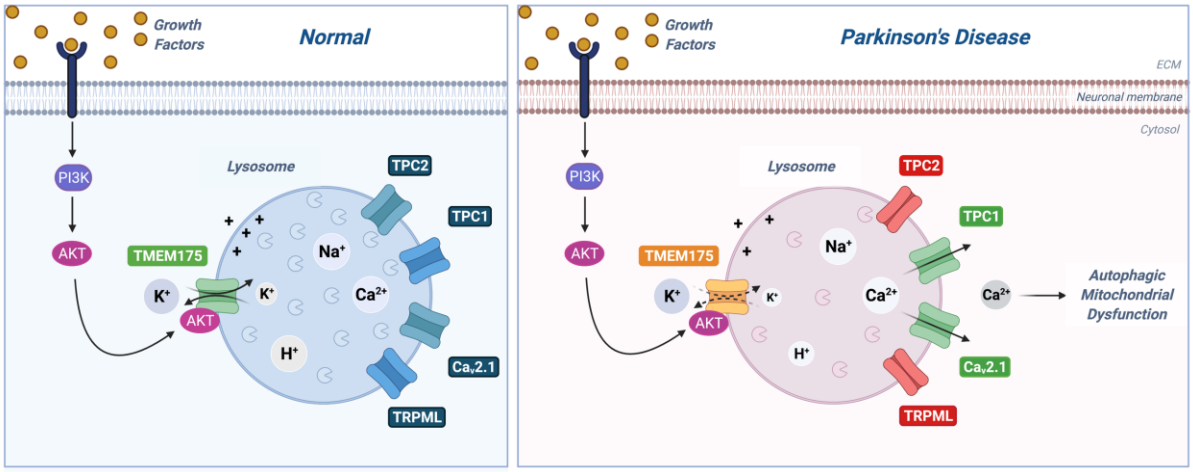

Figure 1. Deviant lysosomal K^+ fluxes and Parkinson's. A calci-centric point of view. Proposed model whereby reduced activity of TMEM175 in Parkinson's depolarises the lysosomal membrane, activates select lysosomal Ca^{2+}-permeable channels and induces Ca^{2+}-dependent dysfunction.