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Lysosomal dysfunction in Parkinson disease

ATP13A2 gets into the groove

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Mutations in *ATP13A2* (PARK9) cause an autosomal recessive form of early-onset parkinsonism with pyramidal degeneration and dementia called Kufor-Rakeb Syndrome (KRS). The *ATP13A2* gene encodes a transmembrane lysosomal P5-type ATPase (ATP13A2) whose physiological function in mammalian cells, and hence its potential role in Parkinson disease (PD), remains elusive. In this context, we have recently shown that KRS-linked mutations in ATP13A2 leads to several lysosomal alterations in ATP13A2 KRS patient-derived fibroblasts, including impaired lysosomal acidification, decreased proteolytic processing of lysosomal enzymes, reduced degradation of lysosomal substrates and diminished lysosomal-mediated clearance of autophagosomes (AP). Similar alterations are observed in stable ATP13A2-knockdown dopaminergic cell lines, which are associated with cell death. Restoration of ATP13A2 levels in ATP13A2-mutant/depleted cells is able to restore lysosomal function and attenuate cell death. Relevant to PD, we have determined that ATP13A2 levels are decreased in dopaminergic nigral neurons from sporadic PD patients. Interestingly in these patients, the main signal of ATP13A2 is detected in the Lewy bodies. Our results unravel an instrumental role of ATP13A2 in lysosomal function and in cell viability. Altogether, our results validate ATP13A2 as a likely therapeutic target against PD degeneration.

Keywords: Parkinson disease, ATP13A2, autophagy, lysosome, neurodegeneration

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Emerging evidence indicates that alterations in autophagic degradation pathways may contribute to the pathogenesis of several neurodegenerative diseases, including PD. For instance, depletion of intraneuronal lysosomes, accumulation of undegraded AP and decreased levels of lysosomal-associated proteins have been observed in post-mortem PD brain samples and in different mouse models of PD-related neurodegeneration. In the latter, pharmacological or genetic restoration of lysosomal-mediated degradation results in increased AP clearance and attenuated dopaminergic cell death. Among the various genes implicated in familial forms of parkinsonism, the *ATP13A2* gene may represent the first genetic link between lysosomal pathways and parkinsonism. Missense or truncation mutations in the *ATP13A2* gene are believed to exert their pathogenic effect by causing a loss of ATP13A2 function due to impaired targeting of ATP13A2 to lysosomes. Indeed, mutant ATP13A2 is retained in the endoplasmic reticulum, thereby resulting in a deficiency of ATP13A2 in lysosomes (**Fig. 1**). Since its discovery, research efforts on *ATP13A2* have sought to define the range of phenotypes involving mutations in *ATP13A2*. However, a functional link between ATP13A2 mutations/defects and the lysosome has not been formally established.

To this end, we have investigated whether defects in ATP13A2 may cause lysosomal dysfunction. We first explored

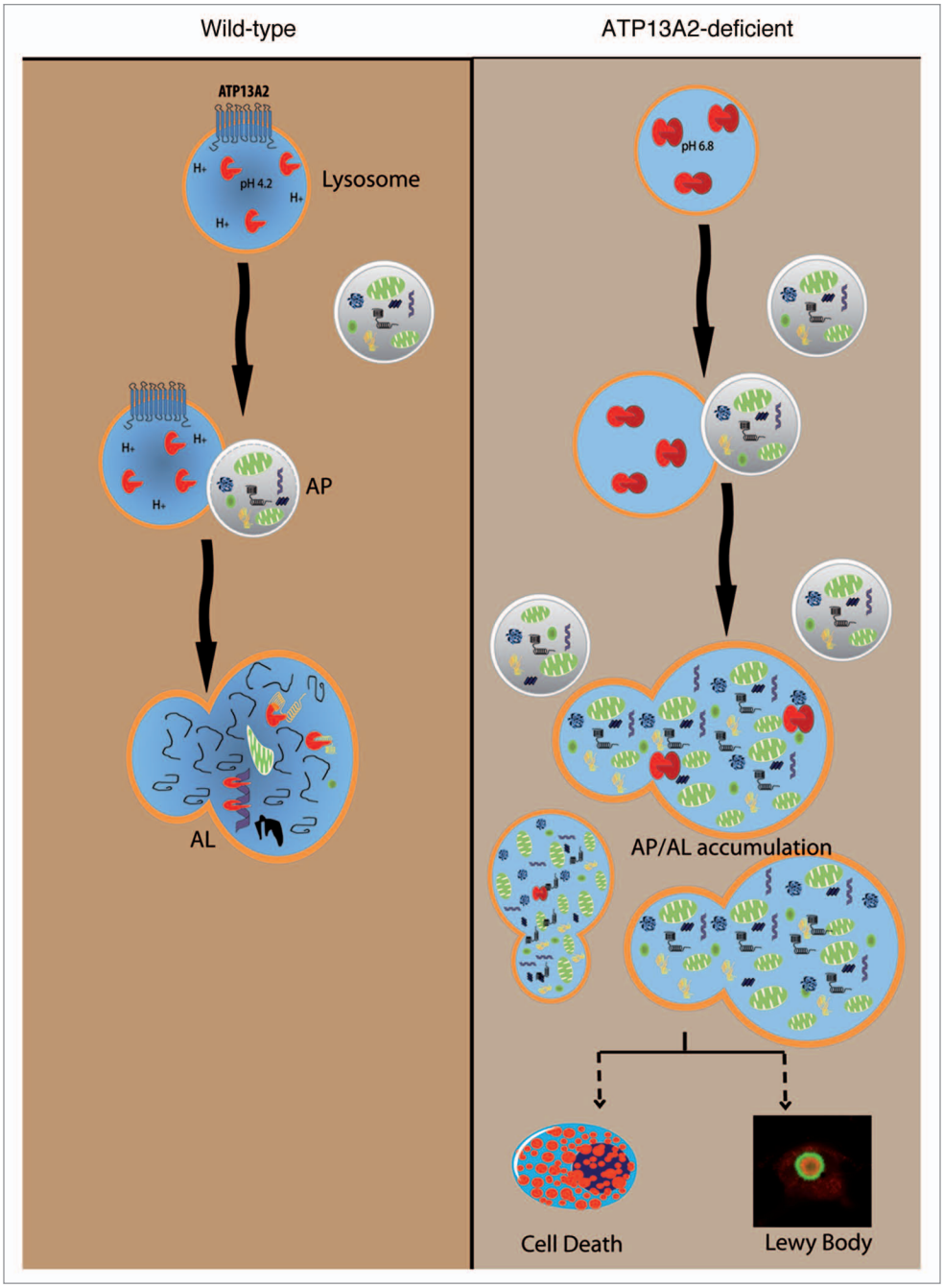

Figure 1. Pathogenic consequences of ATP13A2 deficiency. In wild-type cells, ATP13A2 localizes in the lysosomal membrane where it is associated with adequate lysosomal acidification and function. In contrast, mutations or genetic depletion of ATP13A2 leading to ATP13A2-defective lysosomes result in decreased lysosomal acidification, impaired proteolytic processing of lysosomal proteases, reduced degradation of lysosomal substrates (e.g., SNCA) and diminished clearance and subsequent accumulation of undegraded AP and/or AL. These alterations are associated with cell death and might contribute to the formation of Lewy bodies (Bottom right image: in green, SNCA; in red, ATP13A2 immunofluorescence in a substantia nigra dopaminergic neuron from a post-mortem brain of a PD patient). AP, autophagosome; AL, autolysosome.

potential defects in the autophagy-lysosomal pathway in cultured fibroblasts derived from KRS patients harboring *ATP13A2* mutations. Compared with control fibroblasts, mutant ATP13A2 fibroblasts exhibit several lysosomal alterations, such as impaired lysosomal acidification, decreased maturation of lysosomal enzymes, reduced degradation of lysosomal substrates, increased susceptibility to lysosomal membrane permeabilization and defective clearance and subsequent accumulation of undegraded AP and/or autolysosomes (AL) (**Fig. 1**). Similar alterations were also observed in human dopaminergic BE-M17 neuroblastoma cells depleted of ATP13A2 by an shRNA approach to mimic ATP13A2 deficiency/loss-of-function. In both ATP13A2 mutant or defective cells, impaired lysosomal proteolysis results in

a marked accumulation of macroautophagy and chaperone-mediated autophagy (CMA) substrates, including SNCA/α-synuclein. Moreover, in ATP13A2-depleted cells, lysosomal alterations are associated with cell death. Remarkably, all lysosomal deficits and the cell death phenotype induced by ATP13A2 knockdown are rescued by overexpression of wild-type ATP13A2. Interestingly, lentiviral vector-mediated ATP13A2 knockdown in primary mesencephalic dopaminergic neurons results in specific dopaminergic neurodegeneration, further confirming a deleterious effect of ATP13A2 loss of function on cell viability. In addition, cell death induced by ATP13A2 knockdown is greatly enhanced by overexpression of SNCA. The latter observation concurs with previous reports in *C. elegans* in which ATP13A2 knockdown enhances SNCA misfolding, whereas ATP13A2 overexpression protects dopaminergic neurons against SNCA-induced neurodegeneration. Also in agreement with our results, a link between ATP13A2 deficiency, lysosomal dysfunction, SNCA accumulation and neurotoxicity has been independently demonstrated in a parallel study by the Krainc group. Overall, our data highlight the peculiar sensitivity of dopaminergic neurons to loss of ATP13A2 function, either on its own or in combination with toxic or genetic stressors.

Relevant to PD, we found that ATP13A2 protein levels are decreased in dopaminergic nigral neurons from post-mortem PD brains, thus suggesting that dose-dependent defects in ATP13A2 may also occur in PD patients. Furthermore, we identified ATP13A2 as a component of Lewy bodies. In particular, ATP13A2 is located in the core of Lewy bodies where it is surrounded by more peripherally located SNCA. The presence of ATP13A2 within Lewy bodies raises the possibility that these intracellular inclusions, whose mechanisms of formation and significance for the disease process remain unclear, may seed around lysosomes or undegraded AP and grow in size by the continuous deposition of undegraded AP as the disease progresses. Consistent with this hypothesis, some patients with Gaucher disease, the most common lysosomal storage disorder resulting from the inherited deficiency of the lysosomal enzyme glucocerebrosidase (GBA), exhibit clinical parkinsonism and SNCA-immunoreactive Lewy bodies. In addition, similar to ATP13A2, GBA is present in the core of Lewy bodies in Gaucher-linked PD as well as in sporadic PD cases.

In summary, our study unravels a pivotal role of ATP13A2 defects in lysosomal function and cell viability and underscores the therapeutic potential of modulating ATP13A2 levels in the context of PD-related neurodegeneration.