TFEB
Pathogenic role and therapeutic target in Parkinson disease

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Parkinson disease (PD) is characterized by the progressive loss of nigral dopamine neurons and the presence of accumulations containing the disease-causing protein SNCA/α-synuclein. Here we review our recent findings describing how SNCA impairs the function of the master regulator of the autophagy-lysosomal pathway (ALP), the transcription factor EB (TFEB), and that genetic or pharmacological stimulation of its activity promotes protection of dopamine neurons. These findings suggest that strategies aimed at enhancing autophagy-mediated degradation of SNCA may hold great promise for disease intervention in PD.

Parkinson disease is a neurodegenerative disorder characterized by the progressive death of nigral dopamine neurons and the presence of α-synucleinopathy, i.e., Lewy bodies and neurites. Histological and genetic observations have established a strong link between SNCA and PD. In physiological conditions, SNCA is continually degraded by chaperone-mediated autophagy, macroautophagy (Fig. 1A) and the ubiquitin-proteasome system, while in pathological conditions SNCA can impair all three pathways. In addition, studies showing reduced expression of autophagic markers in the human PD brain or nigral neurodegeneration following conditional ablation of key autophagic protein support the idea that PD pathogenesis may result, at least in part, from a progressive defect in protein clearance pathways. A dominating but yet unproven hypothesis is that upregulation of macroautophagy might provide protection when chaperone-mediated autophagy and the proteasome system are already compromised, but no major neuronal loss or symptoms have developed. To try to address this question, we used an adenovirus-associated viral (AAV) vector to induced high or low expression of SNCA in nigral dopamine neurons in vivo resulting in marginal or profound cell loss, respectively. We demonstrated that survival of dopamine neurons is associated with an increased expression of autophagic markers (Fig. 1B). As SNCA continues to accumulate, the autophagy pathway starts to fail, leading to the aggregation of the disease-causing protein and subsequently to neurodegeneration (Fig. 1C). These dynamic changes prompted us to examine the subcellular localization of the key ALP regulator TFEB. Strikingly, we found that when high levels of SNCA are reached, TFEB is no longer able to translocate into the nucleus and is bound to SNCA (Fig. 1C). We found that this cytoplasmic retention of TFEB occurs not only in our rodent model, but its expression is altered also in nigral dopamine neurons in the human PD brain such that it is less frequently observed in the nucleus and it colocalizes with SNCA in Lewy bodies. These observations strongly suggest that the ALP-related gene program regulated by TFEB may be progressively compromised in response to elevated levels of SNCA.

These findings led us to hypothesize that strategies aimed at compensating for the defect in protein clearance may promote sustained stimulation of autophagy and provide neuroprotection. Consistent with this idea, we showed that AAV-mediated overexpression of
TFEB prevents the loss of nigral dopamine neurons and that this is accompanied by a reduced accumulation of toxic SNCA oligomers (Fig. 1D). In line with a previous report, a similar effect was also achieved with gene delivery of the autophagy initiator BECN1. In order to study the therapeutic effect of TFEB in a clinically relevant situation, we used a pharmacological approach to induce autophagy through inhibition of the mechanistic target of rapamycin (MTOR) using the FDA-approved derivate of rapamycin, temsirolimus (CCI-779). Delayed treatment, starting 3 weeks after AAV-SNCA delivery, effectively blocks the progression of the disease. Notably, this disease-modifying action is mediated by an increased nuclear translocation of TFEB, and prevents further accumulation of toxic SNCA aggregates (Fig. 1D).

Previous studies have shown that specific ablation of key autophagy-related proteins in dopamine neurons in vivo results in PD-like neurodegeneration, but the consequence of TFEB manipulation on the survival of dopamine neurons has so far not been explored. Since micro-RNAs (miRs) are endogenous regulators of many physiological functions, we examined the effect of MIR128 overexpression, a negative regulator of TFEB. We used an AAV vector to overexpress MIR128 in midbrain dopamine neurons, and showed that MIR128-mediated repression of TFEB exacerbates the toxicity of SNCA by repressing autophagy and consequently favoring the formation of toxic oligomers.
We then raised the interesting question of whether autophagy may be one of the mechanisms underlying the differential vulnerability between the dopamine neurons in the substantia nigra and those located in the ventral tegmental area (VTA). Similar to the reduced vulnerability of VTA neurons observed in the human PD brain, this neuronal population is more resistant to SNCA toxicity. We found that the VTA neurons maintain an enhanced autophagic function also in the presence of high SNCA levels, and that \textit{MIR128}-mediated repression of TFEB is sufficient to make SNCA toxic for this neuronal population. In support of this hypothesis, we made the interesting observation that TFEB expression is unchanged in the VTA neurons in the human PD brain, which is in contrast to the reduced nuclear localization seen in the nigral dopamine neurons.

In conclusion, our data highlight the link between the failure of the autophagic-lysosomal system and impaired TFEB function in PD pathogenesis and point to TFEB as an interesting therapeutic target for neuroprotection and disease intervention in PD. The results moreover provide initial evidence for the involvement of autophagy in the resistance of VTA dopamine neurons, and suggest the possibility that miRs may act as important players in the pathogenic process of PD, as well as in the regulation of autophagy in response to toxic insults.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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