PERSPECTIVE

Targeting BPOZ-2 in Lewy body disease

Formation of Lewy body inclusions (LBs) in the substantia nigra (SN) is a very well-characterized pathological hallmark of Parkinson's disease (PD). LBs are aggregates of many biologically inactive proteins including structural elements, alpha-synuclein (asyn)-binding proteins, synphilin-1-binding proteins, and components of the ubiquitin-proteasome system, proteins implicated in cellular responses, proteins associated with phosphorylation and signal transduction, cytoskeletal proteins, cell cycle proteins, cytosolic proteins and many more. So far almost 76 proteins have been found to be associated with the formation of LBs (Beyer et al., 2009). Deposition of LBs in the cell bodies and neurites often leads to the metabolic impairment and eventually the death of dopaminergic (DA) neurons. Therefore, lowering the burden of LBs is a potential challenge to restore the health and function of DA neurons. The most important strategy for minimizing the accumulation of LBs is preserving mitochondrial homeostasis. In a healthy neuron, mitochondrial biogenesis (mitogenesis) is required for the production of ATP, neurotransmitter release, calcium buffering, and augmentation of synaptic strength. However, during neurodegenerative condition, increased mitogenic response might bring opposite effect as it leads to the production of more depolarized mitochondria, reactive oxgen species (ROS), different inflammatory mediators including p38MAPK, Ras, Raf; and eventually the development of LB pathology. Therefore, tight regulation between mitochondrial biosynthesis and its breakdown is critical for the maintenance of neuronal health and function (Palikaras and Tavernarakis, 2014). Recently, our lab has delineated the role of a novel ankyrin-rich, BTB domain containing protein BPOZ-2 or ABTB2 in LB disease (Roy et al., 2016), which can also be a crucial regulator of mitochondrial quality control process. Our current perspective highlights the importance of BPOZ-2 inregulating mitochondrial damage control process and its therapeutic prospect in the treatment of LB disease.

BPOZ-2; a traditional Cullin3(Cul3)-activating protein: BPOZ-2 is a 1,025 amino acid-long scaffold protein with C-terminal 110 *aa*-long *BTB* domain (836th–946th *aa*) and four consecutive ankyrin-rich "*ANK*" motifslocated between 498th to 670th *aa*. Each *ank* motif is composed of two anti-parallel helices with one beta hairpin and serves as an important mediator of protein-protein interaction. Early literatures suggest that BTB domain of BPOZ-2 binds and activates Cul3 ubiquitin ligase (Furukawa et al., 2003; Xu et al., 2003), which in turn ubiquitinates different proteins involved in cell-cycle progression. Therefore, the activation of BPOZ-2-Cul3 pathway has been implicated ingrowth suppression and anti-tumorigenic response (Wilkins et al., 2004). However, its role in neurodegenerative disease had never been explored.

Recently, the role of Cul3-ubiquitin ligase proteins had also been highlighted in Parkinson's disease as a major ubiquitinating enzyme of nuclear respiratory factor 2 (*nrf2*) protein. A similar BTB-domain containing protein, Keap-1 complexed with Cul3 ubiquitin ligase, ubiquitinates and degrades nrf2 (Furukawa and Xiong, 2005) in DA neuron (Ramsey et al., 2007). The function of nrf2 has been shown to stimulate mitochondrial biogenesis by maintaining its redox and energy homeostasis. Therefore, the activation of *Keap-1*/Cul3 complex seems to have anti-mitogenic response in neurons. On the contrary, keap1-knockout mice unexpectedly displayed significantly higher rates of ROS production compared to non-transgenic animals (Dinkova-Kostova and Abramov, 2015) suggesting that BTB-domain containing protein is an essential component of mitochondrial damage control process. The deletion of BTB-containing protein followed by excessive mitogenic response could backfire, which triggers more ROS production, inflammation, and eventually paralysis of the essential cellular metabolic processes (**Figure 1**). Nevertheless, the deletion of BTB-containing protein and the associated risk of mitochondrial damage would further worsen if there is pre-existing LB pathology (**Figure 1**). Augmentation of uncontrolled mitogenic response in this case would simply generate more number of depolarized or damaged mitochondria and accelerate neuronal injury. According to our recent finding, we identified a BTB-domain containing protein BPOZ-2 in the nigral dopaminergic neurons (Roy et al., 2016). We observed that the expression of this protein was strongly downregulated in the presence of MPP^+, an etiological toxin of PD (Roy et al., 2016) as well as with the established asyn pathologies in the nigra of 8-9 months old A53T-tg mouse brain (Roy et al., 2016). Interestingly, lentiviral overexpression of BPOZ-2 significantly ameliorated asyn pathology in A53T-tg nigra suggesting that re-establishing BPOZ-2 expression has been beneficial in restoring health of degenerative DA neurons. Moreover, while analyzing the effect of nullifying endogenous *bpoz-2* gene in the nigra of 8–9 months old A53T-tg mouse brain, we confirmed that the depletion of endogenous level of *bpoz-2* gene by administration of lentiviral *bpoz-2* shRNA had worsened the asyn pathology indicating that endogenous *bpoz-2* could be an important factor of cellular damage control process. Next, we monitored the effect of *bpoz-2* gene delivery on the synthesis and release of dopamine level in the nigra of A53T-tg mouse brain. Consistently, we observed that the depletion of endogenous *bpoz-2* inhibited and the overexpression of *bpoz-2* gene significantly restored the level of dopamine in the degenerating nigral neurons. Since, dopamine production and release is directly correlated with the mitochondrial health (Stack et al., 2008), our observation implies that *bpoz-2* could play an important role in preventing mitochondrial injury. Therefore our observation regarding the downregulation of BPOZ-2 expression inthe presence of asyn pathology simply could be a compensatory response to restore the health and number of mitochondria. However, this response would eventually end up producing more depolarized mitochondria because of established LB pathology and exacerbates alpha-synucleinopathy. Overexpression of *bpoz-2* gene could be involved in the inhibition of faulty mitogenic response and thereby halt the pathology progression.

BPOZ-2; a novel PINK1-regulating protein: We also established a novel and even more direct role of BPOZ-2 in controlling mitochondrial damage control process. Although the precise role is not known, our detailed immunoprecipitation (IP) followed by immunoblot (IB) assay revealed that BPOZ-2 has a direct association with PTEN-induced kinase 1 or PINK1. In healthy mitochondria, PINK-1 had been shown to be involved in ATP production by stimulating complex I of mitochondrial electron transport system (ETS) (Morais et al., 2009). PINK1 is transported from cytosol to mitochondrial inner membrane by a synchronized action of outer and inner membrane-associated translocase enzymes known as TOM and TIM respectively (Jin and Youle, 2012). However, the loss of inner membrane potential of mitochondria due to neuronal injury leads to the accumulation of PINK1 in the outer membrane

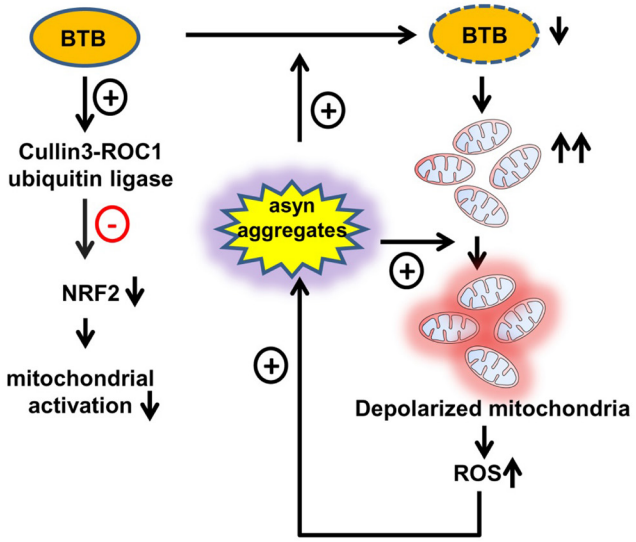

Figure 1 Mitochondrial damage control process by BTB-proteins.
BTB-domain containing protein is a conventional regulator of ubiquitin ligase cul3. Activation of cul3 inhibits nrf2 and subsequent mitochondrial activation and mitogenesis. Emergence of asyn pathology inhibits the expression of BTB-domain containing protein, upregulates mitochondrial activation, may induce the production of more depolarized mitochondria and subsequent ROS production, which again might aggravate asyn pathology. ROS: Reactive oxgenspecies; BTB: for BR-C, ttk and bab.

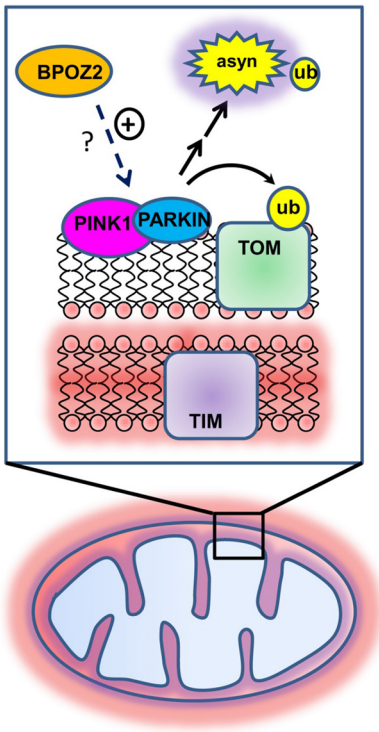

Figure 2 Cross-talk between BPOZ-2-PINK1 in neurodegenerative condition.
PINK1-Parkin complex ubiquitinates mitochondrial outer membrane proteins and triggers mitochondrial breakdown during neurodegenerative condition. In adult A53T-tg brain, we observed that there was a loss of interaction between PINK1 and BPOZ-2. Overexpression of *bpoz*-2 restores its interaction with PINK1, which might play a crucial role in the amelioration of asyn pathology. TIM: Translocase of the inner mitochondrial membrane; TOM: translocase of the outer mitochondrial membrane.

followed by a complex formation with parkin and ubiquitination of multiple outer membrane-associated proteins (**Figure 2**). Finally, these events lead to the turn-over of mitochondria *via* autophagic pathway. Recent studies have shown that mitophagic upregulation is often associated with the amelioration of asyn. It is not known how crosstalk between BPOZ-2 and PINK1 is important in the mitochondrial autophagy process. However, we observed that BPOZ-2-PINK1 association is directly involved in the amelioration of alpha-synucleinopathy. Our immunoprecipitation studies successfully established that there was an association of PINK1 with asyn *in vivo* in the nigra of A53T-tg mice and that association increased with the overexpression of *bpoz-2* gene suggesting that BPOZ-2 might be involved in the increased interaction between PINK1 and asyn and the resulting amelioration of alpha-synucleinopathy. In order to understand BPOZ-2-mediated regulation of PINK1, our future aim is to understand how BPOZ-2-PINK1 association is crucial for the induction of mitophagic process and subsequent amelioration of alpha-synucleinopathy.

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Avik Roy*

Department of Neurological Sciences, Rush University Medical Center, Chicago, IL, USA

***Correspondence to:** Avik Roy, Ph.D., avik_roy@rush.edu.*
***Accepted:** 2016-05-24*
***orcid:** 0000-0003-0523-8504 (Avik Roy)*
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