Emerging roles of NRBF2/PI3KC3 axis in maintaining homeostasis of brain and guts

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NRBF2 has been identified as the fifth component of PI3KC3 complex and is required for maintaining the kinase activity to promote autophagy. However, the physiological and pathological roles of NRBF2 are largely unknown. In our recent studies, we have revealed that NRBF2 plays an important role in preventing the Alzheimer's disease (AD) and inflammatory bowel disease (IBD) development, via the mechanisms involving regulating autophagosome maturation and phagosome maturation. The findings expand our understanding towards the physiological role of PI3KC3 complex and provide a potential strategy for AD and IBD treatment by regulating PI3KC3 complex activity.

PI3Ks are a family of lipid kinases capable of phosphorylating the 3-position hydroxyl group of the inositol ring of phosphatidylinositol (PI). PIK3C3 is the catalytic subunit of the class III PI3K (PI3KC3) which phosphorylates PI to generate phosphatidylinositol 3-phosphate (PI(3)P). Membrane-bound PI(3)P recruits its binding proteins to regulate membrane trafficking processes including autophagy and endocytosis. Mammalian PI3KC3 consists of three core component proteins PIK3C3, PIK3R4 and BECN1. The core components bind different proteins such as Atg14L, UVRAG, Rubicon and Ambra 1 to form subcomplexes of PI3KC3 and regulate distinct cellular functions. For example, complex 1 (PIK3C3-PIK3R4-BECN1-ATG14L) specifically regulates autophagy initiation, while complex 2 (PIK3C3-PIK3R4-BECN1-UVRAG) is required for autophagosome maturation as well as endocytosis (Funderburk et al., 2010). Three reports have independently revealed that NRBF2 exists in the ATG14L-containing PI3KC3 complex that regulates autophagy biogenesis (Cao et al., 2014; Lu et al., 2014; Zhong et al., 2014).

NRBF2 was initially identified as a BECN1 interacting protein which forms stable complex with PIK3C3-PIK3R4-BECN1-ATG14L (Lu et al., 2014). Functional studies revealed that NRBF2 is involved in regulating autophagy biogenesis (Cao et al., 2014; Lu et al., 2014; Zhong et al., 2014). NRBF2 contains 287AA with two noticeable domains: the MIT (microtubule interacting and trafficking) and CCD (coiled-coil domain) domain (Lu et al., 2014). Functional study revealed that MIT domain of NRBF2 is indispensable for PIK3C3 kinase activity and autophagy, while the CCD domain is not essential. Biochemically, NRBF2 directly interacts with ATG14L via MIT domain and probably also interact with PIK3R4 to facilitate the assembly of PI3KC3 complex I by holding ATG14L-BECN1 and PIK3C3-PIK3R4. In yeast, through the similar manner, Atg38 stabilizes the interaction between Atg14-Vps30 and Vps34-Vps15 by directly interacting with Atg14 and Vps34. The structure of PI3KC3 complex 1 without NRBF2 has been resolved and a V-shape like structure is revealed. NRBF2 presents at the basis of the V-shaped PI3KC3 complex I and enhances the kinase activity roughly by 10 folds (Young et al., 2016). NRBF2 forms homodimer via CCD domain to drive the dimerization of PI3KC3 complex I, in a similar way like yeast Atg38. Importantly, NRBF2 was revealed as an allosteric activator of PIK3C3 by releasing the PIK3C3 kinase domain from its inhibitory conformation, and MIT domain alone is sufficient to enhance the activity of PIK3C3 kinase. Combining the cell biology, biochemistry and structural biology data, NRBF2 is a stable component and allosteric enhancer of PI3KC3 complex I to positively regulate autophagosome biogenesis.

The PI3KC3 complex is involved in both autophagosome initiation and autophagosome maturation by binding with diverse partners to form distinct complexes. Our previous study has revealed that NRBF2 regulates ATG14-associated PI3KC3 activity for autophagosome initiation (Lu et al., 2014). Recently, we reported a pivotal role for NRBF2 in controlling autophagosome maturation. We found that *NRBF2* KO increased both LC3-II and SQSTM1 levels in neuronal cells. Starvation dramatically enhanced the degradation of SQSTM1 levels in wild type cells, but not in *NRBF2* KO cells. Additionally, there was no obvious increase of SQSTM1 levels in *NRBF2* KO cells after treatment with chloroquine. By using the tandem fluorescent (tf)-LC3 probe, we showed that *NRBF2* KO causes the increase of yellow puncta (immature autophagosomes) and decrease of red-only puncta (mature autophagosomes). To understand how does NRBF2 affect the autophagosome maturation process, we examined the lysosome function as well as the direct fusion between autophagosome and lysosome in *NRBF2* KO cells and animals. However, neither does *NRBF2* KO impair lysosome function nor autophagosome-lysosome fusion. There are 3 steps to execute autophagosome maturation: autophagosome trafficking, autophagosome-lysosome fusion and lysosomal degradation. After excluding the possibility that *NRBF2* regulates autophagosome-lysosome fusion and lysosomal degradation, it is reasonable to speculate that NRBF2 is required for autophagosome trafficking to lysosome. Interestingly, transmission electron microscope images showed that the size of autophagosomes is increased in *NRBF2* KO cells (Cai et al., 2020), implying the impaired autophagosome trafficking may lead to autophagosome enlargement.

AD is the most common neurodegenerative disorder featured by accumulation of amyloid-β (Aβ) and hyperphosphorylated tau. Accumulation of evidence shows that dysfunction of autophagy is implicated in the AD pathogenesis. Our previous study has revealed that NRBF2 is reduced in brains of 5XFAD mice. Moreover, NRBF2 interacts with APP, and overexpression of NRBF2 decreases APP-CTFs and Aβ levels via autophagy (Yang et al., 2017). In fact, we recently reported that NRBF2 expression was reduced in parahippocampal gyrus and hippocampus in late-onset AD postmortem brains (Lachance et al., 2019). *NRBF2* KO mice displayed multiple phenotypes associated with AD: impaired working memory as shown by fear conditioning test and object-location task, damaged synaptic plasticity as indicated by electrophysiology analysis and accumulation of APP-CTFs and Aβ in hippocampus. We also observed that loss of NRBF2 expression in 5XFAD mice enhances their memory deficits, while overexpression of NRBF2 into hippocampus can rescue memory impairments and reduce Aβ deposits in 5XFAD mice. Finally, our recent study revealed a potential mechanism by which NRBF2 regulates AD pathology: regulating CCZ1-MON1A GEF activity on APP containing vesicles to activate RAB7 for lysosome degradation. Besides, nervous system-specific knockout of *NRBF2* was sufficient to induce learning and memory deficits in mice (Ouyang et al., 2020), further supporting our finding that NRBF2 plays a vital role in maintain neuronal homeostasis.

Autophagy plays an important role in restricting inflammation. Functional abrogation of Vps34 in zebrafish developed into an IBD-like features (Zhao et al., 2018), revealing the role of PI3KC3 complex in maintaining intestinal homeostasis. Our study also revealed the physiological role of NRBF2 in apoptotic cell clearance for the maintenance of intestinal homeostasis (Wu et al., 2020). NRBF2 deficiency increased susceptibility of mice to DSS-induced colitis, featured by large amount of apoptotic cells accumulation in the colon tissue and dramatic leukocytes infiltration. NRBF2 deficiency in macrophages or in mice caused impaired clearance of apoptotic cells. While adoptive transfer of macrophage from wild type mice can attenuate the DSS-induced colitis and apoptotic cell accumulation in *NRBF2* KO mice. Clinical evidence further

proved the regulatory roles of NRBF2 in ulcerative colitis (UC). Higher NRBF2 expression and well colocalization of NRBF2 with macrophage marker CD68 have been observed in colon tissues from UC patients, implying the important functions of NRBF2 on macrophage in UC. Furthermore, apoptotic cell count in colonic biopsy is well correlated with the UC severity (Mayo Score), indicating that apoptotic cell accumulation is associated with UC development. Interestingly, apoptotic cell debris can be observed in NRBF2 positive cells, implying that NRBF2 can potentially facilitate apoptotic cell clearance in UC patients. Collectively, NRBF2 exert a critic role in regulating intestinal inflammation, probably via promoting macrophage-mediated apoptotic cell clearance, thus modulation of NRBF2 function by small molecule may provide therapeutic opportunity for colitis treatment by eliminating apoptotic cells.

Taken together, our two studies not only expanded current understanding towards the biochemical and physiological function of NRBF2 and PI3KC3 complex (**Figure 1**), but also highlighted the potential value of *NRBF2* KO mouse as a mild and systematic autophagy defect model to investigate the role of chronic autophagy defect in the development of chronic human diseases.

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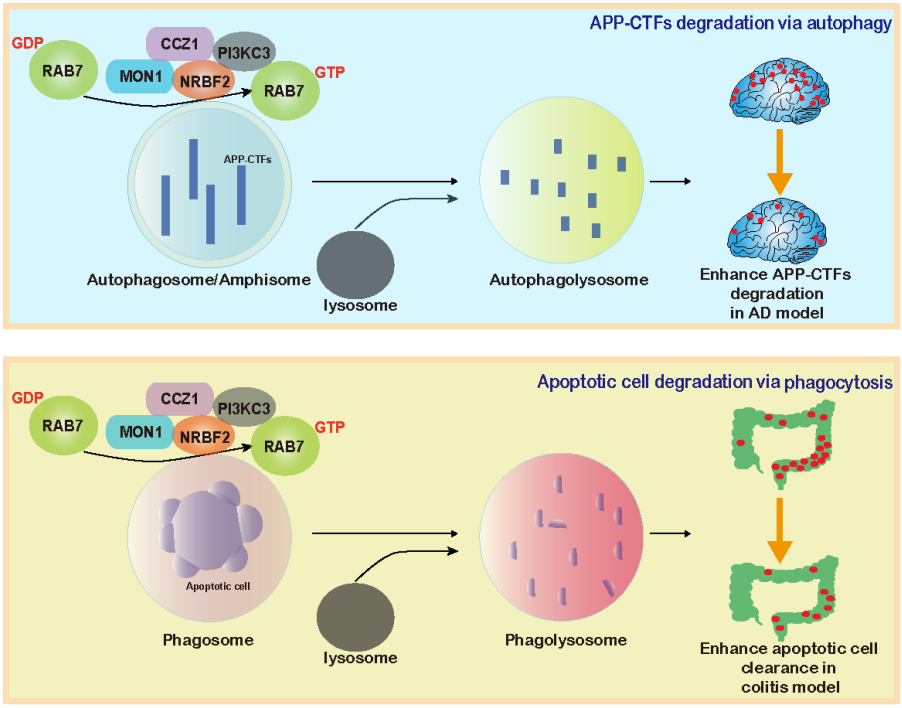

Figure 1 | **Schematics depicting the proposed biochemical activities of NRBF2 in the brain and gut homeostasis.**
NRBF2 promotes APP-CTF in autophagosome and apoptotic cell in phagosome degradation via enhancing MON1/CCZ1 activity to activate RAB7-mediated autophagosome and phagosome maturation in AD and colitis models. AD: Alzheimer's disease; APP-CTF: amyloid precursor protein-C-terminal fragment.

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