Micro Report

Autophagy activity contributes to the impairment of social recognition in Epac2−/− mice

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Abstract

Autophagy is a lysosomal degradation pathway that regulates cellular homeostasis. It is constitutively active in neurons and controls the essential steps of neuronal development, leading to its dysfunction in neurodevelopmental disorders. Although mTOR-associated impaired autophagy has previously been reported in neurodevelopmental disorders, there is lack of information about the dysregulation of mTOR-independent autophagy in neurodevelopmental disorders. In this study, we investigated whether the loss of Epac2, involved in the mTOR-independent pathway, affects autophagy activity and whether the activity of autophagy is associated with social–behavioral phenotypes in mice with Epac2 deficiencies. We observed an accumulation of autophagosomes and a significant increase in autophagic flux in Epac2-deficient neurons, which had no effect on mTOR activity. Next, we examined whether an increase in autophagic activity contributed to the social behavior exhibited in Epac2−/− mice. The social recognition deficit observed in Epac2−/− mice recovered in double transgenic Epac2−/−:Atg5+/− mice. Our study suggests that excessive autophagy due to Epac2 deficiencies may contribute to social recognition defects through an mTOR-independent pathway.

Keywords: Autophagy, Epac2, Social recognition, Neurodevelopmental disorders

Macroautophagy (hereafter autophagy) is a dynamic cellular pathway that regulates the lysosomal degradation of cytosolic components, including organelles, proteins, lipids, DNA, RNA, or unwanted materials within cells [1]. Autophagy is a tightly regulated process, conducted by several autophagy-related (ATG) proteins in neurons. Knockout of key ATG components, like ATG5 or ATG7, causes accumulation of ubiquitinated proteins and neurodegeneration, suggesting its importance in neuronal health [2, 3]. Regarding signaling pathways, autophagy is regulated by mTOR (mammalian target of rapamycin), which senses and integrates several intracellular and environmental cues to orchestrate major processes, including cell growth and metabolism, or mTOR-independent pathways, like cAMP (3′–5′-cyclic adenosine monophosphate), Ca2+, or IP3 (Inositolphosphoinositide-3) [4]. Thus far, most reports indicate that mTOR pathway dysregulation, which regulates neurodevelopment or synaptic plasticity, is linked to impaired autophagy, leading to mTOR-associated brain diseases, including autism spectrum disorders (ASD) [5, 6]. However, to better understand the role of autophagy in neurodevelopment, synaptic function, or neurological disorders, it is also important to investigate

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mTOR-independent autophagy in brain function using in vitro and in vivo models.

Previous investigations have reported that elevated intracellular cAMP inhibits autophagy and is mediated by exchange protein activated by cAMP (Epac) [7, 8]. Epac2 is highly expressed in the brain and is an upstream activator of the small GTPase Ras family [9]. Several animal studies have identified behavioral phenotypes in Epac2 knockout (Epac2−/−) mice consistent with the link to ASD susceptibility [10], including impaired memory, behavioral inflexibility, and altered social interactions [11, 12]. However, whether the loss of Epac2 affects autophagy activity and whether autophagy is associated with social–behavioral phenotypes in Epac2−/− mice remains unclear. Therefore, we investigated autophagy in Epac2−/− mice to assess whether it affects the social–behavioral phenotype observed in these models.

First, we investigated the involvement of autophagy in Epac2−/− mice by transfecting HyD-LIR-based autophagosome sensors (HyD-LIR-GFP), which could detect endogenous LC3 or GABARAP family proteins in autophagosomes, into cultured cortical neurons (div1) [13]. Two days after transfection, the number of HyD-LIR-GFP-positive autophagosomes in Epac2 deficient neurons was significantly accumulated compared with that of the wild-type cortical neurons, in the presence of a lysosomal inhibitor (chloroquine, CQ), indicating that autophagic activity was higher in Epac2-deficient neurons than in wild-type neurons (Fig. 1A, B).

To further investigate whether autophagy was upregulated due to Epac2 deficiencies, we performed an autophagic flux assay by Western blot, using anti-LC3B or anti-GABARAP1 antibodies in the presence or absence of a lysosomal inhibitor in Epac2+/+ or Epac2−/− cortical neurons. As shown in Fig. 1C–E, the expression levels of LC3-II and GABARAP1-II were significantly increased with lysosomal inhibition in Epac2−/− neurons compared with Epac2+/+ neurons. These results indicate that Epac2 deficiencies abnormally upregulated autophagy activity.

Next, we examined whether the mTOR pathway affected abnormal autophagic activity in Epac2−/− mice. Therefore, the protein levels of mTOR, p70S6 kinase, and phosphorylated p70S6 kinase (p-p70S6 kinase) in the cultured cortical neurons of Epac2+/+ and Epac2−/− mice (n.s., no significance) showed a significant preference for exploring a stranger mouse rather than an empty cage. Contrary to Epac2+/+, Epac2−/− mice exhibited no difference in durations exploring a stranger mouse vs. a familiar one. Epac2−/−/Atg5−/+ mice showed sociability and preference for social novelty similar to Epac2+/+ mice, suggesting the rescue of deficit in social novelty recognition of Epac2−/− mice (n.s., no significance; St, stranger mouse; Nov, novel mouse). J Epac2−/− mice can detect and discriminate nonsocial and social olfactory cues with normal dishabituation to novel social odor and habituation to repeated same social odor. K M Western blotting and quantitative analysis indicating the protein levels of ATG5, ATG7, LC3B, and β-actin in cultured cortical neurons in Epac2+/+ and Epac2−/− mice in the presence or absence of siRNAs against ATG5 or ATG7. (M, N) Representative confocal images and quantitative analysis show p62-positive aggregates in wild-type, Epac2−/−, and Epac2−/−/Atg5−/+ cortical neurons. Values are presented as a mean ± standard error of the mean (SEM). *p < 0.05, **p < 0.01, ***p < 0.001. Scale bar, 10 μm.
Fig. 1 (See legend on previous page.)
Next, we performed ATG5 knockdown in vivo using Epac2−/− mice and generated Epac2−/−:Atg5+/− mice to downregulate abnormally enhanced autophagic activity. We confirmed that within the cortical neurons of Epac2−/− mice, aggregation of p62 protein was reduced and was restored in the cortical neurons of Epac2−/−:Atg5+/− mice in vivo (Fig. 1M, N).

Interestingly, normal sociability and preference for social novelty was observed in the Epac2−/−:Atg5+/− mice, with the normal ability of olfactory discrimination similar to Epac2+/+ mice, suggesting that the deficit in social recognition of Epac2−/− mice was rescued through crossing with Atg5+/− mice (Fig. 1I, J). Altogether, our results suggest that Epac2 contributes to the maintenance of basal autophagy activity and normal social recognition as a basis for normal social behavior by suppressing autophagy over activation.

Thus far, impaired or insufficient autophagy has mostly been described in neurological disorders. However, the role of abnormal autophagy upregulation without prominent cell death in brain function or neurological disorders remains unclear [1, 16]. Moreover, the role of mTOR-independent autophagy in brain functioning and the relationship between hyperactive autophagy and social–behavioral defects remain largely unknown. Epac2 negatively regulates autophagy in an mTOR-independent manner [7, 8]. Therefore, to elucidate the role of mTOR-independent autophagy in brain functioning and the relationship between hyperactive autophagy and social–behavioral impairments, we investigated the functional roles of autophagy pathways in Epac2−/− mice with social recognition deficiencies. Although the loss of microglial autophagy can be associated with social–behavioral impairments [17], in this study, we focused on neuronal autophagy because no alterations in morphology and number of microglia in the cortex of Epac2−/− mice were observed (Additional file 1: Fig. S1).

Epac2 deficiencies affect autophagic activity because autophagy is negatively regulated by mTOR and cAMP [18]. Although we examined the cAMP levels, which play an important role in regulating neural autophagic activity and directly activates Epac2 [19], we could not find a significant difference in cAMP levels in the cultured cortical neurons (Additional file 1: Fig. S2). Moreover, when we examined the Rap1 protein expression and enzymatic activity as a downstream signaling pathway of Epac2 activation, we found that the protein expression (Additional file 1: Fig. S3) and enzymatic activity (Additional file 1: Fig. S4) of Rap1 were unchanged in the cortical tissues of Epac2−/− mice in vivo compared with Epac2+/+ mice. However, calcineurin is activated by lysosomal calcium signaling, which is an endogenous serine/threonine phosphatase that dephosphorylates TFEB, leading to an upregulation of autophagy [6]. We found that a reduction in phosphorylated levels of TFEB in Epac2−/− mice cortex (Additional file 1: Fig. S5). Therefore, altogether, these data suggest that other signaling molecules that are affected by Epac2, such as Ca2+, may be involved in the autophagic activity changes of cortical neurons in Epac2−/− mice via indirect pathways, which are related to neither CAMP nor Rap signaling.

To the best of our knowledge, this is the first report that demonstrates the excessive activity of mTOR-independent autophagy, and Epac2 deficiencies could contribute to defects in social behaviors in mice models. In addition, our study provides therapeutic insights into neurodevelopmental disorder treatment, including ASD with excessive autophagic activity, through suppressing autophagic activity.

Abbreviations
ASD: Autism spectrum disorders; ATG: Autophagy-related gene; cAMP: 3′-5′-Cyclic adenosine monophosphate; Epac2: Exchange protein activated by cAMP 2; GABARAPL1: Gamma-aminobutyric acid receptor-associated like protein 1; LC3: Microtubule-associated proteins 1A/1B light chain 3; mTOR: Mammalian target of rapamycin.

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Authors’ contributions
All authors read and approved the final manuscript. JAL and KL designed the study, analyzed the data, and wrote the paper. JHK, MHJ, YKL, MR, JL, and HS performed the experiments.

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Availability of data and materials
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Declarations
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References
1. Levine B, Kroemer G. Biological functions of autophagy genes: a disease perspective. Cell. 2019;176:11–42.
2. Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature. 2006;441:885–9.
3. Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, et al. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature. 2006;441:880–4.
4. Nikoletopoulou V, Tavernarakis N. Regulation and roles of autophagy at synapses. Trends Cell Biol. 2018;28:646–61.
5. Tang G, Gudsnuk K, Kuo SH, Cotrina ML, Rosoklija G, Sosunov A, et al. Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. Neuron. 2014;83:1131–43.
6. Yan J, Porch MW, Court-Vazquez B, Bennett MWL, Zukin RS. Activation of autophagy rescues synaptic and cognitive deficits in fragile X mice. Proc Natl Acad Sci U S A. 2018;115:E9707–16.
7. Holen I, Gordon PB, Stromhaug PE, Seglen PO. Role of cAMP in the regulation of hepatocytic autophagy. Eur J Biochem. 1996;236:163–70.
8. Chu KY, O'Reilly L, Mellot N, Meikle PJ, Bartley C, Biden TJ. Oleate disrupts cAMP signaling, contributing to potent stimulation of pancreatic beta-cell autophagy. J Biol Chem. 2019;294:1218–29.
9. Kawasaki H, Springett GM, Mochizuki N, Toki S, Nakaya M, Matsuda M, Housman DE, Graybiel AM. A family of CAMP-binding proteins that directly activate Rap1. Science. 1998;282:2275–9.
10. Bacchelli E, Blasi F, Biondolillo M, Lamb JA, Bonora E, Barnby G, et al. Screening of nine candidate genes for autism on chromosome 2q reveals rare nonsynonymous variants in the cAMP-GEFII gene. Mol Psychiatry. 2003;8:916–24.
11. Lee K, Kobayashi Y, Seo H, Kwak JH, Masuda A, Lim CS, Lee HR, Kang SJ, Park P, Sim SE, et al. Involvement of cAMP-guamine nucleotide exchange factor II in hippocampal long-term depression and behavioral flexibility. Mol Brain. 2015;8:38.
12. Srivastava DP, Jones KA, Woolfrey KM, Burgdorf J, Russell TA, Kalmbach A, et al. Social, communication, and cortical structural impairments in Epac2-deficient mice. J Neurosci. 2012;32:11864–78.
13. Lee YK, Jun YW, Choi HE, Huh YH, Kaang BK, Jang DJ, et al. Development of LC3/GABARAP sensors containing a LIR and a hydrophobic domain to monitor autophagy. EMBO J. 2017;36:1100–16.
14. Bicks LK, Koike H, Akbarian S, Morishita H. Prefrontal cortex and social cognition in mouse and man. Front Psychol. 1805;2015:6.
15. Yang M, Crawley JN. Simple behavioral assessment of mouse olfaction. Curr Protoc Neurosci 2009, Chapter 8: Unit 8 24.
16. Menzies FM, Fleming A, Rubinsztein DC. Compromised autophagy and neurodegenerative diseases. Nat Rev Neurosci. 2015;16:345–57.
17. Kim HJ, Cho MH, Shin WH, Kim JK, Jeon EY, Kim DH, et al. Deficient autophagy in microglia impairs synaptic pruning and causes social behavioral defects. Mol Psychiatry. 2017;22:1576–84.
18. Sarkar S, Ravikumar B, Floto RA, Rubinsztein DC. Rapamycin and mTOR-independent autophagy inducers ameliorate toxicity of polyglutamine-expanded huntingtin and related proteinopathies. Cell Death Differ. 2009;16:46–56.
19. Sugawara K, Shibasaki T, Takahashi H, Seino S. Structure and functional roles of Epac2 (Rapgef4). Gene. 2016;575:577–83.

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