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Original Article

Experimental Neurobiology

PINK1 Deficiency Enhances Inflammatory Cytokine Release from Acutely Prepared Brain Slices

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Parkinson's disease (PD) is the second most common neurodegenerative motor disease caused by degeneration of dopaminergic neurons in the substantia nigra. Because brain inflammation has been considered a risk factor for PD, we analyzed whether PTEN induced putative kinase 1 (PINK1), an autosomal recessive familial PD gene, regulates brain inflammation during injury states. Using acutely prepared cortical slices to mimic injury, we analyzed expression of the pro-inflammatory cytokines tumor necrosis factor-α, interleukin (IL)-1β, and IL-6 at the mRNA and protein levels. Both mRNA and protein expression of these cytokines was higher at 6-24 h after slicing in PINK1 knockout (KO) slices compared to that in wild-type (WT) slices. In serial experiments to understand the signaling pathways that increase inflammatory responses in KO slices, we found that IκB degradation was enhanced but Akt phosphorylation decreased in KO slices compared to those in WT slices. In further experiments, an inhibitor of PI3K (LY294002) upstream of Akt increased expression of pro-inflammatory cytokines. Taken together, these results suggest that PINK1 deficiency enhance brain inflammation through reduced Akt activation and enhanced IκB degradation in response to brain injury.

Key words: Parkinson's disease, PINK1, inflammation

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder caused by loss of dopaminergic neurons in the substantia nigra. However, despite intensive basic and clinical studies it is still unclear why dopaminergic neurons die in patients with PD.

PTEN induced putative kinase 1 (PINK1) is a familial PD-related gene whose mutation causes autosomal recessive and early-onset PD [1]. PINK1-knock down and -knockout (KO) cells, including neurons, are more vulnerable to various insults than wild-type (WT) cells [2, 3]. However, animal models that carry a PINK1 mutation do not develop PD-like symptoms such as degeneration of dopaminergic neurons and Lewy body formation [4]. Therefore, the emerging concept of the onset and progression of dopaminergic neuronal degeneration *in vivo* is that certain environmental factors must cooperate with genetic factors in the development of PD [5, 6]. As environmental factors, toxins including pesticides and herbicides have been considered [7]. However, the most important environmental factor that regulates

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neuronal function and survival is glia (astrocytes and microglia). Accordingly, glia have recently been suggested as a turning point in the therapeutic strategy for PD [6].

In response to brain injury, microglia as well as neurons die in injury sites [8-10], and microglia in the penumbra region rapidly isolate injury sites and produce cytokines such as interleukin-1β (IL-1 β), which are not harmful to brain cells [9, 10]. However, it is not known how PINK1 deficiency changes microglial inflammatory response. It has been reported that expression of pro-inflammatory cytokines increases in cerebrospinal fluid and brain parenchyma of patients with PD [11]. Inflammatory responses including microglia activation and expression of inflammatory cytokines increase in animal models of PD [12, 13]. Furthermore, brain inflammation is a risk factor for neurodegenerative diseases including PD [14, 15], and anti-inflammatory drugs such as dexamethasone, ibuprofen, and rofecoxib show neuroprotective effects against MPTP toxicity [16, 17]. A recent study reported that abnormal expression of innate immunity genes precedes dopaminergic neuronal death in PINK1-deficient mice [18].

In this study, we hypothesized that a PINK1 mutation alters brain inflammation, which, in turn, affects the onset and progression of PD. We found that a PINK1 deficiency enhanced brain inflammation using acutely prepared organotypic brain slices from PINK1 KO and WT mice.

MATERIALS AND METHODS

Animals

PINK1-KO mice were a gift from Dr. UJ Kang in Chicago University. PNIK1-KO mice were generated by replacing a 5.6-kb genomic region of the PINK1 locus, including exons 4-7 and the coding portion of exon 8, with a PGK-neo-polyA selection cassette flanked by FRT sequences [19, 20].

Organotypic cortical slice cultures

Cortical slices were prepared using a modified Stoppini method [21]. Briefly, postnatal day 7 (P7) WT and PINK1 KO mice were decapitated. Their brains were removed, and coronal slices (400-μm thick) were prepared using a McIlwain tissue chopper (Mickle Laboratory Engineering, Goose Green, UK). Slices were placed into 24-well plates and each well was filled with 500 μl culture medium (MEM containing 25% v/v Hank's balanced salt solution, 25% v/v heat-inactivated horse serum [Hyclone, Logan, UT, USA], 6.5 mg/ml glucose, 1 mM L-glutamine, 10 U/ml penicillin-G, and 10 mg/ml streptomycin).

Reverse transcriptase-polymerase chain reaction (PCR) and quantitative real-time PCR (qPCR)

Total RNA was isolated using an easy-BLUE RNA Extraction kit (iNtRON, Sungnam, Korea), and cDNA was prepared using Reverse Transcription Master Premix (ELPIS Bio, Taejeon, Korea). The primers (Bionneer, Deajeon, Korea) used for the RT-PCR were: tumor necrosis factor-α (TNF-α) (5'-GTAGCCCACGTCGTAGCAAA 3'-CCCTTCTCCAGCTGGGAGAC), IL-1β (5'-TGATGTTC CCATTAGACAGC 3'-GAGGTGCTGATGTACCAGTT), IL-6 (5'-AAAATCTGCTCTGGTCTTCTGG 3'-GGTTTGCC GAGTAGACCTCA), and GAPDH (5'-TCCCTCAAGATTGT CAGCAA 3'-AGATCCACAACGGATACATT). The amplified products were verified by electrophoresis on 1.5% agarose gels with GelRed (Biotium, Hayward, CA, USA). Band intensities were analyzed using Quantity One 1-D analysis software, v 4.6.5 (BioRad Laboratories, Inc., Hercules, CA). cDNA was analyzed using a KAPA SYBR FAST qPCR kit (KAPA Biosystem, Woburn, MA, USA). qPCR was performed using the RG-6000 real-time amplification instrument (Corbett Research, Sydney, Australia). The qPCR conditions were 40 cycles of 95°C for 3 sec, 55°C for 20 sec, and 72°C for 3 sec. The threshold cycle number of each gene was calculated and normalized compared to that of GAPDH.

Enzyme-linked immunosorbent assay (ELISA)

TNF-α levels in the media were measured using an ELISA kit according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA).

Western blot analysis

Brain slices were washed three times with cold PBS and lysed on ice in modified RIPA buffer (50 mM Tris-HCl pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM Na_3VO_4, and 1 mM NaF) containing protease inhibitors (2 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml pepstatin, and 2 mM EDTA). The lysates were centrifuged at 13,000 × g for 10 min at 4°C, and the supernatant was collected. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were incubated with antibodies specific for signal transducers and activators of transcription 1 (STAT1), phospho-STAT1 (p-STAT1), p-STAT3, total STAT3 (Upstate Biotechnology, NY, USA), p-p38, total p38 (Cell Signaling Technology, Beverly, MA, USA), p-Akt, total Akt (Cell Signaling Technology), IκB (Cell Signaling Technology), and actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C and then washed three times with PBS. Membranes were incubated with peroxidase-conjugated secondary antibodies (Zymed, San Francisco, CA,

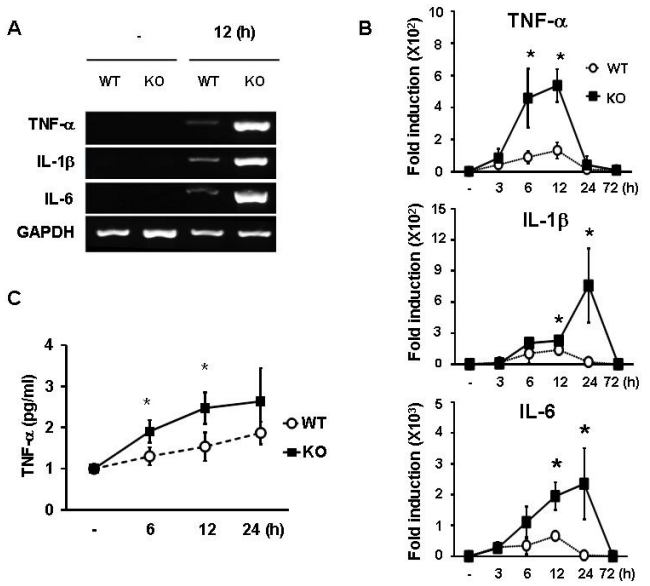

Fig. 1. PINK1 deficiency increased expression of pro-inflammatory cytokines in cortical slice cultures. Cortical slices were prepared from 7 d old PINK1 wild-type (WT) and knockout (KO) mice. (A, B) mRNA was isolated at the indicated times after preparing slices, and expression levels of tumor necrosis factor-α (TNF-α), interlukin-1β (IL-1β), and IL-6 mRNA were analyzed by reverse transcription polymerase chain reaction (RT-PCR) (A) and quantitative PCR (qPCR) (B). (C) Culture media were obtained at the indicated times, and TNF-α was measured by enzyme-linked immunosorbent assay. Values are means ± standard error of at least three samples. * $p<0.05$ vs. WT.

USA), and proteins were visualized using a EZ-Western Detection kit (Daeillab, Seoul, Korea).

RESULTS

PINK1 deficiency increases expression of pro-inflammatory cytokines

To examine how PINK1 regulates the inflammatory response in the injured brain, we measured expression levels of TNF-α, IL-1β, and IL-6 in organotypic brain slices prepared from WT and PINK1 KO mice because the slicing process mimics brain injury [22, 23]. mRNA levels of these cytokines were measured at 3, 6, 12, and 24 h after the preparation of slices using RT-PCR (Fig. 1A) and qPCR (Fig. 1B). PINK1 KO slices expressed higher levels of all cytokines compared to those of WT. TNF-α protein production was also higher in PINK1 KO slices than that in WT slices (Fig. 1C).

Altered activation patterns of STAT3, IκB, and Akt in PINK1 KO slices

Next, we examined the signaling pathways responsible for increased expression of the pro-inflammatory cytokines in the PINK1 KO slices. In serial experiments, we examined various signaling pathways such as mitogen activated protein kinase (MAPK), STATs, and nuclear factor (NF)-kB pathways that regulate brain inflammation [24-27]. Although extracellular signal-regulated kinase (ERK) was activated within 30 min after slicing, there was no difference in the activation levels in WT and

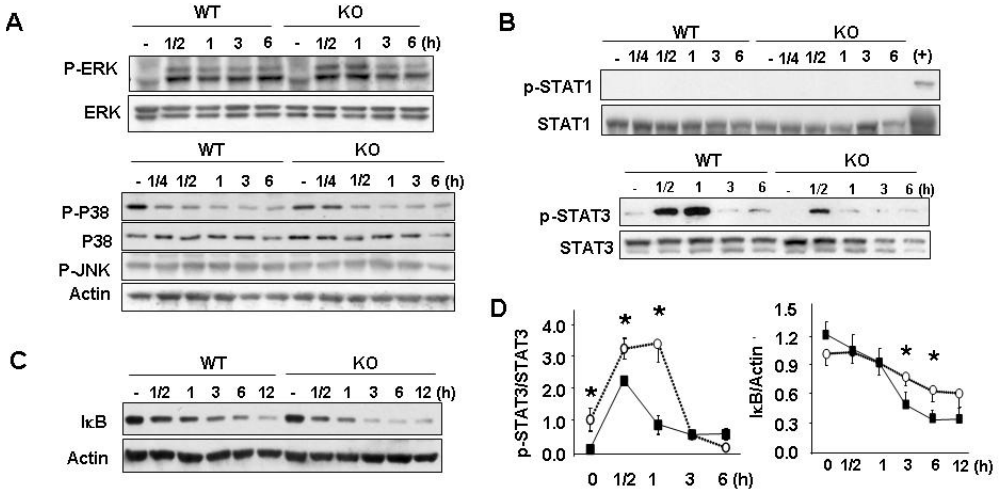

Fig. 2. PINK1 deficiency affected activation of STAT3 levels and IκB degradation but not activation of STAT1 and MAPKs. Proteins were obtained from slice cultures at the indicated times. Levels of p-extracellular regulated kinase (p-ERK), ERK, pp38, p38, pJNK, and actin (A), p-STAT1, STAT1, p-STAT3, and STAT3 (B), IκB (C) were analyzed by Western blot. (+, positive control) in STAT1 blot: BV-2 microglia treated with interferon-γ (IFN-γ) for 30 min. (D) Levels of p-STAT3 and IκB were quantified at the indicated times, normalized with STAT3 and actin, respectively, and plotted. Values are means ± standard error of at least three samples. * $p<0.05$ vs. wild-type (WT).

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KO slices (Fig. 2A). Phosphorylation levels of these molecules (p-p38, p-JNK, and p-STAT1) were not different in WT or KO slices (Fig. 2A, B). In contrast, activation of the STAT3 and IκB-NFκB pathways was different in WT and KO slices. Phosphorylation levels of STAT3 (p-STAT3) were attenuated in KO slices (Fig. 2B, D). IκB degradation, which represents NF-kB activation [28], was detected within 30 min in WT and KO slices but was much faster in KO slices (Fig. 2C, D).

Previously, we found that phosphorylation levels of Akt (p-AKT), a downstream regulator of phosphatidylinositol 3-kinase (PI3K), decreased in astrocytes cultured from KO mice [20]. Since PI3K negatively regulates expression of pro-inflammatory cytokines [29], we examined p-Akt levels in WT and KO slices. p-Akt levels time-dependently decreased after slicing in WT and KO but more rapidly in KO (Fig. 3A). Next, we examined whether reduced p-Akt levels were related to enhanced expression of pro-inflammatory cytokines in KO slices. WT slices were treated with a PI3K inhibitor, LY294002, and TNF-α and IL-1β mRNA levels were assayed. In both RT-PCR and qPCR, LY29402 significantly increased TNF-α and IL-1β mRNA levels (Fig. 3B, C). We further examined the effect of LY204002 on p-STAT3 levels and IκB degradation. However, LY294002 had no effect on p-STAT3 levels and IκB degradation (Fig. 3D), suggesting that Akt negatively regulates the inflammatory responses in slices independent of the STAT3 and NFκB degradation pathways. Taken together, these results suggest that PINK1 deficiency increases inflammatory responses in the injured brain through decreased Akt activation and enhanced NF-κB activation. Although p-STAT3 levels decreased in PINK1 KO slices, we could not determine whether reduced STAT3 activation contributed to enhance the inflammatory response in KO slices.

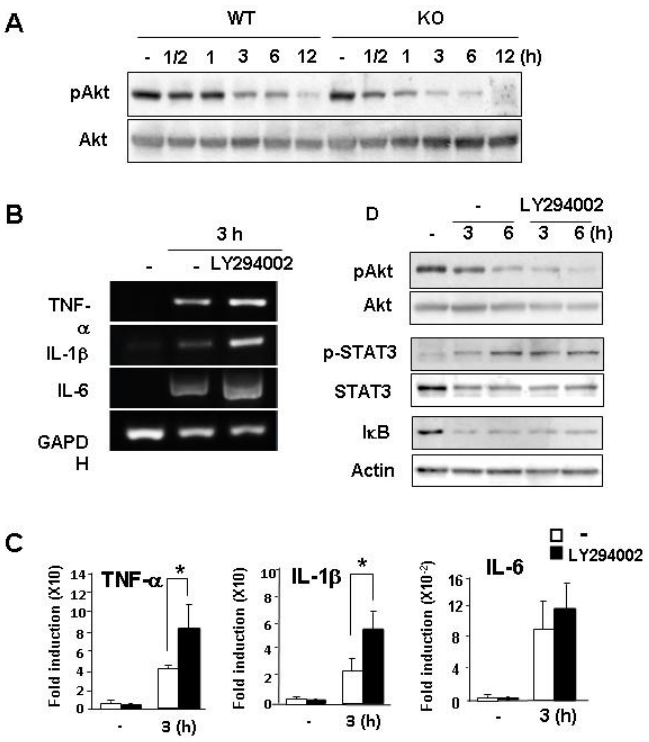

Fig. 3. PINK1 deficiency attenuated Akt activation and a PI3K inhibitor, LY204002, increased cytokine expression in cortical slices. (A) Proteins were obtained at the indicated times after preparation of slices, and p-Akt levels were measured by Western blot. (B-D) Slices obtained from wild-type (WT) mice were cultured with or without LY294002 (20 μM) for the indicated times, and then mRNA and protein levels were obtained. mRNA levels of tumor necrosis factor-α (TNF-α), interlukin-1β (IL-1β), and IL-6 were analyzed by reverse transcription polymerase chain reaction (RT-PCR) (B) and quantitative PCR (qPCR) (C). Levels of p-Akt, Akt, p-STAT3, STAT3, IκB, and actin were analyzed by Western blot (D). Values are means±standard error of three samples. * $p<0.05$.

DISCUSSION

The major findings in this study were that PINK1 deficiency increases expression of pro-inflammatory cytokines and that attenuated Akt activation and enhanced IκB-NF-κB pathways may be involved in increased production of pro-inflammatory cytokines in PINK KO mice.

We used organotypic cortical slice cultures prepared from the early postnatal period (P7) to mimic brain injury since organotypic slice cultures have been used in many studies to investigate mechanisms and treatment strategies for neurodegenerative diseases, such as stroke, Alzheimer's disease, PD, and Huntington's disease [30-33]. We found that the expression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 was more highly induced in PINK1 KO mice than those in WT mice using slices (Fig. 3). These results agree with the results of a recent study in which PINK1 KO mice showed higher striatal levels of IL-1β, IL-12, and IL-10 in response to lipopolysaccharide [18].

It has been reported that PINK1-deficient cells are vulnerable to apoptosis compared to that in WT cells [34, 35]. In some experiments, we also found that more cells died in KO slices and that cytokine release rather decreased in KO slices (data not shown). However, in cases where WT and KO slices did not show differences in the extent of cell death confirmed by Live/Dead and lactate dehydrogenase assays (data not shown), we found increased inflammatory cytokine expression in KO slices compared to that in WT slices (Fig. 1).

The most important signaling pathways regulating brain inflammation are MAPKs and STATs [24-27]. However, activation patterns of STAT1 and MAPKs including ERK, p38, and JNK were not different in WT and KO slices (Fig. 2A, B).

Instead, degradation of IκB, and levels of p-STAT3 and p-Akt were different in WT and KO slices (Figs. 2B-D, 3A). It has been reported that STAT3 blocks NF-κB activation by preventing IκB phosphorylation and degradation [36]. Therefore, we speculate that IκB degradation may be related to STAT3 activation in KO slices, which results in increased pro-inflammatory cytokines in PINK1 KO slices. However, we do not have any evidence to support this speculation. The PI3K/Akt pathway may be another factor regulating the inflammatory responses in KO animals. The importance of the PI3K/Akt pathways in the inflammation has been reported in rheumatoid arthritis, multiple sclerosis, and asthma [37-39]. Inhibiting PI3K increases TNF-α and IL-6 expression in macrophages [29]. In this study, we found that p-Akt levels were attenuated in PINK1 KO slices (Fig. 3A). Furthermore, LY294002, which indirectly inhibits Akt activation by inhibiting PI3K, enhanced expression of TNF-α and IL-1β in WT slices (Fig. 3B, C). However, treatment with LY294002 did not affect p-STAT3 levels or IκB degradation (Fig. 3D). These results suggest that activating Akt increases pro-inflammatory cytokines in PINK1 KO slices independently of the STAT3 or IκB pathway.

The results of this study could shed a light on the unsolved question of why none of the PD-like symptoms occur in animal models that carry mutant PD-related genes and/or whose PD-related genes are knocked out. Because the PINK1 defect enhances brain inflammation in response to injury, the function of PINK1 may be more important in injury states rather than in normal physiological states. Thus, a defect in PINK1 could exaggerate brain inflammation in the injured brain, which increases brain damage and results in dopaminergic neuronal death.

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REFERENCES

1. Valente EM, Salvi S, Ialongo T, Marongiu R, Elia AE, Caputo V, Romito L, Albanese A, Dallapiccola B, Bentivoglio AR (2004) PINK1 mutations are associated with sporadic early-onset parkinsonism. Ann Neurol 56:336-341.
2. Deng H, Jankovic J, Guo Y, Xie W, Le W (2005) Small interfering RNA targeting the PINK1 induces apoptosis in dopaminergic cells SH-SY5Y. Biochem Biophys Res Commun 337:1133-1138.
3. Haque ME, Thomas KJ, D'Souza C, Callaghan S, Kitada T, Slack RS, Fraser P, Cookson MR, Tandon A, Park DS (2008) Cytoplasmic Pink1 activity protects neurons from dopaminergic neurotoxin MPTP. Proc Natl Acad Sci U S A 105:1716-1721.
4. Gispert S, Ricciardi F, Kurz A, Azizov M, Hoepken HH, Becker D, Voos W, Leuner K, Müller WE, Kudin AP, Kunz WS, Zimmermann A, Roeper J, Wenzel D, Jendrach M, García-Arencíbia M, Fernández-Ruiz J, Huber L, Rohrer H, Barrera M, Reichert AS, Rüb U, Chen A, Nussbaum RL, Auburger G (2009) Parkinson phenotype in aged PINK1-deficient mice is accompanied by progressive mitochondrial dysfunction in absence of neurodegeneration. PLoS One 4:e5777.
5. Calne DB, Eisen A, McGeer E, Spencer P (1986) Alzheimer's disease, Parkinson's disease, and motoneurone disease: abiotrophic interaction between ageing and environment? Lancet 2:1067-1070.
6. L' Episcopo F, Tirolo C, Testa N, Caniglia S, Morale MC, Marchetti B (2010) Glia as a turning point in the therapeutic strategy of Parkinson's disease. CNS Neurol Disord Drug Targets 9:349-372.
7. Dawson TM, Ko HS, Dawson VL (2010) Genetic animal models of Parkinson's disease. Neuron 66:646-661.
8. Ji KA, Yang MS, Jeong HK, Min KJ, Kang SH, Jou I, Joe EH (2007) Resident microglia die and infiltrated neutrophils and monocytes become major inflammatory cells in lipopolysaccharide-injected brain. Glia 55:1577-1588.
9. Jeong HK, Ji KM, Kim B, Kim J, Jou I, Joe EH (2010) Inflammatory responses are not sufficient to cause delayed neuronal death in ATP-induced acute brain injury. PLoS One 5:e13756.
10. Min KJ, Jeong HK, Kim B, Hwang DH, Shin HY, Nguyen AT, Kim JH, Jou I, Kim BG, Joe EH (2012) Spatial and temporal correlation in progressive degeneration of neurons and astrocytes in contusion-induced spinal cord injury. J Neuroinflammation 9:100.
11. Mogi M, Harada M, Riederer P, Narabayashi H, Fujita K, Nagatsu T (1994) Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. Neurosci Lett 165:208-210.
12. Beal MF (2003) Mitochondria, oxidative damage, and inflammation in Parkinson's disease. Ann N Y Acad Sci 991:120-

 http://dx.doi.org/10.5607/en.2013.22.1.38

131.
13. Hald A, Lotharius J (2005) Oxidative stress and inflammation in Parkinson's disease: is there a causal link? Exp Neurol 193:279-290.
14. Wilms H, Zecca L, Rosenstiel P, Sievers J, Deuschl G, Lucius R (2007) Inflammation in Parkinson's diseases and other neurodegenerative diseases: cause and therapeutic implications. Curr Pharm Des 13:1925-1928.
15. Whitton PS (2007) Inflammation as a causative factor in the aetiology of Parkinson's disease. Br J Pharmacol 150:963-976.
16. Teismann P, Tieu K, Choi DK, Wu DC, Naini A, Hunot S, Vila M, Jackson-Lewis V, Przedborski S (2003) Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration. Proc Natl Acad Sci U S A 100:5473-5478.
17. Kurkowska-Jastrzebska I, Litwin T, Joniec I, Ciesielska A, Przybyłkowski A, Członkowski A, Członkowska A (2004) Dexamethasone protects against dopaminergic neurons damage in a mouse model of Parkinson's disease. Int Immunopharmacol 4:1307-1318.
18. Akundi RS, Huang Z, Eason J, Pandya JD, Zhi L, Cass WA, Sullivan PG, Büeler H (2011) Increased mitochondrial calcium sensitivity and abnormal expression of innate immunity genes precede dopaminergic defects in Pink1-deficient mice. PLoS One 6:e16038.
19. Xiong H, Wang D, Chen L, Choo YS, Ma H, Tang C, Xia K, Jiang W, Ronai Z, Zhuang X, Zhang Z (2009) Parkin, PINK1, and DJ-1 form a ubiquitin E3 ligase complex promoting unfolded protein degradation. J Clin Invest 119:650-660.
20. Choi I, Kim J, Jeong HK, Kim B, Jou I, Park SM, Chen L, Kang UJ, Zhuang X, Joe EH (2013) PINK1 deficiency attenuates astrocyte proliferation through mitochondrial dysfunction, reduced AKT and increased p38 MAPK activation, and downregulation of EGFR. Glia 61:800-812.
21. Stoppini L, Buchs PA, Muller D (1991) A simple method for organotypic cultures of nervous tissue. J Neurosci Methods 37:173-182.
22. Skibo GG, Nikonenko IR, Savchenko VL, McKanna JA (2000) Microglia in organotypic hippocampal slice culture and effects of hypoxia: ultrastructure and lipocortin-1 immunoreactivity. Neuroscience 96:427-438.
23. Huuskonen J, Suuronen T, Miettinen R, van Groen T, Salminen A (2005) A refined in vitro model to study inflammatory responses in organotypic membrane culture of postnatal rat hippocampal slices. J Neuroinflammation 2:25.
24. Pyo H, Jou I, Jung S, Hong S, Joe EH (1998) Mitogen-activated protein kinases activated by lipopolysaccharide and beta-amyloid in cultured rat microglia. Neuroreport 9:871-874.
25. Pyo H, Joe E, Jung S, Lee SH, Jou I (1999) Gangliosides activate cultured rat brain microglia. J Biol Chem 274:34584-34589.
26. Ryu J, Pyo H, Jou I, Joe E (2000) Thrombin induces NO release from cultured rat microglia via protein kinase C, mitogen-activated protein kinase, and NF-kappa B. J Biol Chem 275:29955-29959.
27. Kim OS, Park EJ, Joe EH, Jou I (2002) JAK-STAT signaling mediates gangliosides-induced inflammatory responses in brain microglial cells. J Biol Chem 277:40594-40601.
28. Kretz-Remy C, Mehlen P, Mirault ME, Arrigo AP (1996) Inhibition of I kappa B-alpha phosphorylation and degradation and subsequent NF-kappa B activation by glutathione peroxidase overexpression. J Cell Biol 133:1083-1093.
29. Medina EA, Morris IR, Berton MT (2010) Phosphatidylinositol 3-kinase activation attenuates the TLR2-mediated macrophage proinflammatory cytokine response to Francisella tularensis live vaccine strain. J Immunol 185:7562-7572.
30. Zhang X, Smith DL, Meriin AB, Engemann S, Russel DE, Roark M, Washington SL, Maxwell MM, Marsh JL, Thompson LM, Wanker EE, Young AB, Housman DE, Bates GP, Sherman MY, Kazantsev AG (2005) A potent small molecule inhibits polyglutamine aggregation in Huntington's disease neurons and suppresses neurodegeneration in vivo. Proc Natl Acad Sci U S A 102:892-897.
31. Larsen TR, Rossen S, Gramsbergen JB (2008) Dopamine release in organotypic cultures of foetal mouse mesencephalon: effects of depolarizing agents, pargyline, nomifensine, tetrodotoxin and calcium. Eur J Neurosci 28:569-576.
32. Cui HS, Matsumoto K, Murakami Y, Hori H, Zhao Q, Obi R (2009) Berberine exerts neuroprotective actions against in vitro ischemia-induced neuronal cell damage in organotypic hippocampal slice cultures: involvement of B-cell lymphoma 2 phosphorylation suppression. Biol Pharm Bull 32:79-85.
33. Wei W, Nguyen LN, Kessels HW, Hagiwara H, Sisodia S, Malinow R (2010) Amyloid beta from axons and dendrites reduces local spine number and plasticity. Nat Neurosci 13:190-196.
34. Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, Ali Z, Del Turco D, Bentivoglio AR, Healy DG, Albanese A, Nussbaum R, González-Maldonado R, Deller T, Salvi S, Cortelli P, Gilks WP, Latchman DS, Harvey RJ, Dallapiccola B, Auburger G, Wood NW (2004) Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 304:1158-1160.
35. Wood-Kaczmar A, Gandhi S, Yao Z, Abramov AY, Miljan EA, Keen G, Stanyer L, Hargreaves I, Klupsch K, Deas E,

Downward J, Mansfield L, Jat P, Taylor J, Heales S, Duchen MR, Latchman D, Tabrizi SJ, Wood NW (2008) PINK1 is necessary for long term survival and mitochondrial function in human dopaminergic neurons. PLoS One 3:e2455.

36. Yu H, Pardoll D, Jove R (2009) STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer 9:798-809.
37. Busse WW, Lemanske RF Jr (2001) Asthma. N Engl J Med 344:350-362.
38. Camps M, Rückle T, Ji H, Ardissone V, Rintelen F, Shaw J, Ferrandi C, Chabert C, Gillieron C, Françon B, Martin T, Gretener D, Perrin D, Leroy D, Vitte PA, Hirsch E, Wymann MP, Cirillo R, Schwarz MK, Rommel C (2005) Blockade of PI3Kgamma suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. Nat Med 11:936-943.
39. Sospedra M, Martin R (2005) Immunology of multiple sclerosis. Annu Rev Immunol 23:683-747.

 http://dx.doi.org/10.5607/en.2013.22.1.38