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AMP-activated protein kinase is activated in Parkinson's disease models mediated by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

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ABSTRACT

The selective loss of dopaminergic neurons in the substantia nigra pars compacta is a feature of Parkinson's disease (PD). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity is the most common experimental model used to investigate the pathogenesis of PD. Administration of MPTP in mice produces neuropathological defects as observed in PD and 1-methyl-4-pyridinium (MPP^+) induces cell death when neuronal cell cultures are used. AMP-activated protein kinase (AMPK) is a key regulator of energy homeostasis. In the present study, we demonstrated that AMPK is activated by MPTP in mice and MPP^+ in SH-SY5Y cells. The inhibition of AMPK by compound C resulted in an increase in MPP^+-induced cell death. We further showed that overexpression of AMPK increased cell viability after exposure to MPP^+ in SH-SY5Y cells. Based on these results, we suggest that activation of AMPK might prevent neuronal cell death and play a role as a survival factor in PD.

Introduction

PD is a neurodegenerative disease characterized by progressive and selective loss of dopaminergic neurons in the substantia nigra pars compacta and striatum [1]. Mitochondrial complex I defect is found in PD patients and triggers a decrease in ATP synthesis and an excess production of reactive oxygen species [2,3]. MPTP induces neurotoxicity and is used to produce an animal model of PD. MPTP is converted to MPP^+ by monoamine oxidase B in glial cells, and MPP^+ accumulation in dopaminergic neurons causes a mitochondrial respiratory defect. MPP^+ also triggers apoptotic cell death and DNA damage [4,5]. The mechanisms regulating neuronal cell death in PD have been extensively investigated [6,7].

AMPK is a key regulator of cellular energy metabolism and a heterotrimeric enzyme consisting of an α catalytic subunit and β and γ regulatory subunits [8]. AMPK is activated by phosphorylation of α subunit at Thr^{172} [9], which is mediated by several kinases, including LKB1 and calmodulin-dependent protein kinase β [10]. This activation is regulated by cellular AMP/ATP ratio, Ca^{2+} concentration and ROS [11–14]. Activated AMPK phosphorylates acetyl-CoA carboxylase (ACC), nitric oxide synthase and peroxisome proliferator-activated receptor gamma coactivator-1-α [15–17]. It has been reported that AMPK activation prevents apoptotic cell death via ceramide accumulation in astrocytes [18]. During brain development in rats, AMPK is highly expressed and induces neuronal survival under glucose deprivation [19]. In contrast, activation of AMPK following ischemia promotes damage of hippocampal and cortical neurons [20].

In the present study, we investigated the relationship between AMPK and PD by examining whether AMPK is activated in PD models *in vitro* and *in vivo*. Moreover, we sought to identify the function of AMPK in neuronal cell death during the progression of PD.

Materials and methods

Materials. MPP^+ and MPTP were purchased from Sigma (St. Louis, MO), and compound C was from CHEMICON (Temecula, CA). Antibodies recognizing a phospho-specific form of AMPKα-Thr^{172} and ACC-Ser^{79} were obtained from Cell Signaling Technology (Beverly, MA). Antibodies for poly (ADP-ribose) polymerase (PARP), β-actin, and c-Myc were from Santa Cruz Biotechnology (Santa Cruz, CA). AMPKα and tyrosine hydroxylase (TH) antibodies were purchased from Upstate Biotechnology (Lake Placid, NY) and BD Biosciences (San Diego, CA), respectively. A mammalian expression vector for the c-myc-tagged wild-type AMPKα subunit and dominant negative AMPKα subunit were kindly provided by Dr. J. Ha (Kyung Hee University).

Cell culture and transfection. Human neuroblastoma SH-SY5Y cells were maintained in Dulbecco's modified Eagle medium (WelGENE, Korea) supplemented with 10% fetal bovine serum (Cellgro, VA) and antibiotics at 37 °C with 95% air and 5% CO_2. Cells were exposed to MPP^+ in culture media containing serum. Transient transfection of SH-SY5Y cells was conducted using the polyethylenimine method [21,22].

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Animals and MPTP mouse model. Male C57BL/6 mice (eight weeks old) were purchased from Daehan bio-link (Chungbuk, Korea). Mice were administrated four i.p. injections of 20 mg/kg MPTP at 2 h intervals (total dosage 80 mg/kg) and sacrificed seven days after the final injection as indicated previously [23]. We adhered to the Guide for Animal Experiments edited by the Korean Academy for Medical Sciences.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. A 0.1 mg/ml treatment of MTT (Sigma, St. Louis, MO) was added to each well and incubated at 37 °C for 2 h. The media was removed from each well, and then dimethyl sulfoxide was added. Optical density was measured at 540 nm on an ELISA plate reader (Bio-Tek Instruments Inc, Winooski, VT) after dissolving. All values were averaged from at least three wells from four independent experiments.

Western blot analysis. Whole cell lysates were harvested in lysis buffer (1 M Tris–Cl, 0.5 M EDTA, 5 M NaCl, 20% NP-40, protease inhibitors, pH 7.4). Western blot analysis using cell lysates was performed as previously described [21].

Immunohistochemistry and statistical analysis. Animals were perfused transcardially with 4% paraformaldehyde. Brains were then removed, frozen, and cut in 30 μm slices on a freezing microtome (Leica Microsystems, Bensheim, Germany). Sections were stained as previously described [24]. Staining intensities of phospho-AMPKα and phospho-ACC were measured using image analysis program, MultiScan-IP software. Results were expressed as the means ± S.E. and Student *t* tests were conducted. Differences were considered significant at a *P* value of <0.05.

Immunocytochemistry. After the treatment with 300 μM MPP^+ for 48 h, SH-SY5Y cells grown on glass coverslips were fixed in 4% paraformaldehyde solution in PBS for 30 min at room temperature, washed three times with PBST, permeabilized with 0.1% triton X-100 in PBST for 40 min at room temperature, and blocked with 1 mg/ml BSA in PBST 1 h. Incubation with antibodies was performed in the blocking solution for overnight at 4 °C. Cy3-conjugated secondary antibody was used for immunodetection. The cells were incubated with Hoechst 33342 for 10 min at room temperature.

Results

Exposure to MPP^+ induces the activation of AMPK in SH-SY5Y cells

When MPP^+ was added to SH-SY5Y cells, the cell viability was gradually decreased with MPP^+ exposure in a dose-dependent manner over a 48-h period (Fig. 1A), as we previously reported [21]. To confirm the effects of MPP^+ on cell death, PARP cleavage was examined. As shown in Fig. 1B, the cleaved form of PARP increased with exposure to MPP^+. To identify the effect of MPP^+ on AMPK activity, the phosphorylation of AMPK and ACC was monitored. MPP^+ increased the phosphorylation level at Thr^{172} in the active site of AMPKα and Ser^{79} of ACC, which is a well characterized AMPK substrate [12] (Fig. 1C and D). These results indicate that AMPK is activated during the progression of cell death mediated by MPP^+.

Activation of AMPK in the midbrain of MPTP-intoxicated mice

To investigate the activation of AMPK in PD, we used a MPTP-injected mouse model which induces specific degeneration of the dopaminergic neurons in substantia nigra pars compacta [5]. As shown in Fig. 2A, MPTP-intoxicated mice produced a significant loss of TH-positive neurons in the substantia nigra. When the immunohistochemical study was performed using the coronal midbrain sections of control and MPTP mice, phosphorylation of AMPK-Thr^{172} was increased 2.8-fold in the degenerated midbrain by MPTP-intoxication (Fig. 2B). In the same condition, AMPK levels remained unchanged in MPTP mice (data not shown). Moreover, phosphorylation of ACC-Ser^{79} was increased 1.8-fold in MPTP-injected mice, indicating that AMPK activation is stimulated in the substantia nigra of MPTP-intoxicated mice as well as in MPP^+-treated SH-SH5Y cells.

AMPK inhibits MPP^+-mediated cell death in SH-SY5Y cells

To ascertain whether activation of AMPK plays a role in MPP^+-induced cell death, we used compound C, which is a potent AMPK inhibitor [12] and has been widely used for studying AMPK signaling. As shown in Fig. 3A, pretreatment of cells with 20 μM

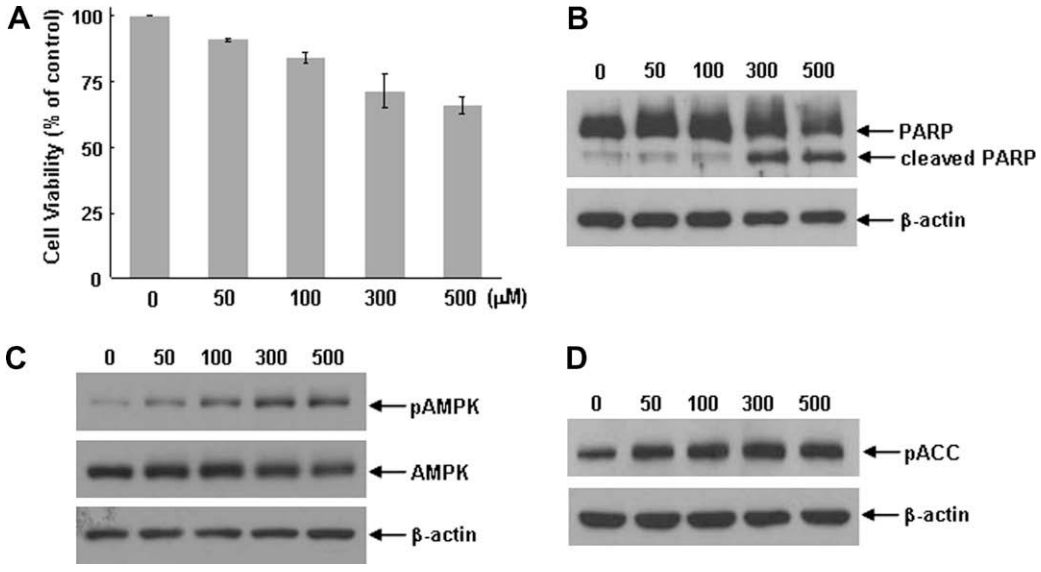

Fig. 1. MPP^+ induces cell death and activation of AMPK in SH-SY5Y cells. (A) SH-SY5Y cells were exposed to MPP^+ with indicated concentrations for 48 h. The viability was examined by MTT assay. The data are expressed as means ± S.E. for three determinations in four independent experiments. (B–D) Whole cell lysates were subjected to Western blot analysis using anti-PARP, anti-phospho-AMPKα and anti-AMPKα, and anti-phospho-ACC antibodies, respectively. β-Actin served as a loading control for all.

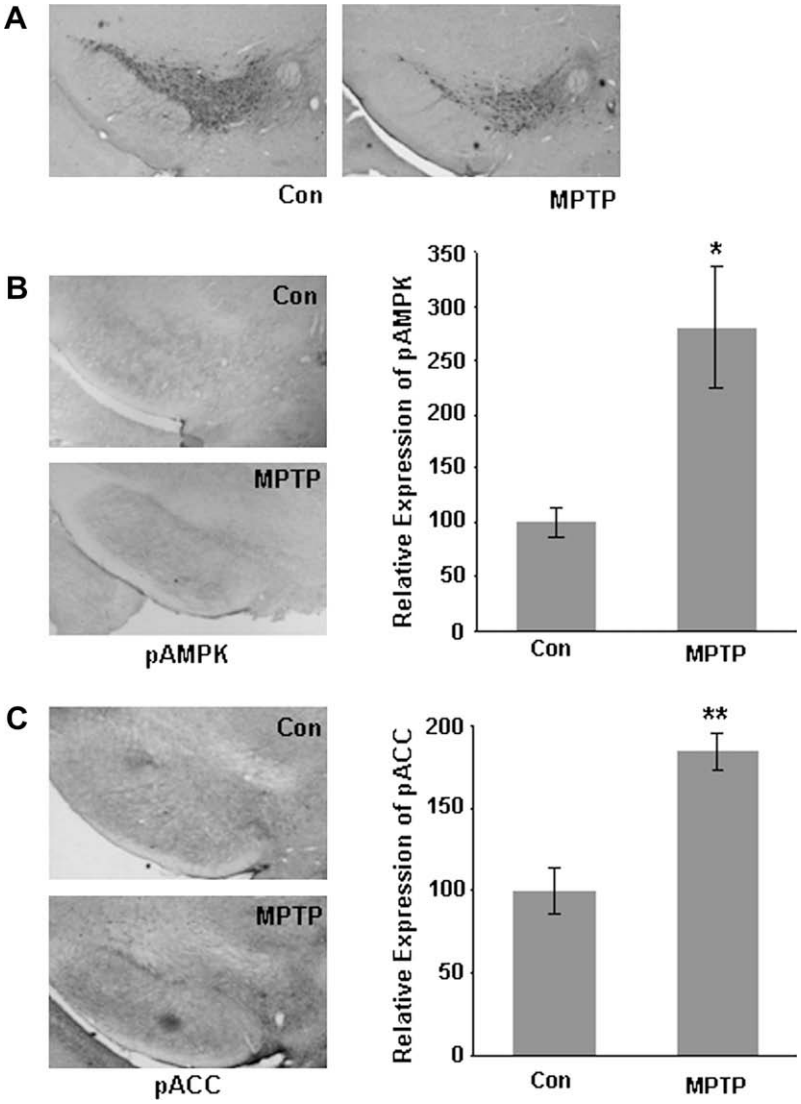

Fig. 2. AMPK is activated in the MPTP-induced PD mouse model. (A) TH was stained in coronal midbrain sections of saline and MPTP-injected mice. The phospho-AMPK (B) and phospho-ACC (C) were stained and statistical analyses were performed. Quantitative values were normalized to 100% of control mice. Data (means ± S.E.) are representative of three experiments with 8–10 mice per group. Con, saline-injected mouse; MPTP, MPTP-injected mouse; $^{*}P < 0.05$; $^{**}P < 0.001$.

compound C for 4 h prevented the AMPK activation by MPP^+ and stimulated MPP^+-induced cell death. To confirm the function of AMPK, wild-type AMPK was transiently transfected into SH-SY5Y cells. When AMPK was overexpressed, MPP^+-induced cell death was significantly inhibited (Fig. 3B), suggesting that activation of AMPK function as a survival factor against MPP^+-induced cell death. To verify the effects of AMPK on MPP^+-induced cell death, we used dominant negative AMPK expression vector. When the function of endogenous AMPK was inhibited by dominant negative form, MPP^+-induced cell death was stimulated like the effects of compound C (Fig. 3C). Finally, we tried to examine that AMPK is activated in dying cells mediated by MPP^+. As shown in Fig. 3D, distinct nuclear condensation was observed in MPP^+-treated cells. The phosphorylated form of AMPK was specifically detected in cells showing nuclear condensation (Fig. 3D), indicating that AMPK is activated in dying cells. Collectively, these results indicate that activation of AMPK functions as a survival signal after MPP^+ treatment in SH-SY5Y cells.

Discussion

Although many PD studies have been conducted, the cause of dopaminergic cell death in the substantia nigra pars compacta of PD patients has still not been clearly identified [5]. Administration of MPTP/MPP^+ is one of the experimental models most commonly used to examine the pathogenesis of PD [4]. Many different molecules have been shown to be involved in neuronal cell death in this model, which makes identification of the underlying stimuli complex. These molecules include nitric oxide, ROS, ceramide and several proteins such as transcription regulators, Bcl-2, caspases, and protein kinases [2,3,6,7]. Rescue and protection studies of PD have involved interfering with the cell death process as well as promoting growth and function of the remaining dopaminergic neurons left within the nigrostriatum [25].

AMPK is a master cellular energy gauge and can be activated by accumulation of AMP under specific conditions such as hypoxia, oxidative stress and glucose deprivation, or by accelerating of ATP consumption as occurs with rapid contraction of skeletal muscle [8]. Mitochondrial dysfunction has been implicated as part of the pathogenesis of PD in patients [3]. Such dysfunction increases free radical production and oxidative stress and decreases ATP production [3]. Here, we showed that MPP^+ activated AMPK in SH-SY5Y cells and induced cell death (Fig. 1). Exposure to MPTP results in parkinsonism symptoms indistinguishable from those of PD and induces a specific loss of dopaminergic neurons in the substantia nigra pars compacta

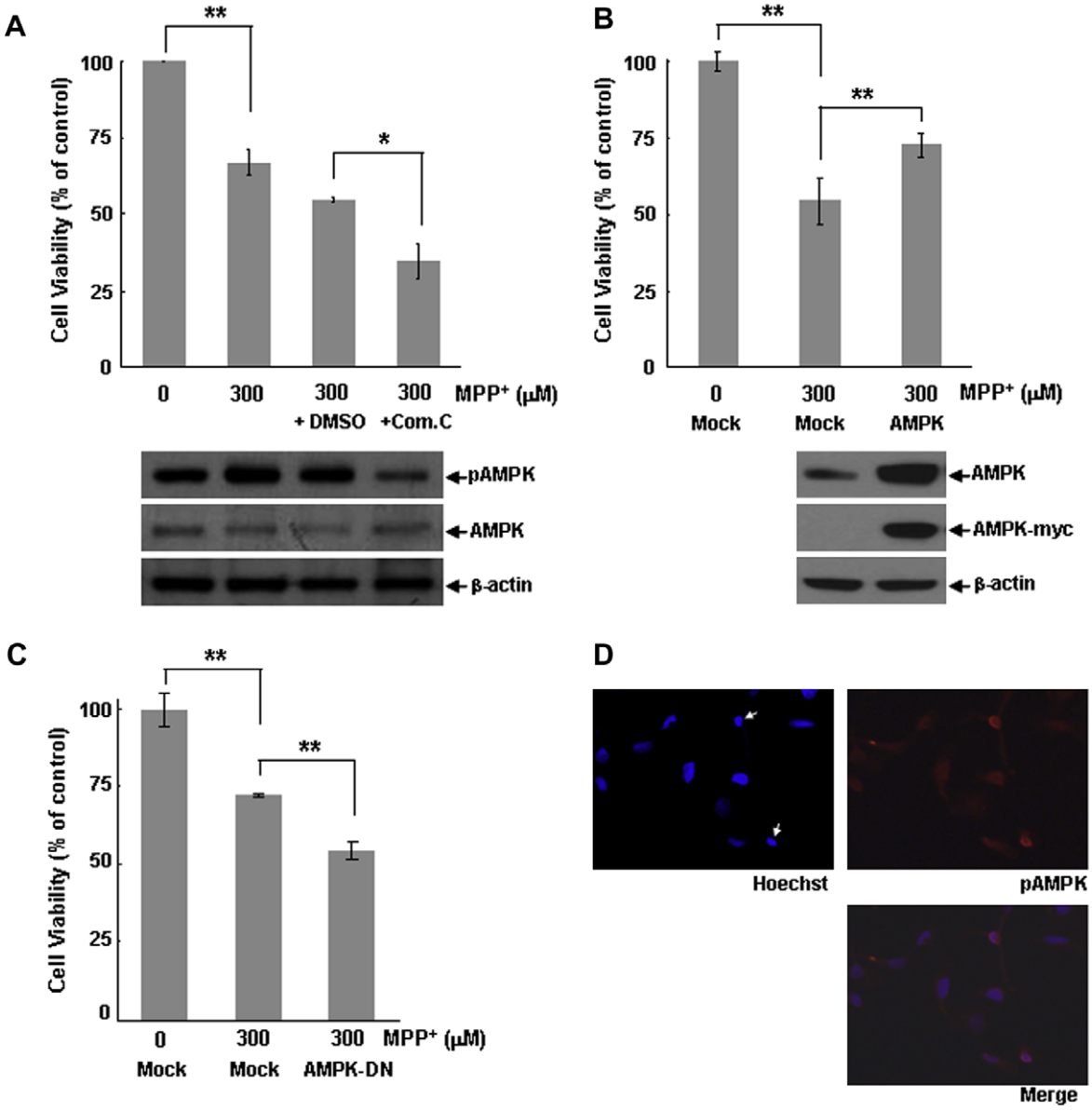

Fig. 3. Effects of AMPK on MPP^+-induced cell death in SH-SY5Y cells. (A) Cells were treated with compound C (20 μM) for 4 h, then exposed to MPP^+ (300 μM) as indicated. The viability was measured by MTT assay and the data are expressed as means ± S.E. for three determinations in three independent experiments. Under the same conditions, the total cell extracts were subjected to Western blot analysis as indicated (bottom). $^*P < 0.05$; $^{**}P < 0.01$; Com.C, compound C. (B,C) Cells were transfected with a myc-tagged wild-type AMPK or dominant negative AMPK expression vector, and then 300 μM of MPP^+ was added for 48 h. The MTT assay data are representative as indicated based on three independent experiments in triplicate. The transfection and expression of AMPK was assessed by Western blot analysis using anti-AMPK and anti-myc antibodies (bottom). β-Actin served as a loading control for all. $^{**}P < 0.01$; mock, empty vector transfected; AMPK, pcDNA-Ad-AMPKα-myc transfected; AMPK-DN, pcDNA-Ad-dominant negative AMPKα-myc transfected. (D) Immunofluorescence analysis was performed. Cy3-conjugated secondary antibody was used to visualize protein by fluorescence microscopy of pAMPK in MPP^+-treated SH-SY5Y cells. After immunostaining, SH-SY5Y cells were also stained with Hoechst 33342, and arrows indicate chromosomal DNA fragmentation.

[5]. TH-positive neurons were decreased by MPTP in the midbrain region, while phospho-AMPK-Thr^{172} and phospho-ACC-Ser^{79} were increased, indicating AMPK was activated (Fig. 2).

AMPK plays a role in cell survival in ceramide-induced apoptosis in astrocyes and glucose deprivation-mediated cell death in developing rat brain [18,19]. On the other hand, active AMPK promotes damage of hippocampal and cortical neurons under ischemic conditions [20]. Thus, the role of active AMPK on cell survival remains controversial. We showed that MPP^+-induced cell death was increased by inhibition of AMPK, but decreased by AMPK overexpression (Fig. 3). Moreover, we also showed that activation of AMPK is occurred in dying cells mediated by treatment with MPP+ (Fig. 3). Our data strongly indicate that the activation of AMPK exerts a protective effect against the neurotoxic effects of MPP^+. Based on our results, it seems rational to view AMPK as a survival factor for dopaminergic neurons in PD. Consequently, further study on the mechanisms underlying the exact role of AMPK in PD will help make AMPK as a valuable molecular target for PD therapy.

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References

[1] L.S. Forno, Neuropathology of Parkinson's disease, J. Neuropathol. Exp. Neurol. 55 (1996) 259–272.
[2] L. Tretter, I. Sipos, V. Adam-Vizi, Initiation of neuronal damage by complex I deficiency and oxidative stress in Parkinson's disease, Neurochem. Res. 29 (2004) 569–577.
[3] P. Jenner, Oxidative stress in Parkinson's disease, Ann. Neurol. 53 (Suppl. 3) (2003) S26–S36 (discussion S36–S38).
[4] B. Dipasquale, A.M. Marini, R.J. Youle, Apoptosis and DNA degradation induced by 1-methyl-4-phenylpyridinium in neurons, Biochem. Biophys. Res. Commun. 181 (1991) 1442–1448.
[5] R.J. Smeyne, V. Jackson-Lewis, The MPTP model of Parkinson's disease, Brain Res. Mol. Brain Res. 134 (2005) 57–66.
[6] T. Borsello, G. Forloni, JNK signalling: a possible target to prevent neurodegeneration, Curr. Pharm. Des. 13 (2007) 1875–1886.
[7] S. Karunakaran, U. Saeed, M. Mishra, R.K. Valli, S.D. Joshi, D.P. Meka, P. Seth, V. Ravindranath, Selective activation of p38 mitogen-activated protein kinase in dopaminergic neurons of substantia nigra leads to nuclear translocation of p53 in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice, J. Neurosci. 28 (2008) 12500–12509.
[8] J.S. Oakhill, J.W. Scott, B.E. Kemp, Structure and function of AMP-activated protein kinase, Acta Physiol. (Oxf.) (2009).
[9] S.A. Hawley, M. Davison, A. Woods, S.P. Davies, R.K. Beri, D. Carling, D.G. Hardie, Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase, J. Biol. Chem. 271 (1996) 27879–27887.
[10] M.J. Birnbaum, Activating AMP-activated protein kinase without AMP, Mol. Cell 19 (2005) 289–290.
[11] S.A. Hawley, D.A. Pan, K.J. Mustard, L. Ross, J. Bain, A.M. Edelman, B.G. Frenguelli, D.G. Hardie, Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase, Cell Metab. 2 (2005) 9–19.
[12] S.N. Jung, W.K. Yang, J. Kim, H.S. Kim, E.J. Kim, H. Yun, H. Park, S.S. Kim, W. Choe, I. Kang, J. Ha, Reactive oxygen species stabilize hypoxia-inducible factor-1 alpha protein and stimulate transcriptional activity via AMP-activated protein kinase in DU145 human prostate cancer cells, Carcinogenesis 29 (2008) 713–721.
[13] I.J. Park, J.T. Hwang, Y.M. Kim, J. Ha, O.J. Park, Differential modulation of AMPK signaling pathways by low or high levels of exogenous reactive oxygen species in colon cancer cells, Ann. N. Y. Acad. Sci. 1091 (2006) 102–109.
[14] J. Weekes, S.A. Hawley, J. Corton, D. Shugar, D.G. Hardie, Activation of rat liver AMP-activated protein kinase by kinase kinase in a purified, reconstituted system. Effects of AMP and AMP analogues, Eur. J. Biochem. 219 (1994) 751–757.
[15] M. Suwa, H. Nakano, S. Kumagai, Effects of chronic AICAR treatment on fiber composition, enzyme activity, UCP3, and PGC-1 in rat muscles, J. Appl. Physiol. 95 (2003) 960–968.
[16] L.G. Fryer, E. Hajduch, F. Rencurel, I.P. Salt, H.S. Hundal, D.G. Hardie, D. Carling, Activation of glucose transport by AMP-activated protein kinase via stimulation of nitric oxide synthase, Diabetes 49 (2000) 1978–1985.
[17] Z.P. Chen, G.K. McConell, B.J. Michell, R.J. Snow, B.J. Canny, B.E. Kemp, AMPK signaling in contracting human skeletal muscle: acetyl-CoA carboxylase and NO synthase phosphorylation, Am. J. Physiol. Endocrinol. Metab. 279 (2000) E1202–E1206.
[18] C. Blazquez, M.J. Geelen, G. Velasco, M. Guzman, The AMP-activated protein kinase prevents ceramide synthesis de novo and apoptosis in astrocytes, FEBS Lett. 489 (2001) 149–153.
[19] C. Culmsee, J. Monnig, B.E. Kemp, M.P. Mattson, AMP-activated protein kinase is highly expressed in neurons in the developing rat brain and promotes neuronal survival following glucose deprivation, J. Mol. Neurosci. 17 (2001) 45–58.
[20] L.D. McCullough, Z. Zeng, H. Li, L.E. Landree, J. McFadden, G.V. Ronnett, Pharmacological inhibition of AMP-activated protein kinase provides neuroprotection in stroke, J. Biol. Chem. 280 (2005) 20493–20502.
[21] Y.S. Kim, K.H. Ahn, S.Y. Kim, J.W. Jeong, Okadaic acid promotes angiogenesis via activation of hypoxia-inducible factor-1, Cancer Lett. 276 (2009) 102–108.
[22] C. Ehrhanrdt, M. Schmolke, A. Matzke, A. Knoblauch, C. Will, V. Wixler, S. Ludwing, Polyethylenimine, a cost-effective transfection reagent, Signal Transduct. 6 (2006) 179–184.
[23] V. Jackson-Lewis, S. Przedborski, Protocol for the MPTP mouse model of Parkinson's disease, Nat. Protoc. 2 (2007) 141–151.
[24] Y. Rong, V.E. Belozerov, C. Tucker-Burden, G. Chen, D.L. Durden, J.J. Olson, E.G. Van Meir, N. Mackman, D.J. Brat, Epidermal growth factor receptor and PTEN modulate tissue factor expression in glioblastoma through JunD/activator protein-1 transcriptional activity, Cancer Res. 69 (2009) 2540–2549.
[25] N.D. Stull, D.P. Polan, L. Iacovitti, Antioxidant compounds protect dopamine neurons from death due to oxidative stress in vitro, Brain Res. 931 (2002) 181–185.