MicroRNA-7 Promotes Glycolysis to Protect against 1-Methyl-4-phenylpyridinium-induced Cell Death*

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Background: The dopaminergic neurotoxin, 1-methyl-4-phenylpyridinium (MPP⁺), kills cells by inhibiting mitochondrial oxidative phosphorylation.

Results: MicroRNA-7-mediated repression of RelA expression and subsequent increase in glucose transporter Glut3 level augments glycolysis in neuronal cells.

Conclusion: MicroRNA-7 protects cells against MPP⁺-induced cytotoxicity by enhancing glycolysis.

Significance: MicroRNA-7 could potentially mitigate the effects of mitochondrial oxidative phosphorylation defects in Parkinson disease.

Parkinson disease is associated with decreased activity of the mitochondrial electron transport chain. This defect can be recapitulated in vitro by challenging dopaminergic cells with 1-methyl-4-phenylpyridinium (MPP⁺), a neurotoxin that inhibits complex I of electron transport chain. Consequently, oxidative phosphorylation is blocked, and cells become dependent on glycolysis for ATP production. Therefore, increasing the rate of glycolysis might help cells to produce more ATP to meet their energy demands. In the present study, we show that microRNA-7, a non-coding RNA that protects dopaminergic neuronal cells against MPP⁺-induced cell death, promotes glycolysis in dopaminergic SH-SY5Y and differentiated human neural progenitor ReNcell VM cells, as evidenced by increased ATP production, glucose consumption, and lactic acid production. Through a series of experiments, we demonstrate that targeted repression of RelA by microRNA-7, as well as subsequent increase in the neuronal glucose transporter 3 (Glut3), underlies this glycolysis-promoting effect. Consistently, silencing Glut3 expression diminishes the protective effect of microRNA-7 against MPP⁺. Further, microRNA-7 fails to prevent MPP⁺-induced cell death when SH-SY5Y cells are cultured in a low glucose medium, as well as when differentiated ReNcell VM cells or primary mouse neurons are treated with the hexokinase inhibitor, 2-deoxy-D-glucose, indicating that a functional glycolytic pathway is required for this protective effect. In conclusion, microRNA-7, by down-regulating RelA, augments Glut3 expression, promotes glycolysis, and subsequently prevents MPP⁺-induced cell death. This protective effect of microRNA-7 could be exploited to correct the defects in oxidative phosphorylation in Parkinson disease.

Parkinson disease (PD)² is a chronic, progressive neurodegenerative disorder characterized by accumulation of misfolded protein aggregates containing α-synuclein and loss of dopaminergic neurons in the substantia nigra pars compacta. It manifests clinically as tremor, rigidity, bradykinesia, and shuffling gait. Multiple mechanisms have been suggested to contribute to the degeneration of dopaminergic neurons in PD. Among them, several lines of evidence indicate dysfunctional mitochondria to be an important contributing factor at the sub-cellular level (1–3). NADH-ubiquinone reductase (complex I of mitochondrial electron transport chain) activity has been found to be significantly reduced in the substantia nigra of PD patients (4). This likely results from oxidative damage and misassembly of the catalytic subunits of complex I (5). In addition, the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) inhibits complex I of the electron transport chain after being converted into its active metabolite 1-methyl-4-phenylpyridinium (MPP⁺) and results in a Parkinsonian phenotype in humans, non-human primates, and mice (6). As MPP⁺-induced mitochondrial dysfunction leads to decreased production of ATP (7), cells attempt to meet their energy demand by enhancing the rate of glycolysis (8). Therefore, strategies to further increase the glycolytic rate could be exploited to sustain ATP production and slow the progression of neuronal degeneration in PD.

MicroRNAs are small, non-coding RNAs that regulate several cellular pathways by altering the expression of their target genes. Dysregulation of microRNA expression and/or function has been associated with neurodegenerative disorders including PD (9–11). MicroRNA-7 (miR-7) was first identified to play a protective role in cellular models of PD by directly targeting

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² The abbreviations used are: PD, Parkinson disease; 2-DG, 2-deoxy-D-glucose; Eno1, enolase1; miR-7, microRNA-7; miR-SC, scrambled microRNA control; MPP⁺, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MTS, 3-(4,5-dimethylazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; NF-κB, nuclear factor κB; PDK4, pyruvate dehydrogenase kinase 4; P-enolpyruvate, phosphoenolpyruvate; PGK1, phosphoglycerate kinase 1; qPCR, quantitative real-time PCR; SCO2, regulator of oxidative phosphorylation cytochrome c oxidase assembly protein; tRFP, turbo red fluorescent protein; siRNA-NT, non-targeting siRNA; lent, lentiviral.
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and down-regulating α-synuclein expression (12, 13). miR-7 also protects against MPP⁺-induced neurotoxicity (14, 15). In the latter model, the cytoprotective role of miR-7 is mediated through down-regulation of its target RelA, a member of the nuclear factor κB (NF-κB) family of transcription factors. In the present study, we show that miR-7 promotes glycolysis as measured by increases in intracellular ATP/ADP ratio, glucose consumption, and lactic acid production. This effect of miR-7 is mediated through down-regulating the expression of its target gene, RelA. In addition, the glycolysis-promoting effect of miR-7 is mediated by increased expression of the cell surface glucose transporter, Glut3, whose expression is regulated by RelA. Decreasing cellular glucose uptake by silencing the Glut3 expression or inhibiting hexokinase, which is the enzyme that catalyzes the first step in glycolysis, diminished the cytoprotective effect of miR-7. These results suggest that miR-7 could help improve cellular bioenergetics and promote survival of dopaminergic neurons in PD.

**EXPERIMENTAL PROCEDURES**

**Materials**—MPP⁺ and 2-deoxy-d-glucose (2-DG), were purchased from Sigma. Non-targeting siRNA (siRNA-NT), siRNA-RelA, and siRNA-Glut3 were purchased from Thermo Scientific. Scrambled microRNA control (miR-SC), miR-7, anti-miR negative control, and anti-miR-7 were purchased from Ambion. Lentiviral vector pLemiR-pri-miR-7-2 (lenti-miR-7) and miR-SC (lenti-miR-SC) containing turboRFP reporter, as well as pGIPz-shRNA-RelA (lenti-shRNA-RelA) and control shRNA (lenti-shRNA-NT) constructs, were purchased from Open Biosystems.

**Animals**—All animals were housed and handled in accordance with the Institutional Animal Care guidelines of Rutgers Robert Wood Johnson Medical School. C57BL/6J mice purchased from The Jackson Laboratory were used in this study.

**Cell Culture**—SH-SY5Y cells and HEK293T cells were cultured in DMEM (Life Technologies) containing 25 mM glucose in the medium was quantified using a lactic acid assay kit (BioVision) according to the manufacturer’s instructions. Glucose and Lactic Acid Assays—Glucose remaining in the medium was quantified using glucose oxidase-peroxidase (Sigma) reaction coupled to oxidation of Amplx Red reagent (Life Technologies). Glucose present in the medium was converted to d-gluconic acid and H₂O₂ by glucose oxidase. The H₂O₂ produced in this reaction was then utilized in the conversion of Amplx Red to resorufin by peroxidase. Resorufin is a colored product whose absorbance, measured at 560 nm, is proportional to the amount of glucose present in the medium. Glucose consumption by cells was calculated by subtracting the amount of glucose present in cell culture medium from that present in the medium without any cells. Lactic acid produced in the medium was quantified using a lactic acid assay kit (Sigma) according to the manufacturer’s protocol.

**Cell Viability Assay**—Cell viability was measured using CellTiter 96 aqueous 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) reagent (Promega) according to the manufacturer’s instructions.

**RNA Extraction and Quantitative Real-time PCR (qPCR)**—RNA was extracted using the miRNeasy mini kit (Qiagen) followed by reverse transcription to synthesize cDNA using SuperScript III (Life Technologies) according to the manufa...
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**RESULTS**

miR-7 Increases Intracellular ATP Production—In a recent study, we demonstrated that miR-7 protects neuronal cells against MPP⁺-induced cytotoxicity by targeting the 3’-UTR of RelA mRNA and repressing its expression (15). MPP⁺ inhibits complex I of the mitochondrial electron transport chain, leading to decreased ATP production. The inability to meet the energy requirements of cellular processes ultimately results in cell death. As overexpression of miR-7 is cytoprotective, we investigated whether miR-7 overexpression can also increase ATP production, thereby helping cells to satisfy their energy demands and improve cell survival.

SH-SY5Y cells were transfected with miR-7 or miR-SC. Forty-eight hours after transfection, cells were treated with two different concentrations of MPP⁺ for 6 h, and intracellular ATP/ADP ratio was measured. As expected, MPP⁺ decreased ATP/ADP ratio in a dose-dependent manner (Fig. 1A). Interestingly, overexpression of miR-7 in un-challenged SH-SY5Y cells resulted in a striking increase of ATP/ADP ratio, indicating that miR-7 promotes ATP production, as compared with cells transfected with miR-SC (Fig. 1A). The increase in ATP/ADP ratio in cells overexpressing miR-7 was maintained even after MPP⁺ treatment. As miR-7 targets RelA expression (15), we next investigated whether RelA knockdown also increases ATP production. Indeed, transfection of siRNA targeting RelA also resulted in a similar increase in ATP/ADP ratio that was...
also maintained after cells were exposed to MPP⁺ (Fig. 1B). To confirm that miR-7 or knockdown of RelA expression can increase ATP production in post-mitotic neuronal cells, differentiated ReNcell VM cells were used. ReNcell VM cells are an immortalized human ventral mesencephalic neural progenitor cell line that can be differentiated into dopaminergic neurons (16). Similar to SH-SYSY cells, overexpression of miR-7 or knockdown of RelA using lentiviral constructs in differentiated ReNcell VM cells led to an increase in ATP/ADP ratio that was sustained even after MPP⁺ challenge (Fig. 1, C and D). Further, the MTS assay showed that cell death during the 6-h MPP⁺ treatment was negligible (data not shown), ensuring that quantification of ATP/ADP ratio was not confounded by differences in cell viability. These results suggest that miR-7 increases ATP production, possibly by down-regulating RelA expression.
**MicroRNA-7 Promotes Glycolysis through Down-regulating RelA Expression**—As MPP⁺ blocks oxidative phosphorylation, miR-7-induced increase in ATP production could result from enhancing other metabolic pathways such as glycolysis. To assess glycolysis, we measured glucose consumption and lactic acid production in SH-SY5Y cells. Indeed, overexpression of miR-7 significantly increased both glucose consumption and lactic acid production (Fig. 2, A and C). Treatment with MPP⁺ also resulted in a concentration-dependent increase in glucose consumption and lactic acid production, which is consistent with previous studies (8), likely representing a defensive mechanism by cells in the face of inefficient mitochondrial ATP production upon MPP⁺ challenge. Cells overexpressing miR-7, however, further increased their glucose consumption and lactic acid production even after MPP⁺ treatment (Fig. 2, A and C). Similarly, knockdown of RelA also increased glucose consumption and lactic acid production, which were maintained after MPP⁺ challenge as well (Fig. 2, B and D).

To confirm our findings in post-mitotic neuronal cell, ReN-cell VM cells were transduced with lentivirus expressing miR-7 or a control lentivirus. Three days after transduction, cells were treated with increasing concentrations of MPP⁺ for 6 h, followed by measurements of glucose consumption and lactic acid in the culture medium. As observed in SH-SY5Y cells, miR-7 overexpression increased glucose consumption and lactic acid production (Fig. 2, E and G), suggesting that miR-7 promotes glycolysis in differentiated neuronal cells as well. Knockdown of RelA by infecting lentivirus expressing shRNA-RelA also promoted glycolysis in ReNcell VM cells (Fig. 2, F and H). Similar to SH-SY5Y cells, miR-7- or shRNA-RelA-induced increase in glucose consumption and lactic acid production was sustained even after exposure of ReNcell VM cells to MPP⁺ (Fig. 2, E–H).

To determine whether miR-7-mediated increase in glycolysis was a result of increase in glycolytic enzyme activity, we measured the pyruvate kinase activity in SH-SY5Y cells. Pyruvate kinase catalyzes the final step of glycolysis and represents an important control site. Pyruvate kinase catalyzes the irreversible conversion of phosphoenolpyruvate (P-enolpyruvate) to pyruvate. Overexpression of miR-7 or knockdown of RelA resulted in statistically significant increase in pyruvate kinase activity (Fig. 3, A and B), although not as dramatic as in other glycolytic parameters (ATP/ADP ratio, glucose consumption, and lactic acid production) (Figs. 1 and 2). Pyruvate kinase activity is allosterically regulated by fructose 1,6-bisphosphate, P-enolpyruvate, and ATP (18, 19). Although fructose 1,6-bisphosphate and P-enolpyruvate increase pyruvate kinase activity (19, 20), high ATP level decreases it (19). The glycolysis-promoting effect of miR-7 leads to increase in fructose 1,6-bisphosphate, P-enolpyruvate, and ATP inside the cell. As these mediators have opposing effects on pyruvate kinase activity, the net result of the end-point assay may, therefore, reflect only a modest change in enzyme activity.

To assess the role of endogenously expressed miR-7 in enhancing glycolysis, anti-miR-7, a single-stranded oligonucleotide designed to specifically bind to and inhibit endogenous miR-7, was used to determine ATP/ADP ratio, glucose consumption, and lactic acid production. Cells transfected with anti-miR-7 showed significantly greater reduction in ATP/ADP ratio upon exposure to MPP⁺, as compared with cells transfected with anti-scrambled control (Fig. 4A). In addition, glucose consumption and lactic acid production were signifi-
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Significantly reduced in anti-miR-7 transfected cells after MPP⁺ treatment (Fig. 4, B and C), suggesting that endogenously expressed miR-7 promotes glycolysis under physiological conditions. Further, it is worth noting that transfection of anti-miR-7 did not block the dose-dependent increase in glucose consumption and lactic acid production upon MPP⁺ treatment (Fig. 4, B and C), suggesting that miR-7 and MPP⁺ promote glycolysis via distinct mechanisms.

To investigate whether miR-7-mediated down-regulation of RelA underlies the glycolysis-promoting effect of miR-7, we overexpressed a RelA construct lacking the 3'-UTR (and therefore unable to be down-regulated by miR-7) along with miR-7 or miR-SC. Transfection of miR-7 along with a control vector resulted in increased glucose consumption (Fig. 5A) and lactic acid production (Fig. 5B), similar to that observed with miR-7 alone. Concomitant overexpression of RelA, however, abrogated the glycolysis-promoting activity of miR-7. These results demonstrate that the glycolysis-promoting effect of miR-7 requires down-regulation of RelA.

miR-7 Increases Expression of the Glucose Transporter Glut3—To elucidate the molecular mechanism by which miR-7 enhances glycolysis, we examined the expression levels of genes that affect glycolytic rate. The following genes that regulate glycolysis were selected for qPCR analysis based on previous studies suggesting their regulation by RelA/NF-κB (21): the glucose transporters Glut1, -3, -4, and -5; the glycolytic enzymes Enol1 and PGK1; the glycolysis regulator PDK4; and SCO2. Among the genes tested, expression of Glut1, Glut4, Enol1, PGK1, and SCO2 did not show any significant change with miR-7 overexpression (Fig. 6A) or RelA knockdown (Fig. 6B). Glut5 expression was negligible in SH-SY5Y cells and was, therefore, not considered for further analysis. Only expression of Glut3 was found to increase significantly following overexpression of miR-7 (Fig. 6A) or knockdown of RelA (Fig. 6B). In addition, successful down-regulation of RelA after transfection of miR-7 or siRNA-RelA was confirmed by qPCR (data not shown). Elevation of Glut3 level might help the efficient uptake of glucose from the medium and increases the rate of glycolysis.
Indeed, knocking down the expression of Glut3, in part, abrogated the cytoprotective effect of miR-7 (Fig. 6C), suggesting that the protective effect of miR-7 exerts partly through up-regulation of Glut3 via repressing RelA expression. Knocking down the expression of Glut3 by siRNA-Glut3 was confirmed by qPCR (Fig. 6D). Inhibition of Glycolysis Results in Loss of miR-7-mediated Protection—Considering that miR-7 protects cells against MPP⁺/H₁₁₀₀₁ through promoting glycolysis, we expect that lowering glucose concentration in the medium will abolish the protective effects of miR-7. To test this hypothesis, we cultured SH-SY5Y cells in medium containing 5 mM glucose (low glucose medium). Reducing glucose concentration alone for several passages was not toxic to cells (data not shown). However, the protective effect of miR-7 against MPP⁺ was abrogated when cells were cultured in low glucose medium (Fig. 7A). In addition, the cytoprotection against MPP⁺ offered by silencing RelA expression was also abrogated by culturing cells in low glucose medium (Fig. 7B). Decreased RelA protein level after transfection of miR-7 or siRNA-RelA in high glucose or low glucose medium was confirmed by Western blot (Fig. 7C–F). To confirm this finding in post-mitotic neuronal cells, we treated differentiated ReNcell VM cells with 2-DG, a glucose analog that inhibits hexokinase and blocks glycolysis. Consistently, the cytoprotection against MPP⁺ seen with miR-7 overexpression (Fig. 8A) or RelA silencing (Fig. 8B) was lost when cells were cultured with 2-DG. To confirm this effect of miR-7 in primary neurons, mouse primary cortical neurons were transduced with lenti-miR-SC or lenti-miR-7, and then cells were exposed to MPP⁺ in the absence or presence of 2-DG. Neurotoxicity was assessed by measuring neurite length and TUNEL assay. Indeed, miR-7-expressing neurons were protected from this neurotoxic effect of MPP⁺ as evidenced by significantly longer neurites, as compared with miR-SC-expressing neurons (Fig. 8, C and D), which is consistent with our previous study (15) as well as with the lower number of TUNEL-positive cells (Fig. 8, E and F). However, when glycolysis was inhibited by 2-DG, a significant decrease in neurite length and a significant increase in the number of TUNEL-positive cells were observed even in the miR-7-expressing neurons (Fig. 8, C–F), indicating that a functional glycolysis pathway is required for miR-7-induced neuroprotection. Exposure to 2-DG alone for 24 h did not alter neuron morphology, neurite length, or cell viability. Taken together, these data indicate that miR-7 promotes glycolysis by targeting RelA, and subsequently leads to protection against MPP⁺.

**FIGURE 7.** Protective effect of miR-7 overexpression or RelA knockdown is lost when glucose availability is limited. A and B, SH-SY5Y cells were cultured in the medium containing 25 mM (high) (H) or 5 mM (low) (L) glucose. Cells were transfected with miR-SC or miR-7 (A) and siRNA-NT or siRNA-RelA (B). After 48 h, cells were treated with the indicated concentrations of MPP⁺ for 24 h. Cell viability was quantified using the MTS assay. C and D, representative Western blots showing that transfection of miR-7 or siRNA-RelA decreases RelA protein level. β-Actin was used as loading control. E and F, quantification of RelA protein level from three independent experiments represented in C and D. Band density was measured to quantify RelA protein level relative to β-actin expression level using ImageJ. Data are presented as means ± S.E. *, p < 0.05, **, p < 0.01, ***, p < 0.001. These results are representative of three separate experiments.
DISCUSSION

In the present study, we have elucidated a novel neuroprotective mechanism of miR-7. We show that miR-7 enhances glycolysis and enables neuronal cells to meet their energy requirement when oxidative phosphorylation is inhibited by MPP⁺. This increase in glycolysis is achieved by the activity of miR-7 to repress RelA expression and subsequently increase Glut3 expression. Furthermore, we demonstrate that the availability of glucose as substrate and a functional glycolytic pathway are both essential for the cytoprotective effect of miR-7.

miR-7 is highly expressed in the brain, including in tyrosine hydroxylase-positive dopaminergic neurons in the substantia nigra (15), and appears to regulate key neuronal functions such as neuronal differentiation and neurite outgrowth (22). Silencing of miR-7 function in the mouse embryonic cortex results in impaired cortical development (23). Additionally, miR-7 has been shown to protect neuronal cells in several models of PD. The first identified neuroprotective mechanism of miR-7 was through down-regulation of α-synuclein by targeting the 3'-UTR of its mRNA (12). Subsequently, miR-7 was found to protect against MPP⁺-induced cell death by activating the mammalian target of rapamycin (mTOR) pathway (14) and by relieving MPP⁺-induced NF-κB suppression via down-regulating the NF-κB subunit, RelA (15).

The glycolysis-promoting activity of miR-7 appears to be particularly important in the brain, where glucose is primarily utilized for the generation of ATP as energy source (24, 25). In healthy and resting neurons, the level of activity of glycolytic
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enzyms is maintained far below their maximum potential. Hexokinase activity, for example, is kept at 97% inhibited state (25). As a result, tricarboxylic acid (TCA) cycle and oxidative phosphorylation can completely catabolize glucose to generate ATP. However, the reported defects in mitochondrial oxidative phosphorylation in PD (4, 5) could suggest that, in this pathological condition, neurons become increasingly dependent on glycolysis for ATP production. Consistently, neuronal cells try to increase their ATP production by enhancing the rate of glycolysis as a self-protective response upon MPP+ exposure (8). Further, MPTP administration to mice also increases glycolysis as evidenced by elevated lactate levels in the striatum (26). However, neuronal cells eventually die despite this self-protective response, possibly due to the limited supply of ATP from inefficient glycolysis in the pathological condition. A larger and more persistent increase in glycolysis would prolong cell survival and, thus, help halt or slow neuronal degeneration. Enhancing glycolysis by miR-7 may, therefore, provide a new therapeutic approach in PD. Our data indicate that miR-7 not only increases the basal rate of glycolysis but also maintains this enhanced glycolytic rate and ATP production in neuronal cells after treatment with MPP+. Additionally, MPP+-induced increase in glycolysis was still observed even after significant increase of glycolytic rate by miR-7 overexpression, suggesting that miR-7 and MPP+ promote glycolysis via independent mechanisms. Inhibition of endogenous miR-7 led to a decrease in ATP production, glucose consumption, and lactic acid production, indicating that endogenous miR-7 also promotes glycolysis. However, inhibition of miR-7 did not block MPP+-induced increase in glycolysis, further supporting our inference that miR-7 and MPP+ enhance glycolysis through separate pathways.

miR-7 promotes glycolysis by repressing its target RelA. In accordance with our findings, previous studies have demonstrated that knockdown of RelA facilitates switchover from oxidative phosphorylation to glycolysis (21). To elucidate the mechanism by which miR-7 and/or down-regulation of RelA increases glycolysis, we determined the level of several RelA-miRNA molecules: role of miRNAs in neurodegeneration research.

REFERENCES
1. Exner, N., Lutz, A. K., Haass, C., and Winklhofer, K. F. (2012) Microdendrology in Parkinson’s disease: molecular mechanisms and pathophysiological consequences. EMBO J. 31, 3038–3062
2. Dass, V., Junn, E., and Mouradian, M. M. (2013) The role of oxidative stress in Parkinson’s disease. J. Parkinsons Dis. 3, 461–491
3. Subramaniam, S. R., and Chesselet, M. F. (2013) Mitochondrial dysfunction and oxidative stress in Parkinson’s disease. Prog. Neurobiol. 106–107, 17–32, 10.1016/j.pneurobio.2013.04.004
4. Schapira, A. H., Cooper, J. M., Dextor, D., Clark, J. B., Jenner, P., and Marsden, C. D. (1990) Mitochondrial complex I deficiency in Parkinson’s disease. J. Neurochem. 54, 832–837
5. Keeney, P. M., Xie, J., Capaldi, R. A., and Bennett, J. P., Jr. (2006) Parkinson’s disease brain mitochondrial complex I has oxidatively damaged subunits and is functionally impaired and misassembled. J. Neurosci. 26, 5256–5264
6. Langston, J. W., Ballard, P., Tetrud, J. W., and Irwin, I. (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 219, 979–980
7. Bates, T. E., Heales, S. J., Davies, S. E., Boakye, P., and Clark, J. B. (1994) Effects of 1-methyl-4-phenylpyridinium on isolated rat brain mitochondria: evidence for a primary involvement of energy depletion. J. Neurochem. 63, 640–648
8. Mazzio, E., and Soliman, K. F. (2003) The role of glycolysis and gluconeogenesis in the cytoprotection of neuroblastoma cells against 1-methyl-4-phenylpyridinium ion toxicity. Neurotoxicology 24, 137–147
9. Junn, E., and Mouradian, M. M. (2012) MicroRNAs in neurodegenerative diseases and their therapeutic potential. Pharmacol. Ther. 133, 142–150
10. Harraz, M. M., Dawson, T. M., and Dawson, V. L. (2011) MicroRNAs in Parkinson’s disease. J. Chem. Neuroanat. 42, 127–130
11. Yelamanchili, S. V., and Fox, H. S. (2010) Defining larger roles for “tiny” RNA molecules: role of miRNAs in neurodegeneration research. J. Neuroimmun. Pharmacol. 5, 63–69
12. Junn, E., Lee, K. W., Jeong, B. S., Chan, T. W., Im, J. Y., and Mouradian, M. M. (2009) Repression of α-synuclein expression and toxicity by microRNA-7. Proc. Natl. Acad. Sci. U.S.A. 106, 13052–13057
13. Doxakis, E. (2010) Post-transcriptional regulation of α-synuclein expression by miR-7 and miR-135. J. Biol. Chem. 285, 12726–12734
14. Frangoulou, A., and Doxakis, E. (2014) miR-7 and miR-153 protect neurons against MPP+-induced cell death via upregulation of mTOR pathway.
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Front. Cell. Neurosci. 8, 182

15. Choi, D. C., Chae, Y. J., Kabaria, S., Chaudhuri, A. D., Jain, M. R., Li, H., Mouradian, M. M., and Junn, E. (2014) MicroRNA-7 protects against 1-methyl-4-phenylpyridinium-induced cell death by targeting RelA. J. Neurosci. 34, 12725–12737

16. Donato, R., Miljan, E. A., Hines, S. J., Aouabdi, S., Pollock, K., Patel, S., Edwards, F. A., and Sindén, J. D. (2007) Differential development of neuronal physiological responsiveness in two human neural stem cell lines. BMC Neurosci. 8, 36

17. Meijering, E., Jacob, M., Sarria, J. C., Steiner, P., Hirling, H., and Unser, M. (2004) Design and validation of a tool for neurite tracing and analysis in fluorescence microscopy images. Cytometry A. 58, 167–176

18. Valenti, G., Chiarelli, L., Fortin, R., Speranza, M. L., Galizzi, A., and Mattevi, A. (2000) The allosteric regulation of pyruvate kinase: a site-directed mutagenesis study. J. Biol. Chem. 275, 18145–18152

19. Jurica, M. S., Mesecar, A., Heath, P. J., Shi, W., Nowak, T., and Stoddard, B. L. (1998) The allosteric regulation of pyruvate kinase by fructose-1,6-bisphosphate. Structure 6, 195–210

20. Mattevi, A., Bolognesi, M., and Valenti, G. (1996) The allosteric regulation of pyruvate kinase. FEBS Lett. 389, 15–19

21. Mauro, C., Leow, S. C., Hines, S. J., Aouabdi, S., Pollock, K., Patel, S., Edwards, F. A., and Sindén, J. D. (2007) Differential development of neuronal physiological responsiveness in two human neural stem cell lines. BMC Neurosci. 8, 36

22. Chen, H., Shalom-Feuerstein, R., Riley, J., Zhang, S. D., Tucci, P., Agostini, M., Aberdam, D., Knight, R. A., Gench, G., Nicotera, P., Melino, G., and Vasa-Nicotera, M. (2010) miR-7 and miR-214 are specifically expressed during neuroblastoma differentiation, cortical development and embryonic stem cells differentiation, and control neurite outgrowth in vitro. Biochem. Biophys. Res. Commun. 394, 921–927

23. Pollock, A., Bian, S., Zhang, C., Chen, Z., and Sun, T. (2014) Growth of the developing cerebral cortex is controlled by microRNA-7 through the p53 pathway. Cell Rep. 7, 1184–1196

24. Chih, C. P., and Roberts Jr., E. L. (2003) Energy substrates for neurons during neural activity: a critical review of the astrocyte-neuron lactate shuttle hypothesis. J. Cereb. Blood Flow Metab. 23, 1263–1281

25. Clarke, D. D., and Sokoloff, S. L. (1999) Circulation and energy metabolism of the brain. In Basic Neurochemistry: Molecular, Cellular and Medical Aspects (Siegel, G. J, Agrawal, B. W., Albers, W. R., et al., eds), 6th Ed., pp. 637–669, Lippincott-Raven, Philadelphia

26. Koga, K., Mori, A., Ohashi, S., Kurihara, N., Kitagawa, H., Ishikawa, M., Mitsumoto, Y., and Nakai, M. (2006) 1H MRS identifies lactate rise in the striatum of MPTP-treated C57BL/6 mice. Eur. J. Neurosci. 23, 1077–1081

27. Planavila, A., Laguna, J. C., and Vázquez-Carrera, M. (2005) Nuclear factor-kB activation leads to down-regulation of fatty acid oxidation during cardiac hypertrophy. J. Biol. Chem. 280, 17464–17471

28. Carter, K. L., Cahir-McFarland, E., and Kieff, E. (2002) Epstein-Barr virus-induced changes in B-lymphocyte gene expression. J. Virol. 76, 10427–10436

29. Sommermann, T. G., O’Neill, K., Pas, D. R., and Cahir-McFarland, E. (2011) IKKß and NF-kB transcription govern lymphoma cell survival through AKT-induced plasma membrane trafficking of GLUT1. Cancer Res. 71, 7291–7300

30. Silva, J. L., Giannococco, F., Furuya, D. T., Lima, G. A., Moraes, P. A., Nachev, S., Bordin, S., Britto, L. R., Nunes, M. T., and Machado, U. F. (2005) NF-kB, MEF2A, MEF2D and HIF1-a involvement on insulin- and contraction-induced regulation of GLUT4 gene expression in soleus muscle. Mol. Cell. Endocrinol. 240, 82–93

31. Hinz, M., Lemke, P., Anagnostopoulos, L., Hacker, C., Knappmann, D., Mathas, S., Döcker, B., Zenke, M., Stein, H., and Scheidegger, C. (2002) Nuclear factor κB-dependent gene expression profiling of Hodgkin’s disease tumor cells, pathogenetic significance, and link to constitutive signal transducer and activator of transcription 5a activity. J. Exp. Med. 196, 605–617

32. Vannucci, S. J., Maher, F., and Simpson, I. A. (1997) Glucose transporter proteins in brain: delivery of glucose to neurons and glia. Glia 21, 2–21

33. Kawachi, K., Araki, K., Tobiume, K., and Tanaka, N. (2008) p53 regulates glucose metabolism through an IKK-NFκB pathway and inhibits cell transformation. Nat. Cell Biol. 10, 611–618

34. Kawachi, K., Araki, K., Tobiume, K., and Tanaka, N. (2008) Activated p53 induces NFκB DNA binding but suppresses its transcriptional activation. Biochem. Biophys. Res. Commun. 372, 137–141

35. Cui, H., Schroering, A., and Ding, H. F. (2002) p53 mediates DNA damaging drug-induced apoptosis through a caspase-9-dependent pathway in SH-SY5Y neuroblastoma cells. Mol. Cancer Ther. 1, 679–686

36. Chan, P., Langston, J. W., Irwin, I., DeLanney, L. E., and Di Monte, D. A. (1993) 2-Deoxyglucose enhances 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced ATP loss in the mouse brain. J. Neurochem. 61, 610–616

37. Knight, A. L., Yan, X., Hamamichi, S., Ajjuri, R. R., Mazzulli, J. R., Zhang, M. W., Daigle, J. G., Zhang, S., Borom, A. R., Roberts, L. R., Lee, S. K., DeLeon, S. M., Viiolet-Djlassi, C., Krainc, D., O’Donnell, J. M., Caldwell, K. A., and Caldwell, G. A. (2014) The glycolytic enzyme, GPI, is a functionally conserved modifier of dopaminergic neurodegeneration in Parkinson’s models. Cell Metab. 20, 145–157