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Review Article

EXPLORING THERAPEUTIC TARGET FOR AMYOTROPHIC LATERAL SCLEROSIS: A REVIEW

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Abstract: ***Amyotrophic Lateral Sclerosis (ALS) is a progressive degeneration disorder, which damages the communication between the brain and voluntary muscles. The progression of neurodegeneration develops various symptoms based on the region of neuronal death. The selective vulnerability of specific neuronal subset {fast fatiguing (ff) motor neuron} followed by the progression of the disease to other motor subunit is the most distinct phenomena in the pathogenesis. However, the leading cause of selective vulnerability is still unclear. Currently, approved medications subside the symptoms and slow down the disease progression, but there is no cure for saving the surviving neuronal population. In order to determine the potential drug targets, it is essential to identify the underlying cause of the disease. From the literature study, we proposed a conceivable mechanism for selective neurodegeneration that shows aberration in blood-brain barrier and astrocytes structural and functional integrity is the primary factor for the energy demand, which leads to the neuronal vulnerability of the energy craving ff motor neuron compared to other neuronal subsets. Restoration of blood-brain integrity resolves the metabolic demand in the selectively vulnerable neuron. the currently known in vivo models showed that inhibiting glycogen synthase kinase 3β and activation of peroxisome proliferator resulted in stabilizing the blood-brain barrier from toxic invaders, which in turn increases ATP production, reduces ER stress, improves neuronal survival and serves as a promising therapeutic target to treat the neuronal vulnerability.***

Key words: ***Amyotrophic lateral sclerosis,***

INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) is the most prevalent motor neuron disorder, affecting more than 450,000 people worldwide, and has a significant mortality rate. In the initial stages, the symptoms varied based on which motor neuron is affected. The limbic onset of ALS resulted in muscle weakness

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Dedication: I am deeply grateful to my mentor, Prof. P. D. Gupta for his constant encouragement and support in my research career. His guidance has helped immensely with my research work. I dedicate this article to him on the occation of his 81 st Birth day. .

and cramps, whereas bulbar onset shows the difficulty in swallowing and speech. At later stages, the deterioration of the lung functionality results in respiratory troubles and choking to death [1-3]. The FDA-approved drugs for ALS reduce the symptoms but cannot reverse the motor neuronal damage. Riluzole (N-Methyl-D-aspartic acid (NMDA) inhibitor) inhibits calcium-induced glutamate receptor activation by interfering with voltage-gated potassium channel and blocks glutamate release so that it reduces the excitotoxicity. The proposed mechanism of Edaravone showed that it reduces the neuronal nitric oxide synthase and removes the toxic reactive oxygen species and decreases the lipid peroxidation [4-6]. However, these drugs exhibit side effects such as gait disturbance, hives, headache, drowsiness, and nausea. This shows that, although current medications reduce the symptoms, there are no permanent solutions to rescue the surviving neuron or regenerate the damaged neuron.

In initial stages of ALS, the selective vulnerability of fast fatiguing motor neurons is followed by other motor neurons. Various factors influence neurodegeneration, which include genetic and environmental factors, mitochondrial stress, dysfunction in oxidative phosphorylation-energy metabolism, calcium load, and glutamate toxicity. Identifying the cause of selective vulnerability will shed light on the disease mechanism. A better understanding of the disease mechanism would provide deeper insights into the cellular pathway that helps in recognizing a potential drug target.

Genetic background: The gene mutation plays a critical role in progressive neurodegeneration. The gene variants present in Fused in Sarcoma (FUS), Superoxide dismutase (SOD1), Transactive response DNA binding protein (TARDBP), and chromosome 9 open reading frame 72 (C9orf72) are involved in disease pathogenesis. The ***in vivo*** models showed that defects in these genes resulted in toxic protein accumulation in the neuronal cell, which in turn resulted in aberrant protein function such as dysregulation of trafficking and signal transmission [7-13].

Selective vulnerability in ALS: The Fast fatigable motor neuron (FF neuron) is a subset of alpha motor neurons (also known as white fiber), which is more vulnerable in ALS compared to other motor neurons [14]. This subgroup of the motor unit requires an enormous amount of force for the movements such as jumping and running [15]. However, the cause of vulnerability for this neuronal subset is still unknown. The anatomical studies of the various motor unit showed that FF motor neuron possesses a larger diameter of axons which includes more number of axon collaterals compared to closely related slow motor units [16,17]. In FF neuron the axonal sprouting and pruning are lower compared to others which lead to a gradual weakening of reinnervation after injury [18]. The gene expression study exhibits that in ALS, neurite outgrowth inhibitor (Nogo) is over expressed in muscle junction, which disrupts the regeneration after injury [19]. Researchers demonstrated that vulnerable motor neuron acquires higher ER stress compared to slow motor neuron [20]. These studies revealed that, in similar stress conditions, FF neuron tend to die rapidly compared to slow motor neuron, and this may be due to its anatomical complexity such as larger arborization and axonal diameter.

Research Question: Why a specific subset of the neuronal population is selectively vulnerable in disease pathogenesis, and what factors are influencing the vulnerability?

Identifying the cellular pathways to reverse vulnerability and improve the neuronal endurance:

Background and significance: the mitochondria are the powerhouse of the cell, which produces an immense amount of energy through oxidative phosphorylation. researchers measured the mitochondrial enzyme activity in various motor neuronal tissue, and they found that cytochrome oxidase (complex-iv), ros scavenging activity, is significantly lower in als affected neurons [21]. due to higher oxidative stress, mtdna is more susceptible to damage and results in aberrant energy, producing enzyme activity as well as substantial mtdna deletions in als patients [22-24] therefore, in general, FF motor neuron requires a more significant amount of energy to maintain its integrity, and even small perturbation in the energy production mechanism can lead to neuronal death. from the literature study, we hypothesized a conceivable mechanism for neuronal degeneration. figure 1 depicts the hypothesized plausible mechanism of FF neuron vulnerability.

The Blood-Brain Barrier (BBB) is essential to preserve the vascular structure, which supplies the

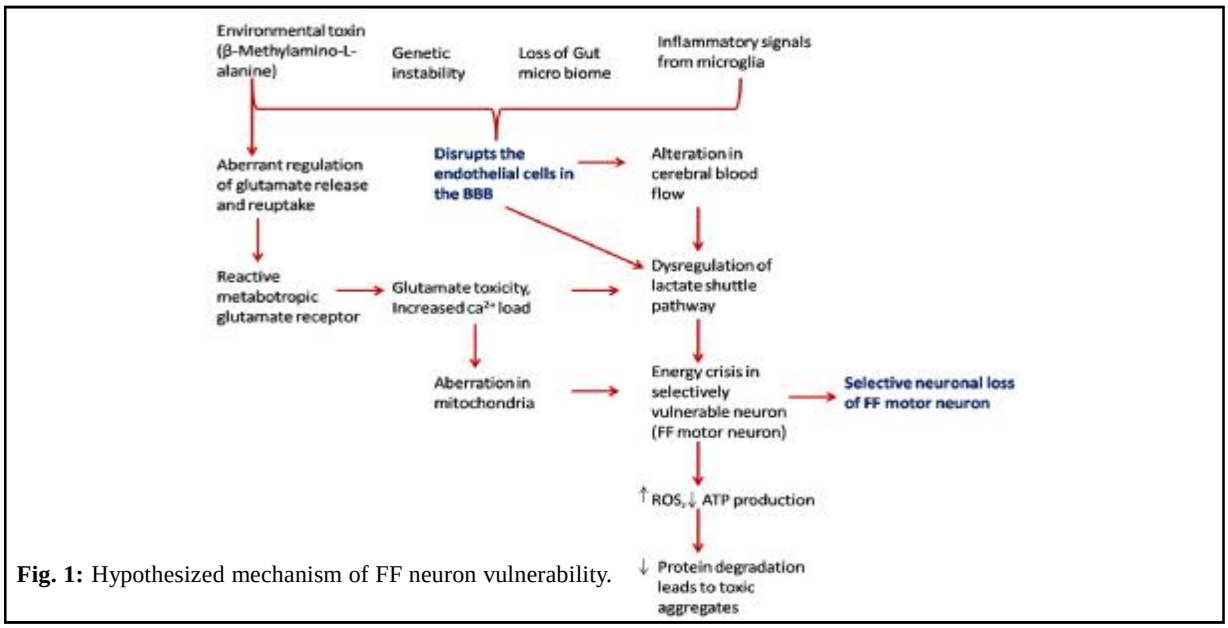

Fig. 1: Hypothesized mechanism of FF neuron vulnerability.

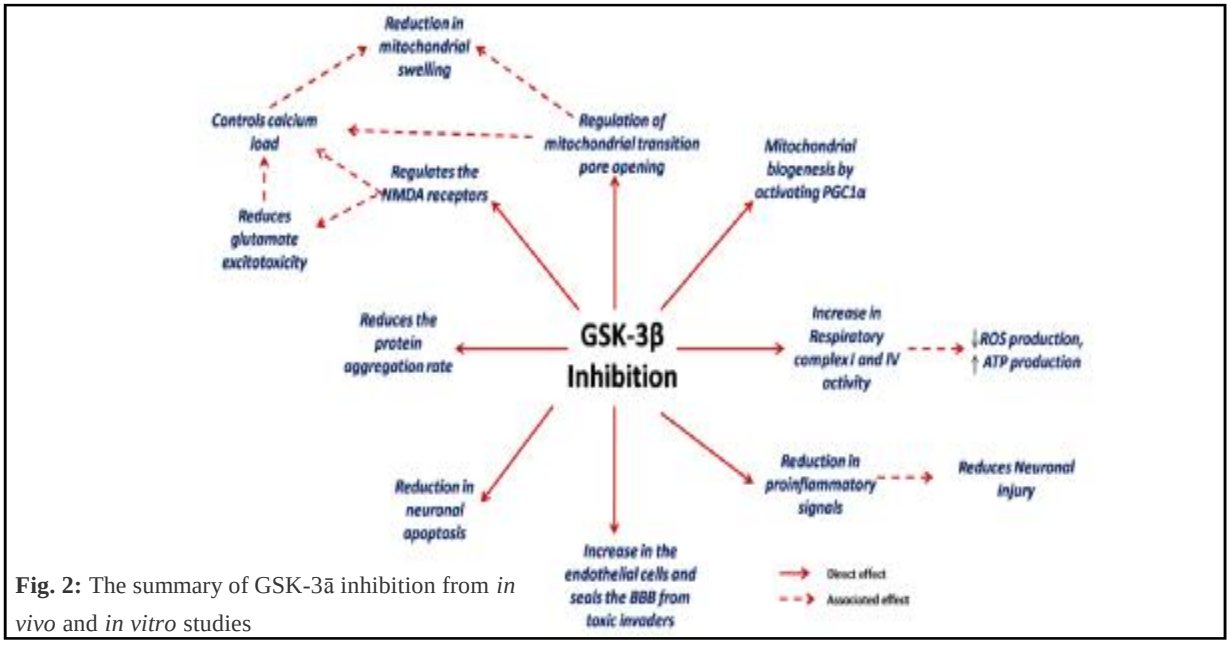

Fig. 2: The summary of GSK-3ā inhibition from *in vivo* and *in vitro* studies

metabolites and neurotrophic factors to energy demanding neurons majorly through glial cells by providing lactate [25]. Therefore, loss of BBB integrity results in disruption of neurovascular communication and cerebral blood flow. In ALS, reduced regional cerebral blood flow was observed in the motor cortex [26]. This leads to impairment in vascular-glial communication resulting in an energy crisis [27] and influences the energy craving (FF) neurons [28]. Due to metabolic stress, these neurons are unable to meet their energy requirement for securing the functional homeostasis.

β-Methylamino-L-alanine (BMAA) is a well-known neurotoxin and serves as a biomarker for ALS [29]. In chemical nature, this neurotoxin is similar to glutamate, it can easily pass through the BBB and activates glutamate receptors. Glutamate is an essential neuromodulator, which participates in various signaling processes. The higher amount of glutamate is toxic to the neuronal cell and glutamate transporter located in the glial cell, which plays an essential role in glutamate regulation in the presynaptic terminal [30]. The gene expression studies showed that the lower expression of glutamate transporters are associated with ALS pathogenesis which results in excessive glutamate toxicity and aberrant activation of NMDA and AMPA receptors [31,32]. Dysregulation of these receptors leads to calcium load inside the cell. In order to regulate the

calcium, mitochondria require a large energy threshold, leading to metabolic stress and the production of reactive oxygen species (ROS), which is toxic for spinal motor neuron [33]. This study showed aberrant glutamate re-uptake and increased glutamate concentration hyper-excites the postsynaptic neuron. This allows greater calcium influx, which disrupts the metabolic functionality of the neuron by promoting energy demand.

Further, this type of metabolic stress causes an aberration in motor protein transport, calcium ion homeostasis, vesicle trafficking, and protein degradation, all these above events require ATP for their specific functionality and this type of metabolic stress results in neuronal apoptosis. Several ***in vivo*** ALS animal models exhibit that impairment in the vascular structure results in motor degeneration [34]. This shows that communication loss between BBB, glia and higher energy craving neuron is the primary cause of energy demand and degeneration. Restoration of BBB and vascular dynamics is a promising therapeutic strategy to treat the disorder.

Identifying therapeutic target for restoration of blood-brain- barrier (BBB): From the brief literature survey, we have identified that restoration of BBB and glial function would provide neurotrophic factors and secondary metabolites to save the surviving neuronal population against various stress stimuli. Glycogen synthase kinase 3 Beta (GSK-3β) plays a dual role in cell survival and death mediated by PI3K/AKT and wnt signaling pathway. Further, it is involved in energy metabolism, ER stress, apoptosis, and neuroinfl-ammation. GSK-3β dysregulation has been reported in various categories of neurological disorders. The gene expression studies showed that GSK-3β expression is upregulated in ALS and results in tau protein aggregation [35-37].

The experimental studies show that inhibition of GSK-3β stabilizes the tight junction protein, which in turn seals the leaky BBB [38]. The proinflammatory signals affect the endothelial cells in BBB. The inhibition of GSK-3β reduces the inflammatory signals [39]. This shows GSK-3β inhibition helps to preserve the BBB structural integrity. Researchers showed that GSK-3β inhibition leads to improved mitochondrial function by activating respiratory complexes, which in turn reduce ROS production and also activate peroxisome proliferator activator receptor gamma coactivator-1alpha (PGC-1α) involved in mitochondrial biogenesis [40-42]. Wei zhoa et al. [43] demonstrated that SOD1 mutant in transgenic mice subjected to overexpression of PGC-1α show increased mitochondrial biogenesis, which improves the motor activity, electron transport energy metabolism and also recovered the spinal neuron. Studies exhibit that inhibition of GSK-3β regulates the transition pore and reduces the permeability that inturn controls the calcium load inside the mitochondria [44]. GSK-3β inhibits the overactive glutamate receptors and reduces glutamate toxicity [45]. ***In vivo*** studies exhibit that GSK-3β inhibition provides neuroprotection against various stress stimuli and delays the disease progression by improving the mitochondrial activity [46-49]. This shows inhibition of GSK-3β restores the BBB and mitochondrial function, which improves the neuronal survival by reducing the metabolic stress. This sugge-sts GSK-3β is an attractive target to treat neurodegeneration. The selected target is not involved in pain and can be modified using potent small molecules.

Gsk- Inhibitor designing strategy: GSK-3β belongs to the family of kinase and is structurally similar to cyclin-dependent kinases 2 and 5. Figure 3 shows the ATP and allosteric binding sites of GSK-3β. The kinase protein comprises three different pockets, i.e., ATP-binding site (yellow), the allosteric site I (blue), and II (red). Designing the potent, selective inhibitors for kinase family is a challenging task due to the similar binding site. The potent ATP-competitive inhibitors (type-I) bind with the active and inactive conformation of kinase but lack selectivity. The currently known allosteric inhibitors (type-II) tend to bind either allosteric site II or I [50-52]. These types of inhibitors are less potent but show high selectivity and likely to bind with the inactive conformation which shows that designing potent and selective allosteric inhibitors is very crucial.

In order to design a specific potent inhibitor, we have to identify conserved water molecules in the binding vicinity. The deigned inhibitor should form hydrogen bond interaction with a stable water molecule, thus boosting the potency by retaining water molecules and removing the unstable water. Forming interaction with the non-conserved hydrophobic binding residues may increase the selectivity in GSK-3β.

Strengths and challenges of the study: The ***in vivo*** and ***in vitro*** experiments showed that GSK-3β acts as a potential therapeutic target for

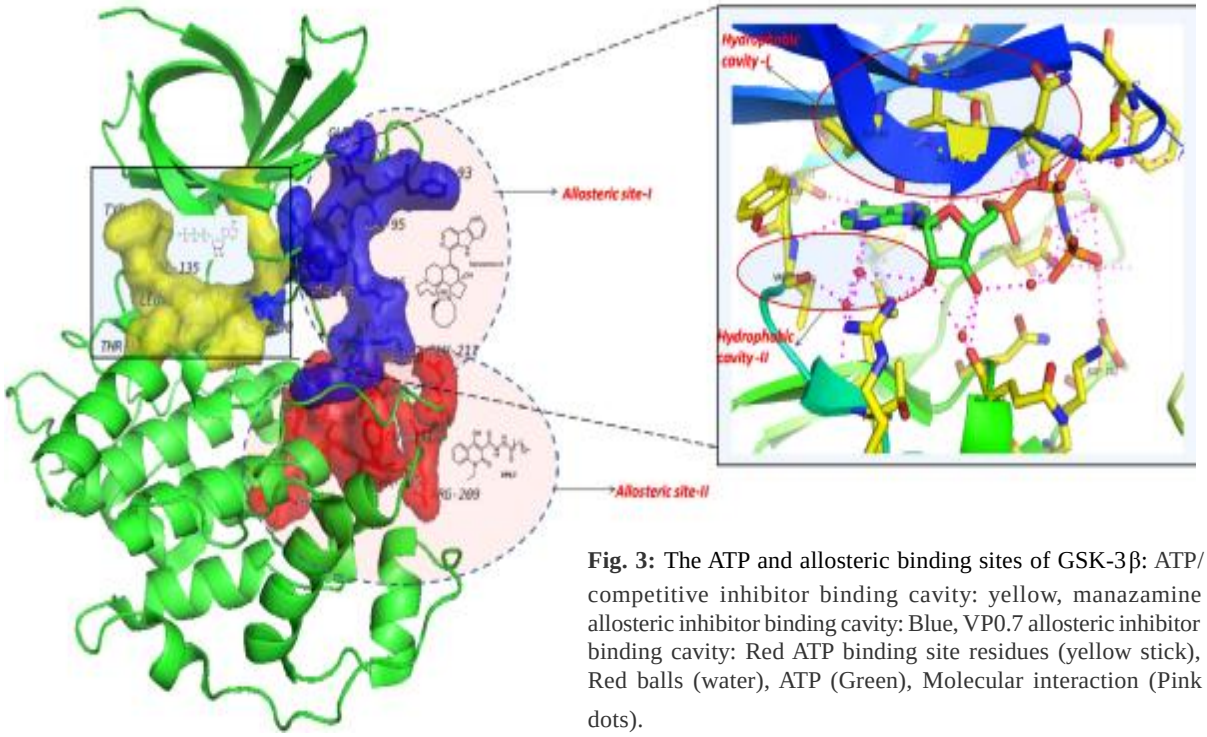

Fig. 3: The ATP and allosteric binding sites of GSK-3β: ATP/ competitive inhibitor binding cavity: yellow, manazamine allosteric inhibitor binding cavity: Blue, VP0.7 allosteric inhibitor binding cavity: Red ATP binding site residues (yellow stick), Red balls (water), ATP (Green), Molecular interaction (Pink dots).

neurodegenerative disorders. The preliminary experiments exhibit that inhibition of GSK-3β resulted in restoring the blood-brain-barrier and mitochondrial integrity and improves the neuronal survival in als animal models. designing a selective and stabilized inhibitor for GSK-3β is very challenging due to its off-target effects. to overcome the selectivity issue of existing kinase inhibitors we have to identify an inhibitor, which shows a specific interaction with GSK-3β using computational and experimental methods.

REFERENCES

[1] Kent, R. D., Kent, J. F., Weismer, G., Sufit, R. L., Rosenbek, J.C., Martin, R.E., Brooks, B.R.: J. Speech Hear. Disord. 55: 721-728 (1990).

[2] Hillel, A.D. and Miller, R.: Head Neck, 11: 51-59 (1989)

[3] Goulon, M. and Goulon-Goėau, C.: Rev. Neurol. (Paris). 145: 293-298 (1989).

[4] Zoccolella, S., Beghi, E., Palagano, G., Fraddosio, A., Guerra, V., Samarelli, V., Lepore, V., Simone, I. L., Lamberti, P., Serlenga, L. and Logroscino, G.: Eur. J. Neurol. 14: 262-268 (2007).

[5] Hubert, J.P., Delumeau, J.C., Glowinski, J., Prémont, J. and Doble, A.: Br. J. Pharmacol. 113: 261-267 (1994).

[6] Takei, K., Watanabe, K., Yuki, S., Akimoto, M., Sakata, T. and Palumbo, J.: Lateral Scler. Front. Degener. 18: 5-10 (2017).

[7] Wang, W.-Y., Pan, L., Su, S. C., Quinn, E. J., Sasaki, M., Jimenez, J. C., Mackenzie, I. R. A., Huang, E. J. and Tsai, L.-H.: Nat. Neurosci.. 16: 1383-1391 (2013).

[8] Nomura, T., Watanabe, S., Kaneko, K., Yamanaka, K., Nukina, N. and Furukawa, Y.J.: Biol. Chem., 289: 1192-1202 (2014).

[9] Gurney, M. E., Pu, H., Chiu, A.Y., Dal Canto, M.C., Polchow, C.Y., Alexander, D.D., Caliendo, J., Hentati, A., Kwon, Y.W. and Deng, H.X.: Science, 264: 1772-1775 (1994).

[10] Bruijn, L.I., Houseweart, M.K., Kato, S., Anderson, K.L., Anderson, S.D., Ohama, E., Reaume, A.G., Scott, R.W. and Cleveland, D.W.: Science 281: 1851-1854 (1998).

[11] Corcia, P., Valdmanis, P., Millecamps, S., Lionnet, C., Blasco, H., Mouzat, K., Daoud, H., Belzil, V., Morales, R., Pageot, N., Danel-Brunaud, V., Vandenberghe, N., Pradat, P.F., Couratier, P., Salachas, F., Lumbroso, S., Rouleau, G.A., Meininger, V., Camu, W.: Neurology, 78: 1519-1526 (2012).

[12] DeJesus-Hernandez, M., Mackenzie, I. R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Flynn, H., Adamson, J., Kouri, N., Wojtas, A., Sengdy, P., Hsiung, G.-Y.R., Karydas, A., Seeley, W.W., Josephs, K.A., Coppola, G., Geschwind, D.H., Wszolek, Z.K., Feldman, H., Knopman, D.S., Petersen, R.C., Miller, B.L., Dickson, D.W., Boylan, K.B., Graff-Radford, N.R. and Rademakers, R.: Neuron 72: 245-256 (2011).

[13] Gitler, A.D. and Tsuiji, H.: Brain Res. 1647: 19-29 (2016).

[14] Conradi, S. and Ronnevi, L.O.: Brain Res. Bull., 30: 369-371 (1993).

[15] Brandstater, M.E. and Lambert, E.H.: Motor Unit Anatomy 1. In New Concepts of the Motor Unit, Neuromuscular Disorders, Electromyographic Kinesiology, S. Karger AG, Vol. 1, pp 14-22 (1973).
[16] Kernell, D. and Zwaagstra, B.: Brain Res., 204: 311-326 (1981).
[17] Kawamura, Y., Dyck, P.J., Shimono, M., Okazaki, H., Tateishi, J. and Doi, H.: J. Neuropathol. Exp. Neurol. 40: 667-675 (1981).
[18] Frey, D., Schneider, C., Xu, L., Borg, J., Spooren, W. and Caroni, P.: J. Neurosci. 20: 2534-2542 (2000).
[19] Bruneteau, G., Bauché, S., Gonzalez de Aguilar, J.L., Brochier, G., Mandjee, N., Tanguy, M.-L., Hussain, G., Behin, A., Khiami, F., Sariali, E., Hell-Remy, C., Salachas, F., Pradat, P.-F., Lacomblez, L., Nicole, S., Fontaine, B., Fardeau, M., Loeffler, J.-P., Meininger, V., Fournier, E., Koenig, J. and Hantaį, D.: Ann. Clin. Transl. Neurol. 2: 362-372 (2015).
[20] Saxena, S., Cabuy, E. and Caroni, P.A.: Nat. Neurosci., 12, 627–636 (2009).
[21] Mattiazzi, M., D'Aurelio, M., Gajewski, C.D., Martushova, K., Kiaei, M., Flint Beal, M., Manfredi, G.J. : Biol. Chem., 277: 29626-29633 (2002).
[22] Vielhaber, S., Kunz, D., Winkler, K., Wiedemann, F.R., Kirches, E., Feistner, H., Heinze, H.J., Elger, C.E., Schubert, W., Kunz, W.S.: Brain, 123 (Pt 7): 1339-1348 (2000).
[23] Menzies, F.M., Ince, P.G. and Shaw, P.J.: Neurochem. Int., 40: 543-551 (2002).
[24] Swerdlow, R.H., Parks, J.K., Cassarino, D.S., Trimmer, P.A., Miller, S.W., Maguire, D.J., Sheehan, J.P., Maguire, R.S., Pattee, G., Juel, V.C., Phillips, L.H., Tuttle, J.B., Bennett, J.P., Davis, R.E. and Parker, W.D. Exp. Neurol. 153, 135-142 (1998).
[25] Philips, T. and Rothstein, J.D.: Exp. Neurol. 262: 111-120 (2014).
[26] Kew, J. J., Leigh, P.N., Playford, E.D., Passingham, R.E., Goldstein, L.H., Frackowiak, R.S. and Brooks, D.J.: Brain 116(Pt 3): 655-680 (1993).
[27] Alvarez, J.I., Katayama, T. and Prat, A.: Glia, 61: 1939-1958 (2013).
[28] Ferraiuolo, L., Higginbottom, A., Heath, P.R., Barber, S., Greenald, D., Kirby, J. and Shaw, P.J.: Brain, 134: 2627-2641 (2011).
[29] Chiu, A.S., Gehringer, M.M., Welch, J.H., Neilan, B.A.: Int. J. Environ. Res. Public Health, 8: 3728-3746 (2011).
[30] Scott, H.L., Pow, D.V, Tannenberg, A.E.G. and Dodd, P.R.: J. Neurosci. 22: RC206 (2002).
[31] Fray, A.E., Ince, P.G., Banner, S.J., Milton, I.D., Usher, P.A., Cookson, M.R. and Shaw, P.J.: Eur. J. Neurosci., 10: 2481-2489 (1998).
[32] Van, L., Bosch, D., Vandenberghe, W., Klaassen, H., Houtte, E. and Van, Robberecht, W.: J. Neurol. Sci. 180: 29-34 (2000).
[33] Carriedo, S.G., Sensi, S.L., Yin, H.Z. and Weiss, J.H.: J. Neurosci., 20: 240-250 (2000).
[34] Garbuzova-Davis, S. and Sanberg, P.R.: Front. Cell. Neurosci., 8: 21 (2014).
[35] Yang, W., Leystra-Lantz, C. and Strong, M.J.: Brain Res. 1196: 131-139 (2008).
[36] Hu, J.-H., Zhang, H., Wagey, R., Krieger, C. and Pelech, S.L:. J. Neurochem. 85: 432-442 (2003).
[37] Kihira, T., Suzuki, A., Kondo, T., Wakayama, I., Yoshida, S., Hasegawa, K.M.G.R.: Neuropathol., Off. J. Japanese Soc. Neuropathol., 29: 548-558 (2009).
[38] Ramirez, S.H., Fan, S., Dykstra, H., Rom, S., Mercer, A., Reichenbach, N.L., Gofman, L. and Persidsky, Y.: PLoS One 8, e55972, doi:10.1371 (2013).
[39] Ramirez, S.H., Fan, S., Zhang, M., Papugani, A., Reichenbach, N., Dykstra, H., Mercer, A.J., Tuma, R.F. and Persidsky, Y.: Am. J. Pathol., 176, 881-892 (2010),
[40] Valerio, A., Bertolotti, P., Delbarba, A., Perego, C., Dossena, M., Ragni, M., Spano, P., Carruba, M.O., De Simoni, M.G. and Nisoli, E.: J. Neurochem., 116: 1148-1159 (2011).
[41] King, T.D., Clodfelder-Miller, B., Barksdale, K.A., Bijur, G.N.: Neurotox. Res., 14: 367-382 (2008).
[42] Yang, K., Chen, Z., Gao, J., Shi, W., Li, L., Jiang, S., Hu, H., Liu, Z., Xu, D., Wu, L.: Cell. Physiol. Biochem., 44: 1445–1459 (2017).
[43] Zhao, W., Varghese, M., Yemul, S., Pan, Y., Cheng, A., Marano, P., Hassan, S., Vempati, P., Chen, F., Qian, X. and Pasinetti, G.: Mol. Neurodegener., 6: 51 (2011).
[44] Juhaszova, M., Zorov, D.B., Kim, S.-H., Pepe, S., Fu, Q., Fishbein, K.W., Ziman, B.D., Wang, S., Ytrehus, K., Antos, C.L., Olson, E.N. and Sollott, S.J.: Glycogen synthase kinase-3β mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. J. Clin. Invest.. 113: 1535-1549 (2004).
[45] Chen, P., Gu, Z., Liu, W. and Yan, Z.: Mol. Pharmacol., 72: 40-51 (2007).
[46] Koh, S.-H., Kim, Y., Kim, H.Y., Hwang, S., Lee, C.H., Kim, S.H.: Exp. Neurol. 205: 336-346 (2007).
[47] Koh, S.-H., Lee, Y.-B., Kim, K.S., Kim, H.-J., Kim, M., Lee, Y.J., Kim, J., Lee, K.W. and Kim, S.H.: Eur. J. Neurosci. 22: 301-309 (2005).
[48] Leng, Y., Liang, M.-H., Ren, M., Marinova, Z., Leeds, P., Chuang, D.-M.: J. Neurosci., 28: 2576-2588 (2008).
[49] Feng, H.-L., Leng, Y., Ma, C.-H., Zhang, J., Ren, M. and Chuang, D.-M.: Neuroscience, 155: 567-572 (2008).
[50] Palomo, V., Soteras, I., Perez, D. I., Perez, C., Gil, C., Campillo, N.E. and Martinez, A.: J. Med. Chem. 2011 (54): 8461-8470 (2011).
[51] Palomo, V., Perez, D.I., Roca, C., Anderson, C., Rodrķguez-Muela, N., Perez, C., Morales-Garcia, J.A., Reyes, J.A., Campillo, N.E., Perez-Castillo, A.M., Rubin, L.L., Timchenko, L., Gil, C. and Martinez, A.: J. Med. Chem. 60: 4983-5001 (2017).
[52] Palomo, V. I., Perez, D., Gil, C. and Martinez, A.: Curr. Med. Chem. 18: 3028-3034 (2011).