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Basic fibroblast growth factor increases the number of endogenous neural stem cells and inhibits the expression of amino methyl isoxazole propionic acid receptors in amyotrophic lateral sclerosis mice☆

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Abstract

This study aimed to investigate the number of amino methyl isoxazole propionic acid (AMPA) receptors and production of endogenous neural stem cells in the SOD1^{G93AG1H} transgenic mouse model of amyotrophic lateral sclerosis, at postnatal day 60 following administration of basic fibroblast growth factor (FGF-2). A radioligand binding assay and immunohistochemistry were used to estimate the number of AMPA receptors and endogenous neural stem cells respectively. Results showed that the number of AMPA receptors and endogenous neural stem cells in the brain stem and sensorimotor cortex were significantly increased, while motor function was significantly decreased at postnatal days 90 and 120. After administration of FGF-2 into mice, numbers of endogenous neural stem cells increased, while expression of AMPA receptors decreased, whilst motor functions were recovered. At postnatal day 120, the number of AMPA receptors was negatively correlated with the number of endogenous neural stem cells in model mice and FGF-2-treated mice. Our experimental findings indicate that FGF-2 can inhibit AMPA receptors and increase the number of endogenous neural stem cells, thus repairing neural injury in amyotrophic lateral sclerosis mice.

Key Words: amino methyl isoxazole propionic acid receptor; amyotrophic lateral sclerosis; basic fibroblast growth factor; endogenous neural stem cells

Abbreviations: AMPA, amino methyl isoxazole propionic acid; FGF-2, basic fibroblast growth factor; ENSCs, endogenous neural stem cells; ALS, amyotrophic lateral sclerosis

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) patients always present neural degeneration in their motor neurons[1], but there is currently no effective treatment. An increasing number of studies have found that endogenous neural stem cells (ENSCs) are a potential therapeutic strategy for the repair of neuronal degeneration and injury[2]. Once affected by disease, ENSCs are thought to be activated and migrate, prior to differentiation into various cells under the induction of the surrounding microenvironment, thus repairing neural injury[3-4]. Environmental change greatly determines the survival of ENSCs and such changes are directly related to cell membrane ion channels[5].

Alpha amino methyl isoxazole propionic acid (AMPA) receptor in motor neurons, is the glutamate receptor (GluR) in the postsynaptic membrane. The receptors mediate fast synaptic transmission and their content in the membrane is highly variable, correlating with synaptic activity[6]. AMPA receptors contribute to alter the membrane permeability of Ca^{2+} and/or Zn^{2+}, which is mainly mediated by the GluR2 subunit and therefore plays an important role in neuronal degeneration following cerebral ischemia[7]. In addition, ADAR2, a ribozyme related to the GluR2 pre-mRNA code, mediates Ca^{2+} membrane permeability *via* the AMPA receptor. GluR2 pre-mRNA-dependent GluR2 Q/R coding exerts a direct effect on hippocampal neurons in cerebral ischemic rats[5]. We therefore hypothesize that AMPA-induced neuronal excitotoxicity may be the leading cause of degeneration in selective motor neurons.

Basic fibroblast growth factor (FGF-2) is a multifunctional protein involved in the neural protection and promotion of ENSC differentiation[8].

In this study, we analyzed the number of AMPA receptors and the proliferation of

ENSCs in the brainstem and sensorimotor cortex in the SOD1^{G93AG1H} transgenic mouse model of ALS[9-10] at different disease stages, following FGF-2 administration. The correlation between ENSC proliferation and AMPA receptor number was investigated and the possible mechanism underlying the FGF-2 neuroprotective effect is discussed.

RESULTS

Quantitative analysis of experimental animals

A total of 60 SOD1^{G93AG1H} transgenic mice at postnatal day 57 were used (transgenic mice developed symptoms at postnatal day 90 but no symptoms were visible at postnatal day 60). Mice were randomly divided into ALS group (mice were intraperitoneally injected with saline from day 60) and FGF-2 group (mice were intraperitoneally injected with FGF-2 from day 60). In addition, 30 wild-type mice were used as a control group (mice were intraperitoneally injected with saline from day 60). Ten mice were harvested in each group at postnatal days 60, 90 and 120 for experiments. Ninety mice were used for analysis.

FGF-2 promoted motor function recovery in ALS mice

Rotarod performance tests showed that the time of 60-day-old mice to remain on the rotating cylinder (rod) was maintained at the same level in each group, with no significant difference ($P > 0.05$). Conversely, the 90-day-old mice in both the control and FGF-2 groups remained on the rotating rod for a significantly prolonged time compared with the ALS group ($P < 0.05$). The length of time for the 120-day-old mice to remain on the rotating rod was significantly shortened in both the ALS and FGF-2 groups compared with the control group ($P < 0.05$), whilst the duration in the FGF-2 group was significantly longer than in the ALS group ($P < 0.05$; Table 1).

Table 1 The length of time (second) for mice remaining on the rotating rod in the rotarod performance test

Group	Postnatal time (day)		
	60	90	120
Control	180.0±0.0	180.0±0.0	180.0±0.0
Amyotrophic lateral sclerosis	180.0±0.0	111.8±16.7[a]	43.5±14.4[a]
Basic fibroblast growth factor	180.0±0.0	165.4±12.4[b]	103.9±16.9[ab]

Data are expressed as mean ± SD of 10 mice in each group. [a]$P < 0.05$, *vs.* control group; [b]$P < 0.05$, *vs.* amyotrophic lateral sclerosis group (one-way analysis of variance). Each rotarod performance test lasted 180 seconds. Longer time length indicates better motor function in mice.

FGF-2 increased the weight of ALS mice

The body weight of mice showed no significant differences at postnatal days 60 and 90 ($P > 0.05$). The weight of 120-day-old mice in the ALS group decreased significantly compared with the control group ($P < 0.05$), while the weight of the mice in the FGF-2 group was significantly increased compared with the ALS group ($P < 0.05$; Table 2).

Table 2 Change in mouse body weight (g) for each group

Group	Postnatal time (day)		
	60	90	120
Control	21.1±1.1	22.7±1.4	24.0±1.2
Amyotrophic lateral sclerosis	20.6±0.6	21.5±0.6	18.2±0.5[a]
Basic fibroblast growth factor	21.3±1.0	22.3±1.0	22.0±1.1[b]

Data are expressed as mean ± SD of 10 mice in each group. [a]$P < 0.05$, *vs.* control group; [b]$P < 0.05$, *vs.* amyotrophic lateral sclerosis group (one-way analysis of variance).

FGF-2 promoted ENSC proliferation in the brainstem and sensorimotor cortex of ALS mice

Anti-nestin immunofluorescence staining showed there were no ENSCs observed in the brainstem and sensorimotor cortex of wild-type control mice. A small number of ENSCs were visible in the brainstem and sensorimotor cortex of the 60-day-old SOD1^{G93AG1H} transgenic mice and the ENSC number gradually increased with age. At postnatal day 120, an abundance of green fluorescent ENSCs was found in the SOD1^{G93AG1H} transgenic mice (Figure 1).

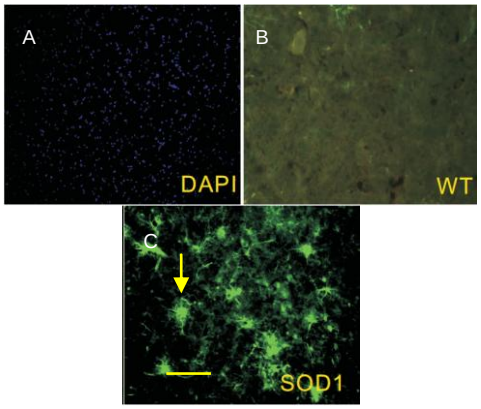

Figure 1 Endogenous neural stem cells in the brainstem of wild-type (WT) and SOD1^{G93AG1H} transgenic mice at postnatal day 120 (immunofluorescence staining, fluorescent microscope, scale bars: 200 μm in A, and 100 μm in B, C).

(A) Amyotrophic lateral sclerosis group, 4', 6-diamidino-2-phenylindole (DAPI) staining alone displays blue labeled cell nuclei.

(B) Control group, green fluorescence labeled cells are rarely seen by immunofluorescence staining.

(C) Amyotrophic lateral sclerosis group, abundant green fluorescence (fluorescein isothiocyanate-labeled) cells (arrow).

Cell counts showed no significant difference in the number of ENSCs in the brainstem and sensorimotor cortex of 60-day-old mice in each group ($P > 0.05$). At postnatal days 90 and 120, the ALS and FGF-2 groups had significantly increased in ENSC number in the

mouse brainstem and sensorimotor cortex compared with the control group ($P < 0.05$), whilst cell number in the 120-day-old mice was significantly higher in the FGF-2 group than in the ALS group ($P < 0.05$; Table 3).

Table 3 Number of endogenous neural stem cells (nestin$^+$) (n/μm^2) in the brainstem and sensorimotor cortex of mice

Group	Postnatal time (day)		
	60	90	120
Brainstem			
Control	2.71±1.24	1.92±2.49	2.25±3.01
Amyotrophic lateral sclerosis	3.11±1.42	62.02±21.68[a]	162.32±58.73[a]
Basic fibroblast growth factor	1.93±0.66	81.76±23.35[a]	385.72±102.56[ab]
Group	Postnatal time (day)		
	60	90	120
Sensorimotor cortex			
Control	0.76±0.13	1.02±0.34	1.13±0.41
Amyotrophic lateral sclerosis	1.43±0.11	34.76±20.19[a]	103.35±38.10[a]
Basic fibroblast growth factor	1.32±0.53	41.48±12.83[a]	296.36±51.74[ab]

Data are expressed as mean ± SD of 10 mice in each group. [a]$P < 0.05$, *vs.* control group; [b]$P < 0.05$, *vs.* amyotrophic lateral sclerosis group (one-way analysis of variance).

FGF-2 inhibitd AMPA receptor expression in motor neurons in the brainstem and sensorimotor cortex of ALS mice

At 3 days after fluorescent gold injection, microscopic detection showed motor neurons in the brainstem and sensorimotor cortex of all mice were clearly labeled with fluorescent gold and radioligand experimental labeling was also visualized (Figure 2).

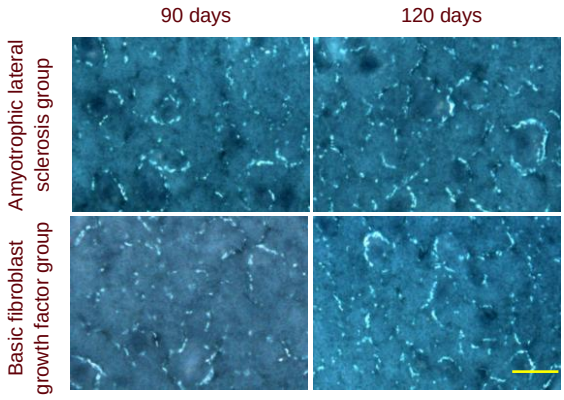

Figure 2 Amino methyl isoxazole propionic acid (AMPA) receptor expression in motor neurons of the mouse brainstem [fluorescent gold labeled motor neurons (blue), ^{3}H radioligand marker AMPA (highlighted), scale bar: 200 μm].

The AMPA receptor numbers gradually increased with age, and it decreased slightly in the basic fibroblast growth factor 2 group compared with amyotrophic lateral sclerosis mice at identical time points.

Statistical analysis showed there was no significant difference in the AMPA receptor number in motor neurons at the brainstem and sensorimotor cortex of 60-day-old mice ($P > 0.05$). At postnatal days 90 and 120, the ALS and FGF-2 groups showed significant increase in numbers of AMPA receptors compared with the control group ($P < 0.05$). The AMPA receptor number in 120-day-old mice of the bFGF-2 group was significantly less than in the ALS group ($P < 0.05$; Table 4).

Table 4 Number of amino methyl isoxazole propionic acid receptors (n/μm^2) in motor neurons of the mouse brainstem and sensorimotor cortex

Group	Postnatal time (day)		
	60	90	120
Brainstem			
Control	0.27±0.03	0.26±0.11	0.29±0.06
Amyotrophic lateral sclerosis	0.32±0.12	0.51±0.08[a]	1.03±0.27[a]
Basic fibroblast growth factor	0.33±0.18	0.44±0.09[a]	0.68±0.24[ab]
Group	Postnatal time (day)		
	60	90	120
Sensorimotor cortex			
Control	0.22±0.03	0.21±0.07	0.27±0.09
Amyotrophic lateral sclerosis	0.20±0.04	0.63±0.25[a]	1.46±0.37[a]
Basic fibroblast growth factor	0.29±0.04	0.52±0.16[a]	0.89±0.23[ab]

Data are expressed as mean ± SD of 10 mice in each group. [a]$P < 0.05$, *vs.* control group; [b]$P < 0.05$, *vs.* amyotrophic lateral sclerosis group (one-way analysis of variance).

Correlation between number of activated ENSCs and AMPA receptor change

Pearson correlation analysis was used to investigate the correlation between ENSC number and AMPA receptor number in the 120-day-old mice of the ALS and FGF-2 groups. Results showed that the number of ENSCs was negatively correlated with the number of AMPA receptors ($r = -0.889\ 3$, $P < 0.01$; Figure 3).

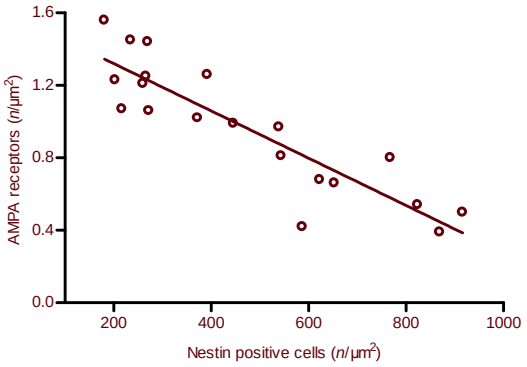

Figure 3 Correlation between the number of amino methyl isoxazole propionic acid (AMPA) receptors and the number of endogenous neural stem cells in the mice of amyotrophic lateral sclerosis and basic fibroblast growth factor groups at postnatal day 120.

DISCUSSION

SOD1^{G93AG1H} transgenic mouse models have achieved increasing recognition for ALS studies, where SOD1^{G93A} can be highly expressed when models are modulated by cytomegalovirus promoter[9-10]. Studies have found that ENSCs are activated to varying degrees in SOD1^{G93AG1H} transgenic mouse models at different disease stages, which accordingly remodel neural functions but cannot treat the disease[4].

FGF-2 is continuously expressed in the neurogenic area of adult brains, allowing regulation of proliferation in neural precursor cells[11]. FGF-2 injected into the lateral cerebral ventricle of adult rats can activate proliferation of subventricular zone precursors and increase the number of neurons migrating from the subventricular zone to the olfactory bulb. FGF-2 can also promote and determine cellular differentiation[12]. The present study demonstrates that, FGF-2 can increase the number of ENSCs in the brainstem and sensorimotor cortex of ALS mice, thus promoting recovery of motor function.

The ALS pathogenesis mechanism may be attributed to the transmission of motor neuron synaptic signals[12]. AMPA receptors, mediating fast synaptic transmission with varying content in the membrane, are shown to correlate with synaptic activity[13]. AMPA receptor overexpression mainly occurs in GluR2-free calcium permeable receptors, so induced neuronal excitotoxicity may be a leading cause of selective motor neuron degeneration[5, 13-14]. Strong evidence suggests a significant reduction in motor neurons and synaptic receptors in ALS mouse models at postnatal day 90[13]. At postnatal day 120, magnetic resonance imaging visualized multiple atrophy lesions in the mouse brain and nerve cells exhibited the symptoms of apoptosis and degeneration[15]. Evidence suggested that the ALS disease course directly correlates with some synapse-associated proteins and determines neuronal degeneration. It is clear that AMPA is correlated with the disease process. In this study, the numbers of ENSCs and AMPA receptors were significantly increased over the disease process. FGF-2 treatment significantly increased the number of ENSCs, but decreased the number of AMPA receptors. There was a negative correlation between ENSC number and AMPA receptor number in 120-day-old mice in both the ALS and FGF-2 groups.

ENSCs are a potential, valuable therapeutic remedy. Not only are they a source of autologous stem cells instead of using the damaged nerve tissue, they also secreted nerve growth and nutritional factors, which can promote cell growth and division[2]. Therefore, further studies are needed to explore the mechanisms underlying ENSCs differentiation in order to induce substantial ENSCs differentiation, increase their migration potential, control their differentiation towards neurons and oligodendrocytes and guide axonal growth, to enable ENSCs to become a suitable alternative or complementary therapy for nervous system disease[3]. Much effort was made in this study to develop a new method to yield disease process or symptoms through FGF-2 administration, thus reducing AMPA damage on neurons, improving the microenvironment and promoting proliferation of ENSCs. The possible mechanism of FGF-2 action has also been discussed, which provides a feasible method for future clinical treatment of ALS.

MATERIALS AND METHODS

Design

A randomized controlled animal experiment.

Time and setting

Experiments were performed from 2009 to 2010 in the laboratory of Tianjin First Center Hospital of China and in Melbourne Medical College of Australia.

Materials

A total of 60 transgenic mice originated from the B6SJL-TgN (SOD1-G93A) and expressing the mutant human superoxide dismutase 1 gene, were included in this study. Mice aged 57 days old with similar weight were evenly divided by sex. Another 30 wild-type mice aged 57 days old and with similar body weight, were used for the control group. All mice were provided by the Jackson Laboratory, USA. Experiments in Australia were performed under the guidance of the Howard Florey Institute Animal Ethics and Experimentation Committee approved all experimental procedures used on mice (permit number: 03-100). Experimental disposals in China were in strict accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of China[16].

Methods

Drug administration

All groups of mice received an intraperitoneal injection, once per day from postnatal day 60. The FGF-2 group was given 25 g/kg FGF-2 (50 μg dissolved in 20 mL; Thermo Fisher Scientific, Boston, MA, USA). Control and ALS groups were given equal volumes of saline.

Neural function detected by rotarod performance test

Mice received adaptive rotarod tests of motor function from postnatal day 57 and were tested at postnatal days 60, 90 and 120. Mouse body weight was recorded when testing. The length of time for each mouse remaining on the rotating rod (Ugo Basile, Schwenksville, PA, USA) was determined with an 180-second limit. Mice were tested three times at each time point and the average value was calculated.

Fluorescent gold labeling and specimen preparation

Mice were injected with 5 μL fluorescent gold at postnatal days 57, 87 and 117, to tag brainstem and cortical motor neurons. Mice were then intraperitoneally injected with sodium pentobarbital at postnatal days 60, 90 and 120. The heart was perfused with saline under anesthesia at

room temperature and subsequently perfused with 4% paraformaldehyde. Brain tissue was harvested and fixed for 24 hours, followed by dehydration in 30% sugar solution for 24 hours and embedding in paraffin. Specimens were cut into serial sections 8 μm thick and 6 out of every 10 pieces were harvested. Two sets were used for the AMPA radioligand assay, one set for nestin immunofluorescence, one set for positive or negative controls and two sets for experimental emergencies.

Number of nestin positive cells in mouse brainstem and sensorimotor cortex detected by immunofluorescence staining

Histological sections were conventionally dewaxed, rinsed and incubated with nestin-fluorescein isothiocyanate polymer (1:50; Chemicon, Santa Cruz, CA, USA) at 4°C overnight. Cell nuclei were stained with DAPI (1:2 000; Chemicon). Sections were observed under a fluorescence microscope. Images of positive cells were processed using Microfire (Life Technologies, Carlsbad, CA, USA) and Imagepro software (Life Technologies). The number of AMPA receptors and ENSCs were calculated using a confocal counting system (Life Technologies) and cell count was expressed as $n/\mu m^2$.

AMPA expression in the brainstem and sensorimotor cortex detected by radioligand assay

Tissue sections and coverslips were rinsed to remove impurities. Coverslips were soaked in photoemulsion liquid (Thermo Fisher Scientific; pre-mixed with an equal amount of distilled water and 1% glycerol) in a dark room. Using the ^3H radiation method[16], coverslips were incubated with 14.3 M AMPA antibody (Thermo Fisher Scientific) at 4°C for 40 minutes, then covered with the photosensitive emulsion solution-coated coverslip and placed in a darkroom. The photosensitive time depended on the intensity of radiation, which was detected with a ^3H radiation tester (Life Technologies). The experimental sensitization time was 6 weeks. Results were analyzed using a confocal counting system (Life Technologies) and the number of AMPA receptors in 10 random fields in each section was calculated ($n/\mu m^2$).

Statistical analysis

Data were expressed as mean ± SD and analyzed using Prism software (GraphPad Software Inc, San Diego, CA, USA) by one-way analysis of variance. Pearson analysis was used for the correlation between the number of activated ENSCs and the AMPA receptors. A $P < 0.05$ value was considered a significant difference.

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Author contributions: Dawei Zang was responsible for the study concept and design, validating the study and heading the funding. Weihui Huang integrated and analyzed the data and wrote the manuscript. Yi Lu and Ping Jiang were responsible for statistical processing and data acquisition.

Conflicts of interest: None declared.

Ethical approval: This pilot was approved by the Experimental Animal Ethics Committee of the Medical School of Melbourne University in Australia.

REFERENCES

[1] Kalra S, Arnold DL, Cashman NR. Biological markers in the diagnosis and treatment of ALS. J Neurol Sci. 1999;165 (Suppl 1): S27-32.
[2] Martino G, Pluchino S, Bonfanti L, et al. Brain regeneration in physiology and pathology: the immune signature driving therapeutic plasticity of neural stem cells. Physiol Rev. 2011;91(4):1281-1304.
[3] Liu J, Zang D. Response of neural precursor cells in the brain of Parkinson's disease mouse model after LIF administration. Neurol Res. 2009;31(7):681-686.
[4] Juan L, Dawei Z, Julie AD. Increased number and differentiation of neural precursor cells in the brainstem of superoxide dismutase 1(G93A) (G1H) transgenic mouse model of amyotrophic lateral sclerosis. Neurol Res. 2007;29(2):204-209.
[5] Peng PL, Zhong X, Tu W, et al. ADAR2-dependent RNA editing of AMPA receptor subunit GluR2 determines vulnerability of neurons in forebrain ischemia. Neuron. 2006;49(5):719-733.
[6] Lee HK, Kirkwood A. AMPA receptor regulation during synaptic plasticity in hippocampus and neocortex. Semin Cell Dev Biol. 2011;22(5):514-520.
[7] Liu S, Lau L, Wei J, et al. Expression of Ca(2+)-permeable AMPA receptor channels primes cell death in transient forebrain ischemia. Neuron. 2004;43(1):43-55.
[8] Coutu DL, Galipeau J. Roles of FGF signaling in stem cell self-renewal, senescence and aging. Aging (Albany NY). In press.
[9] Turner BJ, Atkin JD, Farg MA, et al. Impaired extracellular secretion of mutant superoxide dismutase 1 associates with neurotoxicity in familial amyotrophic lateral sclerosis. J Neurosci. 2005;25(1):108-117.
[10] Zang DW, Cheema SS. Degeneration of corticospinal and bulbospinal systems in the superoxide dismutase 1(G93A G1H) transgenic mouse model of familial amyotrophic lateral sclerosis. Neurosci Lett. 2002;332(2):99-102.
[11] Zang DW, Lopes EC, Cheema SS. Loss of synaptophysin-positive boutons on lumbar motor neurons innervating the medial gastrocnemius muscle of the SOD1G93A G1H transgenic mouse model of ALS. J Neurosci Res. 2005;79(5):694-699.
[12] Lai WT, Krishnappa V, Phinney DG. Fibroblast growth factor 2 (FGF2) inhibits differentiation of mesenchymal stem cells by inducing Twist2 and Spry4, blocking extracellular regulated kinase activation, and altering Fgf receptor expression levels. Stem Cells. 2011;29(7):1102-1111.
[13] Kwak S, Hideyama T, Yamashita T, et al. AMPA receptor-mediated neuronal death in sporadic ALS. Neuropathology. 2010;30(2): 182-188.
[14] Henley JM, Barker EA, Glebov OO. Routes, destinations and delays: recent advances in AMPA receptor trafficking. Trends Neurosci. 2011;34(5):258-268.
[15] Zang DW, Yang Q, Wang HX, et al. Magnetic resonance imaging reveals neuronal degeneration in the brainstem of the superoxide dismutase 1 transgenic mouse model of amyotrophic lateral sclerosis. Eur J Neurosci. 2004;20(7):1745-1751.
[16] Liu J, Zang DW. Reduction of expression of alpha-amino-3-hyddroxy-5-methyl-4-isoxazolepronic acid receptors in the brain stem and sensorimotor cortex of the SOD1G93AG1H transgenic mouse model of amyotrophic lateral sclerosis induced by leukaemia inhibitory factor. Zhongguo Zuzhi Gongcheng Yanjiu yu Linchuang Kangfu. 2006;10(46):126-128.

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