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Ganoderic acid improves 5-fluorouracil-induced cognitive dysfunction in mice

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5-Fluorouracil (5-FU) is a chemotherapeutic drug with a good anti-cancer effect on various types of cancers, such as colorectal cancer and breast cancer. However, previous studies have found that 5-FU could induce cognitive deficit in clinics. As ganoderic acid, isolated from *Ganoderma lucidum*, has a protective effect on neurons, this study investigated the effects of ganoderic acid (GA) against 5-FU-induced cognitive dysfunction with a series of behavioral tests and related indicators. Experimental results showed that GA significantly prevented the reduction of spatial and non-spatial memory in 5-FU-treated mice. In addition, GA not only ameliorated the damage to hippocampal neurons and mitochondrial structure, but also significantly improved abnormal protein expression of mitochondrial biogenesis related marker PGC-1α, and mitochondrial dynamics related markers MFN2, DRP1 and FIS1 in the hippocampi of 5-FU-treated mice. Moreover, GA could up-regulate the expression of neuronal survival and growth-related proteins, such as BDNF, *p*-ERK, *p*-CREB, *p*-Akt, *p*-GSK3β, Nrf2, *p*-mTOR, and *p*-S6, in the hippocampi of 5-FU-treated mice. These results suggest that GA could prevent cognitive dysfunction in mice treated with 5-FU *via* preventing mitochondrial impairment and enhancing neuronal survival and growth, which provide evidence for GA as a promising adjunctive therapy for chemotherapy related cognitive impairment in clinics.

1 Introduction

Chemotherapy is one of the most important strategies in the treatment of cancer and increases the survival rates of cancer patients. The American Cancer Society and the National Cancer Institute reported that more than 16.9 million patients with cancer survived in 2019 and estimated that this number would reach 22.1 million by 2030,[1] indicating that early stage diagnosis and treatment of cancers can extend the lifespan of more and more cancer patients. However, during the chemotherapy treatment, up to 75% of cancer survivors experience cognitive impairments,[2–4] which include attention deficits, decreased executive and multitasking function, and decreased memory functions,[5,6] and these changes affect their quality of life.[7,8] 5-Fluorouracil (5-FU) is a chemotherapeutic drug, which functions as an anti-metabolite,[9] with a good anti-cancer effect on various types of cancers, such as breast cancer and colorectal cancer.[10,11] But previous studies have found that 5-FU could induce cognitive deficits in clinics,[12–14] and has a negative impact on spatial and non-spatial memory in animals.[15–17] However, up to now, the potential mechanism of chemotherapy related cognitive impairment (CRCI) has not been well elucidated, and there was no FDA approved drug for preventing and treating CRCI.

Ganoderma lucidum (*G. lucidum*), a well-known Chinese traditional medicine,[18] has been used in Asian countries for over 2000 years. *G. lucidum* is endowed with various therapeutic properties, such as the ability to improve immunity, promote health, and protect against inflammation and aging.[19–22] A variety of bioactive compounds with pharmacological activities have been isolated from *G. lucidum*, which include triterpenes, polysaccharides, as well as other constituents.[23] Recently, it has been reported that *G. lucidum* triterpenoids improved cognitive function in APP/PS1 transgenic Alzheimer's disease mice.[24] Moreover, researchers found that ganoderic acid (GA), the main bioactive and medicinal compounds of *G. lucidum* triterpenoids, might adjust the synaptic reconstructions through activating the expression of brain derived neuro-

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trophic factor (BDNF) and transient receptor potential canonical 3 (TRPC3), and exert a protective effect on hippocampal neurons *via* improving mitochondrial function, neuronal survival and the recovery of injured neurons,[25,26] which imply that GA may have a moderating effect on CRCI.

In this study, we investigated the effect of GA on 5-FU induced cognitive impairment in mice with a series of behavioral tests and related parameters. Our experimental results demonstrated that GA attenuated cognitive dysfunction in mice treated with 5-FU *via* improving mitochondrial impairment, and neuronal survival and growth, which indicated that GA might be developed as a promising novel drug for CRCI treatment.

2 Materials and methods

2.1 Animals

Male BALB/c mice (7–8 weeks, 18–22 g body weight) were purchased from the Animal Center of Peking University Health Science Center (Beijing, China). The experiments were performed following the National Institutes of Health Guidelines on the Use of Laboratory Animals. The University Animal Care Committee for Animal Research of Peking University Health Science Center approved the study protocol (approval code LA2020485). Mice were maintained on a 12 h light/dark cycle in cages with free access to water and food in a room at 25 ± 1 °C during the whole experimental period. Animals were randomly divided into each experimental group (n = 12 per group).

2.2 Drugs

5-FU was purchased from Sigma (USA). GA was isolated and purified from dried fruiting bodies of *G. lucidum* as reported previously.[20,27] Three monomers accounted for 16.1% (GA-A), 10.6% (GA-B), and 5.4% (GA-C2) of crude GA. The purity of these three monomers was > 98%, as determined by high performance liquid chromatography (HPLC).[20,27]

2.3 Treatment schedule

The mice were randomly divided into three groups by computer based randomization (n = 12): control group, 5-FU group, and 5-FU + GA group. The mice of the 5-FU group were treated with 5-FU (60 mg kg^{-1}, dissolved in normal saline with 5% Tween 80)[28–30] 4 times by an intraperitoneal (i.p.) injection, 3 days apart from day 15 to day 24. The 5-FU + GA group was treated with an equal dose of 5-FU as the 5-FU group and received GA (50 mg kg^{-1} d^{-1}, dissolved in normal saline with 5% Tween 80)[20] starting from day 1 to day 24 intraperitoneally.

As shown in Fig. 1, cognitive dysfunction was evaluated by the novel object location test (NOLT), novel object recognition test (NORT) and Y-maze test (YMT) after drug intervention.

2.4 Novel object location test and novel object recognition test

NOLT and NORT were carried out according to the protocol in the previous study[31] with some modifications. All experimental mice were transferred to the testing room with dimmed illumination and habituated for one day. NOLT was composed of three parts: adaptation trial, familiarization trial and choice trial. In the adaptation trial, all mice were individually placed in the central zone of the open field apparatus (50 × 50 × 50 cm) and allowed to explore for 5 min. The familiarization trial was carried out after one day of adaptation trial, in which each mouse was placed in the middle, far from objects and allowed to freely explore two identical objects placed onto nearest corners for 5 min. After four hours, the choice trial was conducted. In this section, one object stayed in the old location, whereas the other object was moved to a new location. The mouse was once again allowed to explore the objects in the arena for 5 min, and the exploration time was recorded by video. The preference index (%) was calculated following formula: preference index (%) = exploration time in the new location/(exploration time in the old location + exploration time in the new location) × 100%.

NORT was conducted the day after the NOLT. The NORT was also composed of familiarization and choice trials. In the familiarization trial, a mouse was placed at the arena and allowed to explore two identical objects placed onto nearest corners for 5 min. After four hours, the choice trial was conducted. In this section, one object was replaced with a differently colored and shaped object at the same position, and the mouse was allowed to explore the objects in the arena for 5 min. The exploration time was recorded by video. The

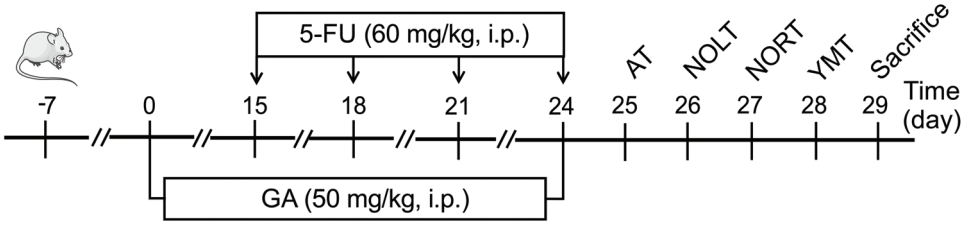

Fig. 1 Experimental flowchart for studying the effect of ganoderic acid (GA) on 5-FU induced cognitive dysfunction in mice. GA (50 mg kg^{-1} d^{-1}) or 5-FU (60 mg kg^{-1} $3d^{-1}$) was intraperitoneally administered to male BALB/c mice for 24 days after randomly grouping. From day 25, cognitive functions were assessed in mice by using adaptation test (AT), novel object location test (NOLT), novel object recognition test (NORT) and Y-maze test (YMT), and the mice were sacrificed on day 29.

preference index (%) was calculated by the following formula: preference index (%) = exploration time with new object/(exploration time with old object + exploration time with new object) × 100%.

2.5 Y-maze test

YMT was conducted as previously reported.[32] In brief, a mouse was placed at the end of one arm of the Y-maze (10 × 50 × 20 cm), in which three arms are symmetrically separated at 120°. The mouse was allowed to freely explore the Y-maze for 8 min, and recorded by video. The number of arm entries was calculated following the animals' activity. A spontaneous alternation was defined as entries into all three arms on consecutive choices. The data were presented as a percentage of spontaneous alternations. The percentage of spontaneous alternations was defined according to the following formula: spontaneous alternation (%) = number of alternations/(total arm entries-2) × 100%.

2.6 Histological HE staining and ultrastructural examination

After the behavioral tests, mice were deeply euthanized by isoflurane inhalation. The mice were perfused with 4% paraformaldehyde. Mouse brains were collected and fixed in 4% paraformaldehyde for 24 h, dehydrated, embedded in paraffin, and cut into 5 μm thick slices. After deparaffinization, the sections were rehydrated and stained with hematoxylin and eosin and examined under a microscope for morphological and pathological evaluation.

For ultrastructural evaluation, the mice were perfused with 2.5% glutaraldehyde, and hippocampi were carefully removed. Tissues of hippocampi were fixed in 2.5% glutaraldehyde, postfixed in osmium tetroxide, and stained with uranyl acetate and lead citrate. The samples were sectioned and observed under a transmission electron microscope (TEM).

2.7 Western blot analysis

Protein was extracted from the hippocampus in tissue protein lysis buffer (Mei5, MF188-01, Beijing, China) supplemented with a protease inhibitor cocktail (Roche, Basel, Kanton Basel-Stadt, Switzerland). The concentration of protein extracts was determined using the bicinchoninic acid (BCA) protein assay reagent kit (Pierce, Rockford, IL, USA). An equal amount of protein was loaded and separated on SDS-PAGE, then transferred into polyvinylidene difluoride (PVDF) membranes (Amersham Biosciences, Boston, MA, USA). After blocking, the membranes were incubated at 4 °C overnight with homologous primary antibodies against β-actin (1 : 10 000, A1011, ABclonal, Wuhan, China), MFN1 (1 : 1000, A9880, ABclonal, Wuhan, China), MFN2 (1 : 1000, A19678, ABclonal, Wuhan, China), DRP1 (1 : 1000, A16661, ABclonal, Wuhan, China), FIS1 (1 : 1000, A19666, ABclonal, Wuhan, China), PGC1-α (1 : 2500, 2178s, Cell Signaling, USA), BDNF (1 : 1000, A18129, ABclonal, Wuhan, China), *p*-Akt (1 : 1000, AP0140, ABclonal, Wuhan, China), *p*-GSK3β (1 : 1000, 9336s, Cell Signaling, USA), Nrf2 (1 : 2500, 12721s, Cell Signaling, USA), *p*-mTOR (1 : 1000, YP0176, ImmunoWay, Beijing, China), mTOR (1 : 1000, YP2913, ImmunoWay, Beijing, China), *p*-S6 (1 : 1000, Ap0538, ABclonal, Wuhan, China), S6 (1 : 1000, A6058, ABclonal, Wuhan, China), *p*-ERK (1 : 1000, 4370s, Cell Signaling, USA), *p*-CREB (1 : 1000, 9198s, Cell Signaling, USA), IL-6 (1 : 1000, A11115, ABclonal, Wuhan, China), IL-1β (1 : 1000, A11370, ABclonal, Wuhan, China), COX2 (1 : 1000, A1253, ABclonal, Wuhan, China), iNOS (1 : 1000, Ab3523, Abcam, UK), *p*-NF-κB (1 : 1000, 3033s, Cell Signaling, USA) and the blots were detected with the ECL plus kit (Biodragon, Beijing, China). The protein band were visualized by a chemiluminescence detection system (Syngene, GeneGnome XRQ, Cambridge, Cambridgeshire, UK) and analyzed using Image J software (NIH, MD, USA).

2.8 Statistical analysis

Statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA, USA). For multiple comparisons, statistical analysis was performed using Student's *t*-test or one-way ANOVA. Significance was declared at $p < 0.05$. All data are expressed as mean ± SEM.

3 Results

3.1 GA significantly improved chemotherapy induced cognitive impairment in mice

As shown in Fig. 1, NOLT, NORT and Y-maze tests were applied to explore the role of GA on chemotherapy related cognitive impairment in mice treated with 5-FU.

3.1.1 GA increased spatial memory in mice treated with chemotherapy. As shown in Fig. 2A, in the familiarization trial of NOLT, mice in all groups spent similar amounts of time exploring each location, indicating that the mice in all groups had no preference for location. During the choice trial of NOLT, mice in the control group spent a longer time exploring the new location than the old location. There was no difference between the time taken to explore the new location and old location in mice treated with 5-FU. However, mice treated with GA tend to explore the new location longer than the old location, and there was no statistical difference in NOLT (Fig. 2B). The total exploration time did not significantly differ among groups (Fig. 2C), indicating that locomotor activity was not impaired in these mice. Compared to the control group, 5-FU decreased the preference index in the NOLT (Fig. 2D) and the spontaneous alteration in the Y-maze test (Fig. 2E), but GA significantly improved the preference index as well as spontaneous alteration in 5-FU treated mice. These experimental results suggest that GA could ameliorate 5-FU induced spatial memory deficits.

3.1.2 GA increased non-spatial memory in mice treated with chemotherapy. In the familiarization trial of NORT, mice in all groups spent a similar amount of time exploring each object (Fig. 2F), indicating that these mice had no preference for objects. During the choice trial of NORT, mice in the control group spent obviously longer time exploring the new object than the old object, but there was no difference between

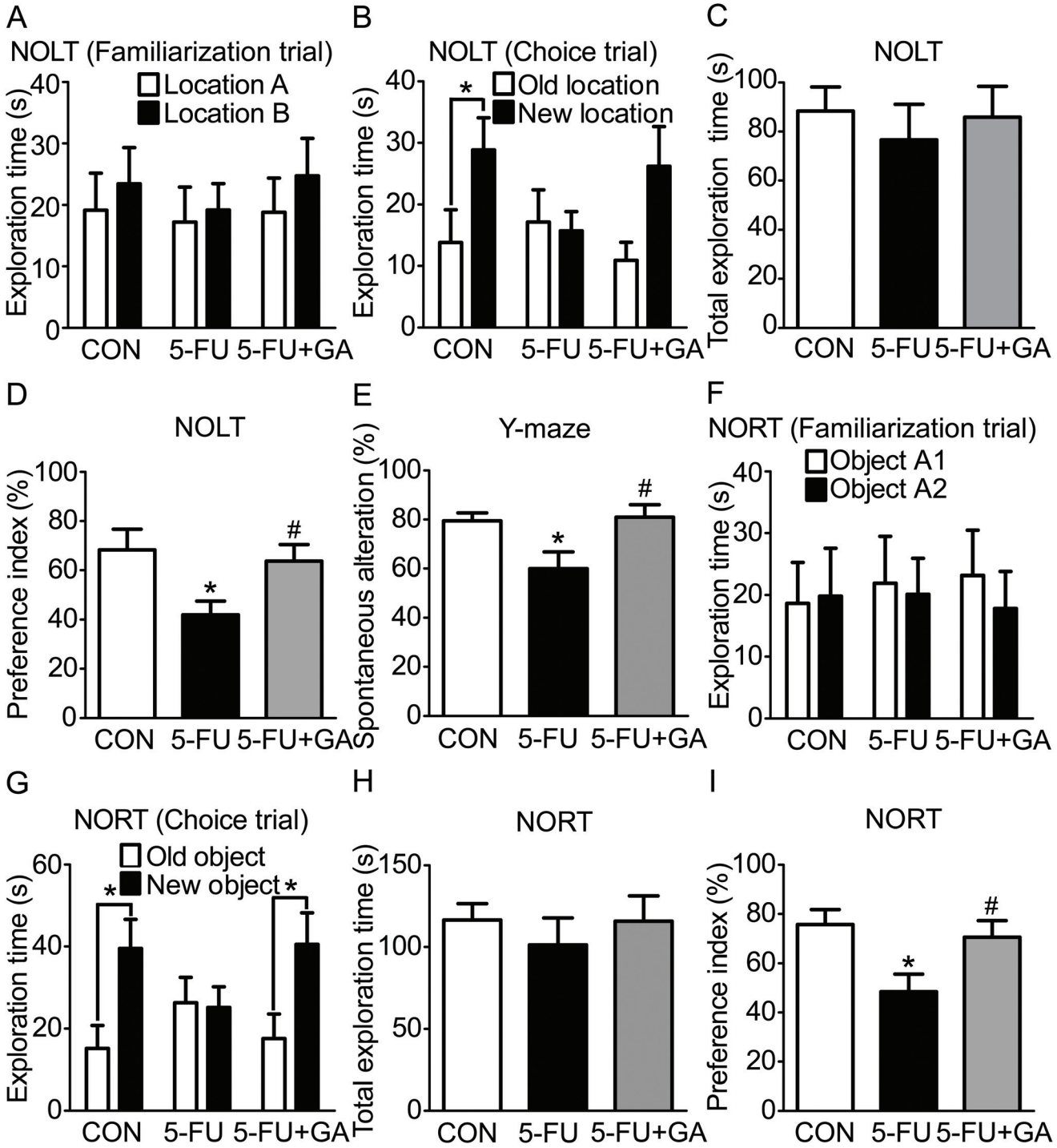

Fig. 2 GA improved chemotherapy induced cognitive impairment in mice. (A) The familiarization trial of novel object location test (NOLT). (B) The choice trial of NOLT. (C) The total exploration time in NOLT. (D) Preference index in NOLT. (E) Spontaneous alteration in the Y-maze test (YMT). (F) The familiarization trial of novel object recognition test (NORT). (G) The choice trial of NORT. (H) The total exploration time in NORT. (I) Preference index in NORT. The values are shown as the mean ± SEM (n = 12). *P < 0.05 *vs*. the CON group. #P < 0.05 *vs*. the 5-FU group. CON, control; 5-FU, 5-fluorouracil; 5-FU + GA, 5-fluorouracil combined with ganoderic acid.

the time taken to explore the new object and old object in mice treated with 5-FU. Mice in the GA group spent significantly longer time exploring the new object than the old object compared to the 5-FU group (Fig. 2G). The total exploration time did not differ among groups (Fig. 2H), indicating that locomotor activity was not impaired. Compared to the control group, 5-FU decreased the preference index in the NORT (Fig. 2I), and GA significantly improved the preference index in

5-FU treated mice. These results imply that GA could ameliorate 5-FU induced non-spatial memory impairment.

3.2 GA alleviated structural damage to the hippocampus in chemotherapy treated mice

Considering that the hippocampus is essential in memory and learning, we measured the morphologic changes of the hippocampi in 5-FU treated mice with or without GA intervention. H&E staining demonstrated lots of tightly packed, deeply dyed neuronal cells in the CA1, CA2, CA3 and DG regions of hippocampi in the control group. 5-FU led to loosely arranged cells, increased tissue space, and a decreased number of cells in the CA1, CA2, CA3 and DG regions of the hippocampus. Compared to the 5-FU group, although GA failed to improve the structural impairment in the CA1 and CA2 regions of 5-FU treated mice, mice treated with GA exhibited a large number of neatly packed pyramidal cells in the CA3 region and granulosa cells in the DG region of the hippocampus (Fig. 3A), indicating that GA could protect 5-FU treated mice from structural injuries of neurons.

We also observed the ultrastructural changes of the hippocampus in the DG region with TEM, and found that 5-FU

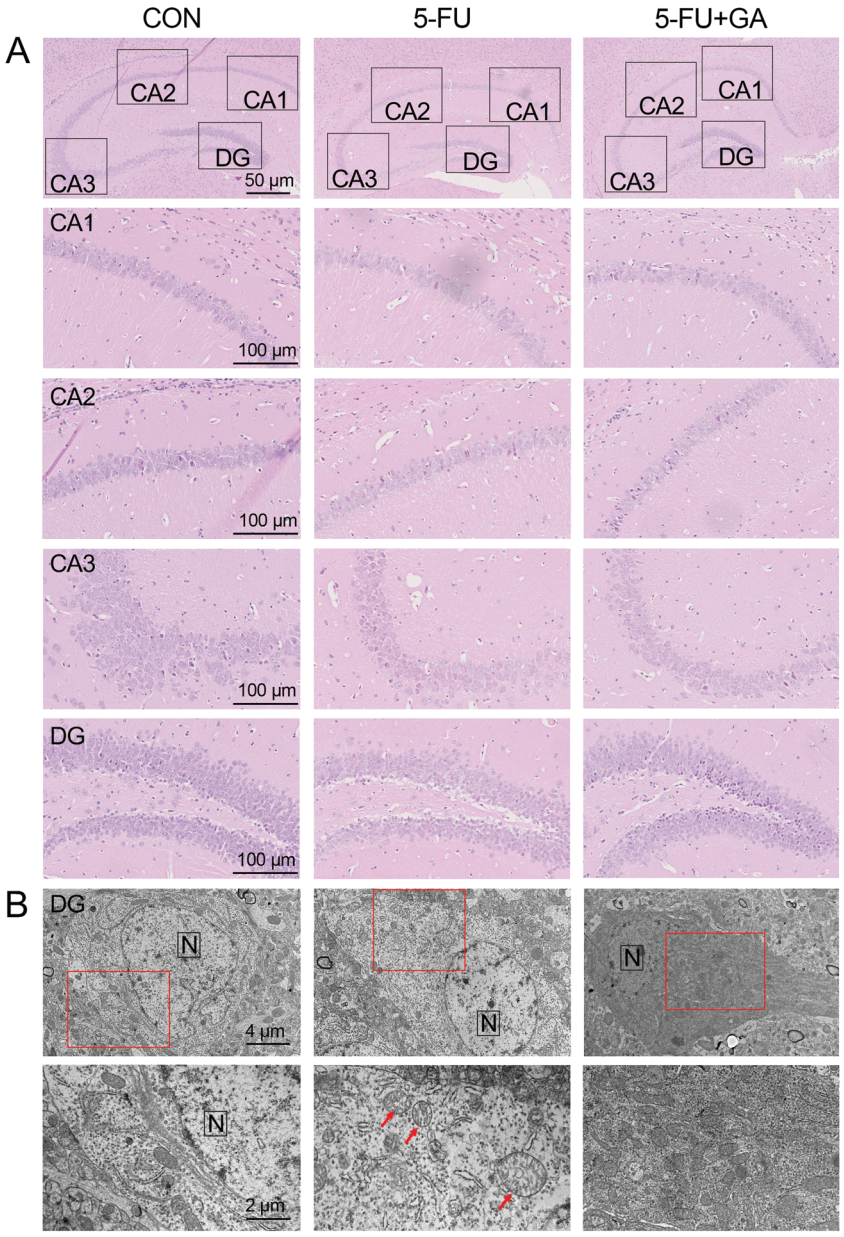

Fig. 3 GA alleviated the damage to hippocampus neurons in chemotherapy treated mice. (A) Representative histological photos of the hippocampus with H&E staining (magnification of 100× or 400×). (B) Representative transmission electron micrographs (TEM) (magnification of 8000× or 20 000×, red arrows showed mitochondrial swelling and crest fracture). CON, control; 5-FU, 5-fluorouracil; 5-FU + GA, 5-fluorouracil combined with ganoderic acid.

decreased electron density in the nucleus and cytoplasm, indicating that edema of cells and intercellular stroma, especially induced intracellular mitochondrial swelling and crest fractures, but GA rescued those structural impairments of mitochondria (Fig. 3B), implicating that GA could prevent mice from hippocampal mitochondrial damage induced by 5-FU.

3.3 GA improved mitochondrial dysfunction induced by chemotherapy

To confirm the protective effect of GA on neurons in the hippocampus, we examined the expression levels of related parameters in the hippocampus of CRCI mice. As shown in Fig. 4A and B, GA significantly increased the expression of MFN2, DRP1 and PGC1-α, and decreased the expression of FIS1 in the hippocampus of 5-FU treated mice, implying that GA retarded CRCI *via* improving the abnormal expression of mitochondrial fusion, fission and biogenesis related proteins in the hippocampus.

3.4 GA regulated the BDNF/Akt/GSK3β/Nrf2 signaling pathway in chemotherapy treated mice

It is reported that BDNF and Nrf2 play an important role in 5-FU induced cognitive impairment. We found that 5-FU down-regulated the expression of BDNF, *p*-Akt, *p*-GSK3β and Nrf2 compared with the control group, and GA significantly improved the decreased expression of proteins above (Fig. 5A and B), indicating that GA could ameliorate 5-FU induced cognitive dysfunction *via* the BDNF/Akt/GSK3β/Nrf2 pathway.

3.5 GA protected neurons from injury *via* ERK and mTOR signaling pathways in chemotherapy treated mice

In order to investigate the neuroprotective effect of GA against 5-FU-induced cognitive dysfunction, we examined the signaling pathways of ERK and mTOR. The results showed that there was a significant down-regulation of *p*-ERK, *p*-CREB, *p*-mTOR and *p*-S6 in the hippocampi of 5-FU treated mice compared to the control group, and GA not only significantly up-regulated the expression of *p*-ERK and *p*-CREB (Fig. 6A and B), but also increased the protein phosphorylation of mTOR and S6 compared to 5-FU treated mice (Fig. 6C and D). These results indicate that GA could protect neurons of CRCI mice *via* ERK and mTOR pathways.

3.6 GA decreased the expression of COX2 and IL-6 in chemotherapy treated mice

Considering that chemotherapy can activate inflammation in the brain, including in the hippocampus, which in turn can lead to cognitive deficits, we investigated the inflammatory cytokines and mediators. Compared to the control group, 5-FU over-activated the expression of COX2, and had no significant effect on the expression of *p*-NF-κB and its downstream proteins like IL-6, IL-1β and iNOS. GA remarkably decreased the levels of COX2 and IL-6 in 5-FU treated mice (Fig. 7A and B).

4 Discussion

5-FU is a chemotherapeutic drug, which functions as an antimetabolite,[9] with a good anti-cancer effect on various types of cancers, such as breast cancer and colorectal cancer.[10,11]

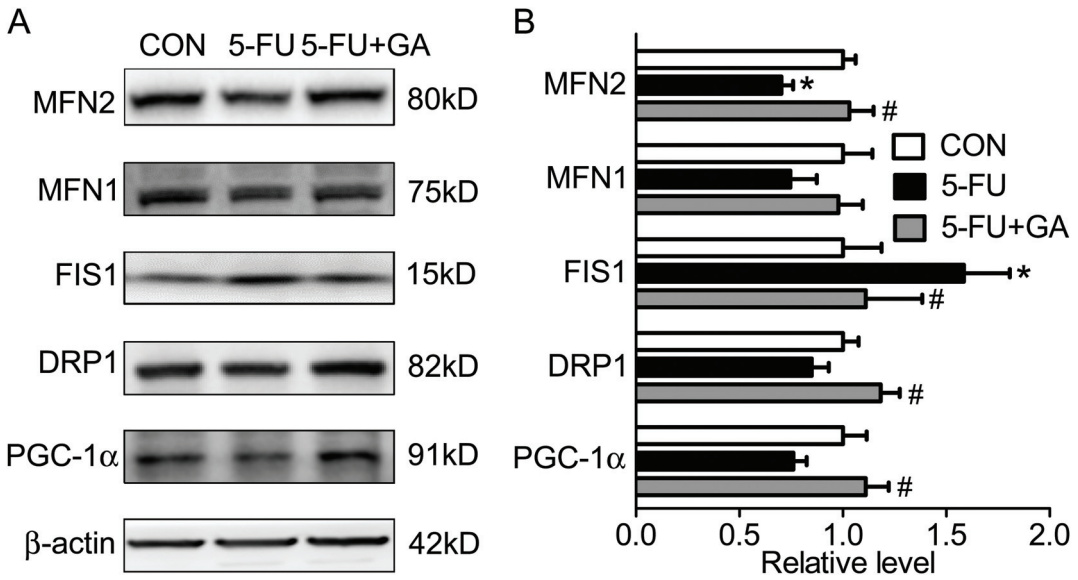

Fig. 4 GA improved the mitochondrial dysfunction induced by chemotherapy. (A) Representative Western blots showing the expression of MFN2, MFN1, FIS1, DRP1, and PGC-1α in the hippocampus. (B) Relative protein levels in the experiments shown in (A). The data were normalized to the intensity of β-actin and are expressed relative to the value of the control group. The values are shown as the mean ± SEM (n = 5). *P < 0.05 *vs*. the CON group. #P < 0.05 *vs*. the 5-FU group. CON, control; 5-FU, 5-fluorouracil; 5-FU + GA, 5-fluorouracil combined with ganoderic acid.

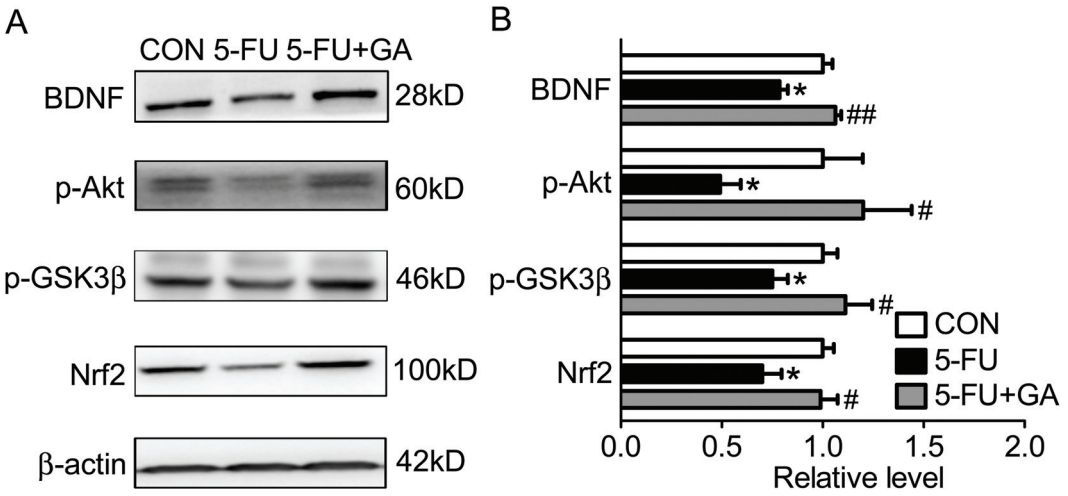

Fig. 5 GA regulated the BDNF/Akt/GSK3β/Nrf2 signaling pathway in chemotherapy treated mice. (A) Representative Western blots showing the expression of BDNF, *p*-Akt, *p*-GSK3β and Nrf2 in the hippocampus. (B) Relative protein levels in the experiments shown in (A). The data are normalized to the intensity of β-actin and are expressed relative to the value of the control group. The values are shown as the mean ± SEM (n = 5). *P < 0.05 *vs*. the CON group. #P < 0.05, ##P < 0.01 *vs*. the 5-FU group. CON, control; 5-FU, 5-fluorouracil; 5-FU + GA, 5-fluorouracil combined with ganoderic acid.

However, previous studies have found that 5-FU could induce cognitive deficit in clinics,[12–14] and has a negative impact on spatial and non-spatial memory in animals.[15–17] In this study, we investigated the neuroprotective effect of GA on 5-FU-induced cognitive impairment using related behavior tests, such as NOLT, NORT and Y-maze tests. Our experimental results showed that GA significantly improved cognitive behavioral alterations in CRCI mice, as evidenced by increasing exploration time of the new object, preference index and spontaneous alteration in 5-FU treated mice.

Cytokine dysregulation is regarded as one of the pathogenesis of CRCI.[33] Proinflammatory cytokines exhibited considerable toxic effects on neuronal cells, especially the hippocampal cells.[33] Recently, it was reported that 5-FU induced cognitive impairment in aging mice by upregulating cytokines.[30] In our experiment, we found that 5-FU treatment over-activated the expression of only cyclooxygenase 2 (COX2), but did not significantly affect the expression of other inflammatory cytokines and mediators, such as nuclear factor kappa-B (NF-κB) and its downstream molecules interleukin-6 (IL-6), interleukin-1β (IL-1β) and inducible nitric oxide synthase (iNOS). GA attenuated the expression of COX2 and IL-6 in CRCI mice. IL-6 is a potent inflammatory cytokine that mediates several important physiological functions, most importantly control of the acute phase response at the beginning of acute inflammation.[34] COX2 is an inducible enzyme that is widely distributed in the brain, including the hippocampus,[35] and largely takes part in regulating the inflammation process following stimuli.[36,37] Recently, some researchers reported that COX2 inhibitors ameliorated memory and learning performance in mice.[38,39] Therefore, we suggest that COX2 plays an important role in 5-FU induced cognitive impairment and GA, to some extent, improved 5-FU induced cognitive dysfunction *via* inhibition of COX2 expression.

Our experimental results demonstrated that damage to the hippocampal mitochondria is a potential mechanism of 5-FU induced cognitive impairment. 5-FU could cause neurotoxicity *via* dysfunction of mitochondrial dynamics, including mitofusin1/2 (MFN1/2).[40] In this study, we found that 5-FU induced structural changes of hippocampal mitochondria. The morphology, quality and quantity of mitochondria are strictly controlled by dynamic biosynthesis, fission and fusion.[41,42] In biosynthesis, mitochondria are generated by proteins encoded by nuclear and mitochondrial DNA, which are regulated by peroxisome proliferator-activated receptor-γ coactivator (PGC-1α).[43] PGC-1α not only mediates mitochondrial biogenesis, but also regulates mitochondrial fusion and fission.[44] It is revealed that the upregulation of neuronal PGC-1α ameliorates cognitive impairment.[45] Mitochondria are the dynamic organelles that are reshaped by the balanced processes of fusion and fission, which are involved in the clearance of damaged mitochondria and the maintenance of normal mitochondrial morphology.[46] MFN1 and MFN2, which are involved in the fusion of the outer mitochondrial membrane, have been revealed to play an essential role in controlling mitochondrial fusion.[47] It is reported that the loss of MFN2 results in aberrant mitochondrial morphology[48] and contributes to pathogenesis in chemotherapy induced neuropathy.[49] In addition, fission 1 (FIS1) and dynamin-like protein 1 (DRP1) are the primary regulator of mitochondrial fission. FIS1 shrinks the mitochondrial membrane in a GTP dependent manner, resulting in the mitochondrial division, and excessive division leads to mitochondrial fragmentation and dysfunction.[50,51] Recently, researchers have found that abnormal mitochondrial dynamics leads to neuron degeneration, and the normalization of mitochondrial dynamics could inhibit cognitive impairment.[52]

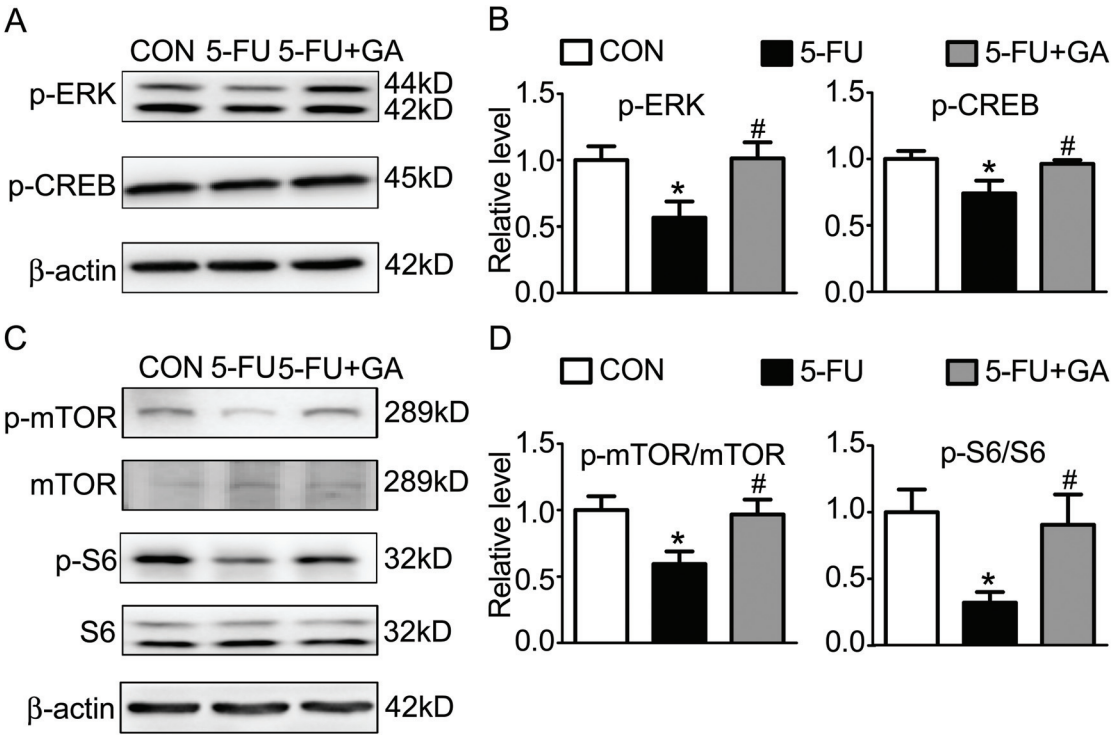

Fig. 6 GA protected neurons from injury *via* the ERK and mTOR signaling pathways in chemotherapy treated mice. (A) Representative Western blots showing the expression of *p*-ERK and *p*-CREB in the hippocampus. (B) Relative protein levels in the experiments shown in (A). (C) Representative Western blots showing the expression of *p*-mTOR, mTOR, *p*-S6 and S6 in the hippocampus. (D) Relative protein levels in the experiments shown in (C). The data were normalized to the intensity of β-actin or the nonphosphorylated form of the protein of interest and are expressed relative to the value of the control group. The values are shown as the mean ± SEM (n = 5). *P < 0.05 *vs*. the CON group. #P < 0.05 *vs*. the 5-FU group. CON, control; 5-FU, 5-fluorouracil; 5-FU + GA, 5-fluorouracil combined with ganoderic acid.

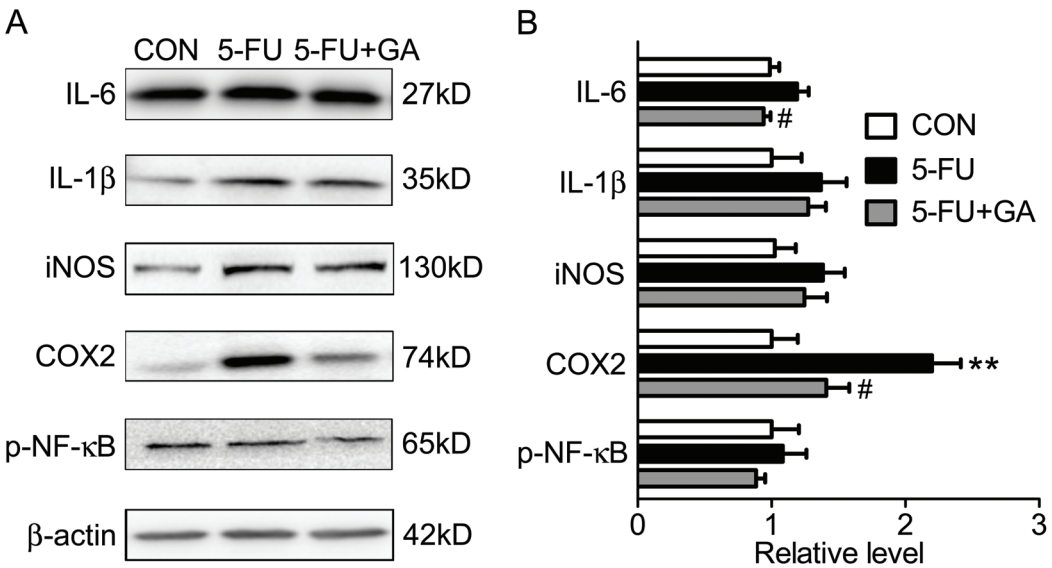

Fig. 7 GA decreased the expression of COX2 and IL-6 in chemotherapy treated mice. (A) Representative Western blots showing the expression of IL-6, IL-1β, iNOS, COX2 and *p*-NF-κB in the hippocampus. (B) Relative protein levels in the experiments shown in (A). The data were normalized to the intensity of β-actin and are expressed relative to the value of the control group. The values are shown as the mean ± SEM (n = 4–5). *P < 0.05, **P < 0.01 *vs*. the CON group. #P < 0.05 *vs.* the 5-FU group. CON, control; 5-FU, 5-fluorouracil; 5-FU + GA, 5-fluorouracil combined with ganoderic acid.

In this study, we found that GA not only improved morphological changes of hippocampal mitochondria in 5-FU treated mice, such as decreased electron density of the nucleus and cytoplasm, and intracellular mitochondrial swelling and crest fractures, but also significantly promoted the expression of mitochondrial biogenesis related protein PGC-1α, and mitochondria dynamics related proteins MFN2 and DRP1 while inhibited the expression of mitochondrial fission related marker FIS1 in the hippocampi of 5-FU-treated mice, indicating that GA could retard 5-FU induced cognitive dysfunction *via* preventing mitochondrial impairment.

Brain derived neurotrophic factor (BDNF) maintains the growth, differentiation and survival of neurons as well as synaptic plasticity.[53,54] Moreover, researchers found that the decreased protein expression of BDNF in chemotherapy induced cognitive impairment.[55] A number of studies have indicated that BDNF activates the extracellular regulated protein kinases (ERK) pathway, one of the best characterized signal transduction pathways, after binding to TrkB.[56,57] The ERK pathway effectively connects external signals with intracellular signals.[58] The cAMP-response element binding protein (CREB), a transcription factor, is reported as the downstream target of ERK. A series of studies have revealed the important roles of the BDNF/ERK/CREB signaling pathway in cognitive impairments.[59–61] In this study, we found that 5-FU significantly reduced the protein expression of BDNF and its downstream molecules *p*-ERK and *p*-CREB, but GA could activate the BDNF/ERK/CREB signaling pathway in the hippocampal tissue to inhibit cognitive impairment in the 5-FU-treated mice.

Moreover, BDNF also activates the mammalian target of the rapamycin (mTOR) signaling pathway in the hippocampus.[62,63] mTOR regulates cell growth, proliferation, protein synthesis and synaptic plasticity.[64] Researchers reported protein kinase B (Akt)/mTOR signaling as the key pathway for neuronal growth and survival.[65,66] Mice with Akt deficiency exhibited impaired cognitive function *via* the inhibition of mTOR signaling.[67,68] As an upstream regulator of glycogen synthase kinase 3 beta (GSK3β), activated Akt suppressed the activation of GSK3β by increasing the phosphorylation of GSK3β and promoted cognitive function.[69] It is reported that Nrf2 is regulated by the phosphorylation of GSK3β.[70] Nrf2, as a stress sensitive transcription factor, plays an important role in mediating redox balance and protecting cells from damage *via* transferring into the nucleus, and subsequently expressing its downstream genes.[71,72] In addition, Nrf2 is closely related to the mTOR signaling pathway. Recent studies showed that downregulation of Nrf2 expression induced the inhibition of

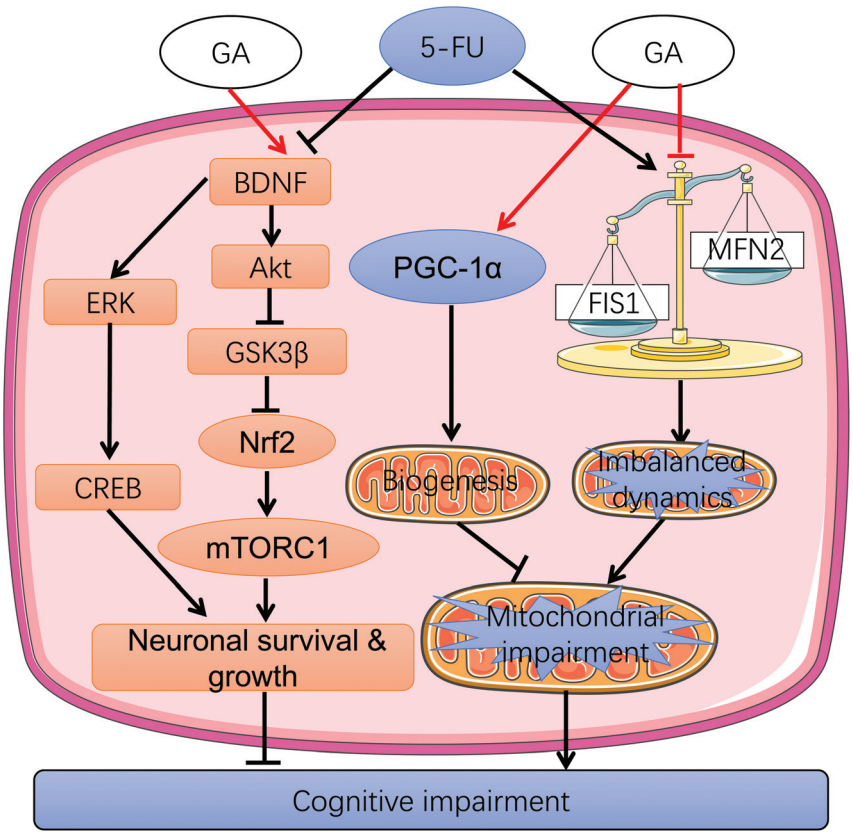

Fig. 8 Schematic diagram of the proposed mechanisms underlying the effect of GA in 5-FU induced cognitive dysfunction. GA ameliorates 5-FU-induced cognitive dysfunction *via* improving mitochondrial impairment and BDNF mediated ERK/CREB and Akt/Nrf2/mTORC1 signaling pathways in mice.

mTOR.[73,74] Thus, the regulation of the BDNF/Akt/GSK3β/Nrf2/mTORC1 signaling plays an important role in memory impairment.[75] In this study, our experimental results showed that GA significantly promotes the expression of BDNF, *p*-Akt, *p*-GSK3β, Nrf2, *p*-mTOR and *p*-S6 in the hippocampi of 5-FU-treated mice, suggesting that GA could ameliorate CRCI *via* improving neuronal survival and growth-related pathways.

However, we have to acknowledge the limitation of our study. We have not used relevant activators or conditional knockout mice to confirm the specific underlying molecular mechanisms, which should be studied in the future.

5 Conclusion

In this study, we, for the first time, provided evidence that GA ameliorated 5-FU induced cognitive dysfunction by improving mitochondrial impairment and BDNF mediated ERK/CREB and Akt/Nrf2/mTORC1 signaling pathways (Fig. 8), suggesting that GA may be developed as a promising novel therapeutic drug for the development of cognitive impairment in patients with chemotherapy treatment.

Author contributions

Abudumijiti Abulizi, Baoxue Yang and Min Li conceptualized and designed this study. Abudumijiti Abulizi, Jianhua Ran, Yuwei Ye, Yongpan An, Yukun Zhang, Zhizhen Huang, Simei Lin, Dongmei Lin and Lianfu Wang performed the experiments and analyzed the data. Audumijiti Abulizi wrote the manuscript. Hong Zhou, Zhibin Lin, Min Li and Baoxue Yang reviewed and revised the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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