



Coenzyme Q10 Protects Astrocytes from Ultraviolet B-Induced Damage Through Inhibition of ERK 1/2 Pathway Overexpression

Qi Zhao¹ · Yan-Mei Ma¹ · Li Jing¹ · Tian-Xiang Zheng¹ · Hai-Feng Jiang^{1,2} · P. Andy Li³ · Jian-Zhong Zhang¹

Received: 30 January 2019 / Revised: 2 May 2019 / Accepted: 4 May 2019 / Published online: 15 May 2019
© Springer Science+Business Media, LLC, part of Springer Nature 2019

Abstract

Overexpression of extracellular signal-regulated kinase 1/2 (ERK 1/2) signaling pathway leads to overproduction of reactive oxygen species (ROS) which induces oxidative stress. Coenzyme Q10 (CoQ10) scavenges ROS and protects cells against oxidative stress. The present study was designed to examine whether the protection of Coenzyme Q10 against oxidative damage in astrocytes is through regulating ERK 1/2 pathway. Ultraviolet B (UVB) irradiation was chosen as a tool to induce oxidative stress. Murine astrocytes were treated with 10 µg/ml and 25 µg/ml of CoQ10 for 24 h prior to UVB and maintained during UVB and 24 h post-UVB. Cell viability was evaluated by counting viable cells and MTT conversion assay. ROS production was measured using fluorescent probes. Levels of p-ERK 1/2, ERK 1/2, p-PKA, PKA were detected using immunocytochemistry and/or Western blotting. The results showed that UVB irradiation decreased the number of viable cells. This damaging effect was associated with accumulation of ROS and elevations of p-ERK 1/2 and p-PKA. Treatment with CoQ10 at 25 µg/ml significantly increased the number of viable cells and prevented the UVB-induced increases of ROS, p-ERK 1/2, and p-PKA. It is concluded that suppression of the PKA-ERK 1/2 signaling pathway may be one of the important mechanisms by which CoQ10 protects astrocytes from UVB-induced oxidative damage.

Keywords Astrocyte · Extracellular signal-regulated kinase · Coenzyme Q10 · Reactive oxygen species · Ultraviolet

Introduction

It is well known that astrocytes are the most abundant cells in the central nervous system (CNS), and they can be found all over the regions of the brain [1]. Astrocytes exert far-ranging functions including guidance of the development of neurons during brain development, supplement of

many growth factors, maintenance of the blood–brain barrier (BBB) integrity, and participating in the immune and repairing responses to injury [2, 3]. In the past few decades, neuronal responses to acute and chronic brain injury events have been extensively studied [4, 5]. In recent years, more attentions have been drawn to astrocyte due to its critical role in the modulation of synaptic transmissions, management of energy metabolism, maintenance of ion homeostasis and integrity of the BBB, and the promotion of tissue repair after

Qi Zhao, Yan-Mei Ma and Li Jing contributed equally to this work.

✉ P. Andy Li
pli@nccu.edu

✉ Jian-Zhong Zhang
zhangjz@nxmu.edu.cn

Qi Zhao
956835074@qq.com

Yan-Mei Ma
13895624275@163.com

Li Jing
1203220205@qq.com

Tian-Xiang Zheng
349710430@qq.com

Hai-Feng Jiang
jh0347@163.com

¹ School of Basic Medical Sciences, Department of Pathology, Ningxia Medical University; Ningxia Key Laboratory of Vascular Injury and Repair, Yinchuan, Ningxia, People's Republic of China

² Department of Pathology, General Hospital of Ningxia Medical University, Yinchuan, Ningxia, People's Republic of China

³ Department of Pharmaceutical Sciences, Biomanufacturing Research Institute and Technological Enterprise (BRITE), North Carolina Central University, Durham, NC, USA

brain injuries [6–8]. Understanding the molecular signaling pathways that initiate astrocyte damage is important for the development of therapeutic approaches that preserve astrocyte morphology and function.

Excessive accumulation of reactive oxygen species (ROS) in cell causes damage to lipid, protein, and DNA, which adversely affects cellular function and results in cell death. In the nervous system, accumulation of ROS has been shown to damage the electron transport chain (ETC) complexes, resulting in more mitochondrial production of toxic oxygen radicals [9]. For the reasons of high oxygen consumption, low antioxidant defense and the presence of abundant oxygen-sensitive lipids, the brain is vulnerable to excessive production of ROS [10]. It is reported that ROS induced by H_2O_2 , changes lipid level of astrocytes plasma membrane and makes it fragile [11]. As astrocytes may be more vulnerable than neurons to ROS following injury, preventing ROS damage in glia is particularly important [12].

Mitogen-activated protein kinase (MAPK) family is an important intracellular signal transduction system, that participates in a series of physiological and pathological processes, including cell death and survival [13]. Extracellular signal-regulated kinase 1/2 (ERK 1/2) is a member of MAPK family. ERK 1/2 signaling pathway plays an important role in the maintenance of neuronal function and plasticity following traumatic brain injury [14]. It has been demonstrated that suppressing ERK 1/2 activation by U0126 could alleviate sevoflurane induced learning and memory malfunction [15]. Our previous study has shown that lactic acid could result in astrocyte death through activation of phosphorylated-ERK 1/2 (p-ERK 1/2) [16] and that increased ERK 1/2 phosphorylation might be associated with neuronal cell death caused by cerebral ischemia [17]. Protein kinase A (PKA) is a member of the Ser/Thr protein kinase family and is a catalytic subunit of cAMP-dependent protein kinase. The stimulation of PKA causes ERK 1/2 phosphorylation [18].

Coenzyme Q10 (CoQ10), a component of the mitochondrial electron transport chain, is well characterized as a neuroprotective antioxidant in animal models of neurodegeneration, including Alzheimer's disease [19] and Parkinson's disease [20]. It is also believed that CoQ10 prevents oxidative stress which induced vascular endothelial cell senescence and dysfunction [21]. CoQ10 protects optic nerve head astrocytes against oxidative stress-caused damage through increasing mitochondrial mass and bioenergetic function [22]. Our previous studies also suggested that CoQ10 could protect neural cells from UVB damage by inhibiting mitochondria-initiated cell death pathway and promotes mitochondrial biogenesis [23]. Previous studies have shown that CoQ10 could block ERK 1/2 MAPK signaling in vascular endothelial cells and articular chondrocytes [24, 25]. The objectives of this study are to define whether

oxidative stress induced cell damage in astrocytes, is related to activation of PKA-ERK signaling pathway, and whether CoQ10 protects astrocytes against oxidative damage, by preventing the over activation of PKA-ERK 1/2 pathway. In this study, murine embryonic astrocytes were exposed to ultraviolet wave B (UVB) irradiation and treated with vehicle or CoQ10 of 10 and 25 $\mu\text{g/ml}$. Cell viability, ROS content, and p-ERK 1/2, ERK 1/2, p-PKA, and PKA levels were measured. The results demonstrated that CoQ10 protected astrocytes against UVB-induced cell death. The protective effect was associated with reduction of ROS content and suppression of UVB-induced activation of PKA-ERK 1/2 signaling pathway.

Material and Methods

Reagents

Dulbecco's modified Eagle medium (DMEM) (Invitrogen, Carlsbad, CA) and fetal bovine serum (FBS, HyClone Cell Culture and Bioprocessing) were used. Trypsin-Versene mixture and Penicillin–Streptomycin solution were purchased from HyClone laboratories (Waltham, MA). CoQ10 was obtained from Sigma-Aldrich (St. Louis, MO USA), dissolved in DMSO (Mediatech, USA) and diluted in culture medium.

Cell Culture, UVB and CoQ10 Treatment

The purchased murine astrocytes from embryo (G355-5, ATCC, USA) were cultured in DMEM supplemented with 10% FBS, 100 mM Penicillin–Streptomycin and maintained at 90% relative humidity in 5% CO_2 at 37 °C. The culture medium was renewed every 2 days. Murine astrocytes (15,000–80,000/well) were seeded in 12 or 96-wells cell culture plates and incubated in the above medium for at least 24 h in CO_2 incubator to 80% cell confluence.

Ultra violet B irradiation was chosen for inducing oxidative stress to the cells. Prior to UVB irradiation, the cultures were washed twice to remove residual serum and nonattached cells. Cells were incubated in serum-free medium for 1 h and then exposed to 7 mJ/cm^2 dose of UVB irradiation with a Fisher UV Transilluminator FB-TI-88A. This UVB dosage energy was chosen based on our previous study showing 50–70% cell death at 24 h post-exposure [26]. After UVB radiation, cells were returned to the culture incubator for 24 h of recovery at 37 °C. The cells were treated with 10 $\mu\text{g/ml}$ and 25 $\mu\text{g/ml}$ of CoQ10 for 24 h prior to UVB and the CoQ10 dose was maintained during and after UVB irradiation [26]. DMSO (0.1%) treated cells were used as control.

Cell Viability Assay

At 24 h after UVB irradiation, numbers of viable cells in 12-well plates were counted using Beckman Coulter Vi-Cell Automated Cell Viability Analyzer (WS-VICELL), which uses trypan blue exclusion principle. Before counting numbers of viable cells using Beckman Coulter Vi-Cell Automated Cell Viability Analyzer (WS-VICELL), cultured cells were digested with trypsin to obtain single cell suspension and stained by trypan blue. The number of viable cells was calculated and presented. All experiments were performed in triplicate and repeated in at least three separate experiments.

Mitochondrial Succinate Dehydrogenase Activity

To measure the mitochondrial succinate dehydrogenase activity in vehicle and CoQ10 treated murine astrocytes, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) conversion assay was used. Conversion of yellow MTT to purple formazan requires succinate dehydrogenase and depends on the activity of mitochondria respiratory chain. The MTT assay can be used to determine mitochondria activity, as well as measure cell viability. The cells were seeded in 96-well plates for MTT staining after 24 h of recovery following UVB injury. The MTT was added to the medium and incubated for 4 h in 37 °C in dark. After incubation, the cells were incubated in DMSO to enable the release of formazan. Absorbency at the wavelength 570 nm was read on an ELISA plate reader (Molecular Device, San Jose, USA).

Measurements of Superoxide

Intracellular ROS (superoxide anion) production was determined using dihydroethidium (DHE) fluorescent probe. Briefly, cells (2×10^6 /mL) were incubated with the DHE (2.5 μ M) for 30 min at 37 °C. The cells were washed, resuspended in phosphate buffered saline (PBS) and analyzed for fluorescence intensity using Fluoromax-4 spectrofluorometer (HORIBA Jobin Yvon Inc, Edison, NJ) at the excitation and emission wavelengths of 480 nm and 590 nm respectively. The results were presented as relative fluorescence intensity (RFI).

Immunocytochemistry

Astrocytes were fixed with 4% paraformaldehyde for 20 min at room temperature and washed with PBS for immunocytochemistry. After permeabilization in 0.5% Triton X-100 for 15 min, the cells were incubated overnight at 4 °C with monoclonal anti-phospho-ERK 1/2 antibody (1:200 dilution, Cell Signaling Technology), monoclonal anti-GFAP antibody (1:300 dilution, Cell Signaling Technology), and

polyclonal anti-phospho-PKA antibody (1:100 dilution, Abcam) followed by incubation with a secondary goat anti-rabbit Alexa Fluor 488 (Invitrogen) or goat anti-mouse Alexa Fluor 568 conjugate (Invitrogen) for 1 h at room temperature. The slides were mounted with Vectashield Mounting Media containing DAPI (Vector Laboratories) and scanned under a fluorescence confocal microscope (Nikon Eclipse C1). Three microscopic fields at 400 \times were captured and number of positively stained cells was counted.

Western Blot Analysis

After UVB irradiation and CoQ10 treatment, cells were collected and lysed on ice in lysis buffer containing 20 mM Tris (pH 7.4), 10 mM KCl, 3 mM MgCl₂, 0.5% NP40 and protease inhibitors (Roche). The lysates were centrifuged at 500 g for 10 min and resulted in a supernatant (S1) and a pellet (P1). The S1 fraction was centrifuged at 20,000 g for 20 min and the resulting supernatant was used as a cytosolic fraction. The P1 fraction was washed twice with lysis buffer, resuspended in lysis buffer containing 1% SDS and sonicated briefly on ice (Misonix, Ultrasonic Cell Disrupter). It was then centrifuged at 20,800 g for 30 min and the resulting supernatants were designated as nuclear fractions. The purity of different cellular fractions has been tested previously [26]. The cell lysates containing 20 μ g proteins were loaded into each lane, separated in 10% NuPAGE BT gels (Invitrogen), transferred to a PVDF membrane (Millipore), and probed with antibodies against phospho-ERK 1/2 (1:2000 dilution, Cell Signaling Technology), ERK 1/2 (1:1000 dilution, Cell Signaling Technology), phospho-PKA (1:2500, Abcam), and PKA (1:500, Abcam).

Statistical Analysis

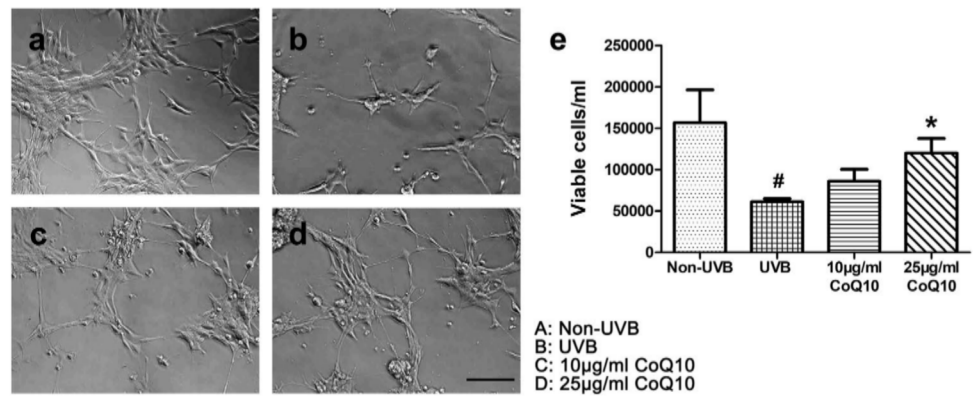
All data were presented as means \pm SD. One-way ANOVA followed by Tukey's test was used for statistical analysis. A value of $p < 0.05$ was considered statistical significant.

Results

Effect of CoQ10 on UVB Induced Astrocytes Injury

To investigate the effect of CoQ10 on UVB-induced damage to astrocytes, numbers of viable cells were counted in non-UVB-challenged (Fig. 1a), UVB-challenged (Fig. 1b), and CoQ10-treated groups (Fig. 1c, d). UVB irradiation reduced the viable cells from $156,667 \pm 39,832.98$ /mL to $61,333 \pm 3723.80$ /mL, nearly a 60% reduction compared with non-UVB treated cells ($p < 0.01$). Treatment with CoQ10 at a concentration of 25 μ g/ml significantly increased the number of viable cells ($p < 0.05$), but there was no

Fig. 1 Cell viability with trypan blue staining. The representative photomicrograms in non-UVB (a), UVB (b), CoQ10 10 $\mu\text{g}/\text{ml}$ (c), and CoQ10 25 $\mu\text{g}/\text{ml}$ (d). Bar=25 μm . **e** Bar graph sums up viable cells. # $p < 0.01$ versus non-UVB and * $p < 0.05$ versus UVB. One-way ANOVA followed by Tukey's test



significant difference the between 10 $\mu\text{g}/\text{ml}$ group and UVB group (Fig. 1c).

Influence of CoQ10 on Mitochondrial Succinate Dehydrogenase Activity

The mitochondrial succinate dehydrogenase enzyme activity was detected using MTT assay (Fig. 2). Compared with Non-UVB control (Fig. 2a), UVB-irradiation resulted in a significant decrease of MTT reduction (Fig. 2b), indicating a decrease of metabolically active mitochondria and cell viability. In contrast, 25 $\mu\text{g}/\text{ml}$ of CoQ10 significantly intensified MTT reduction (Fig. 2d), but significant difference was not found between 10 $\mu\text{g}/\text{ml}$ group (Fig. 2c) and UVB group. A summarized bar graph is given in Fig. 2e.

Effects of CoQ10 on ROS

ROS production was measured using dihydroethidium (DHE) probe. As shown in Fig. 3, compared with Non-UVB control (Fig. 3a), UVB irradiation significantly increased the production of ROS (Fig. 3b). Treatment with both 10 $\mu\text{g}/\text{ml}$ and 25 $\mu\text{g}/\text{ml}$ of CoQ10 prevented the increase of

UVB-induced ROS production (Fig. 3c, d), almost down to the level of ROS in non-UVB irradiated controls. Relative fluorescence intensity (RFI) was summarized in the bar graph (Fig. 3e).

Effect of CoQ10 on p-ERK 1/2 After UVB

To examine whether the protective effect of Coenzyme Q10 on astrocytes is via regulating ERK 1/2 pathways, we performed immunocytochemistry and/or Western blotting using specific antibodies against GFAP and p-ERK 1/2 (Fig. 4). As is shown in Fig. 4a, almost no p-ERK 1/2 positive staining was observed in control cells without UVB challenge (Fig. 4a, upper panel). UVB irradiation induced a significant increase in p-ERK 1/2 positive cells (Fig. 4a, middle panel). The location of the p-ERK 1/2 is in the nuclei. Treatment with CoQ10 (25 μg) dramatically decreased the number of p-ERK 1/2 positive cells (Fig. 4a, lower panel). In addition, all of the cells in every group were labeled by the marker of astrocyte GFAP. In agreement with the immunocytochemical finding, Western blotting using p-ERK 1/2 antibody (Fig. 4b, c) revealed two weak bands of 44 and 42 KD in the nuclear fraction of the non-UVB control group.

Fig. 2 MTT assay of cell viability and mitochondrial succinate dehydrogenase activity. The representative photomicrograms in non-UVB (a), UVB (b), CoQ10 10 $\mu\text{g}/\text{ml}$ (c), and CoQ10 25 $\mu\text{g}/\text{ml}$ (d). Bar=25 μm . **e** Bar graph sums up cell viability. # $p < 0.01$ versus non-UVB and * $p < 0.05$ versus UVB. One-way ANOVA followed by Tukey's test

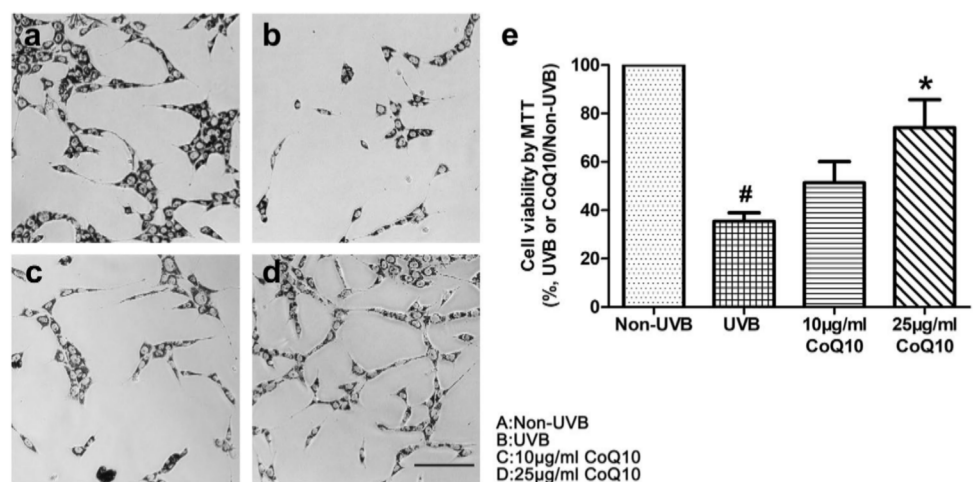


Fig. 3 Measurements of ROS by dihydroethidium probe. The representative photomicrograms of dihydroethidium fluorescent probe in non-UVB (a), UVB (b), CoQ10 10 $\mu\text{g/ml}$ (c), and CoQ10 25 $\mu\text{g/ml}$ (d). Bar = 25 μm . **e** Bar graph sums up the relative fluorescence intensity (RFI). # $p < 0.01$ versus non-UVB and * $p < 0.05$ versus UVB. One-way ANOVA followed by Tukey's test

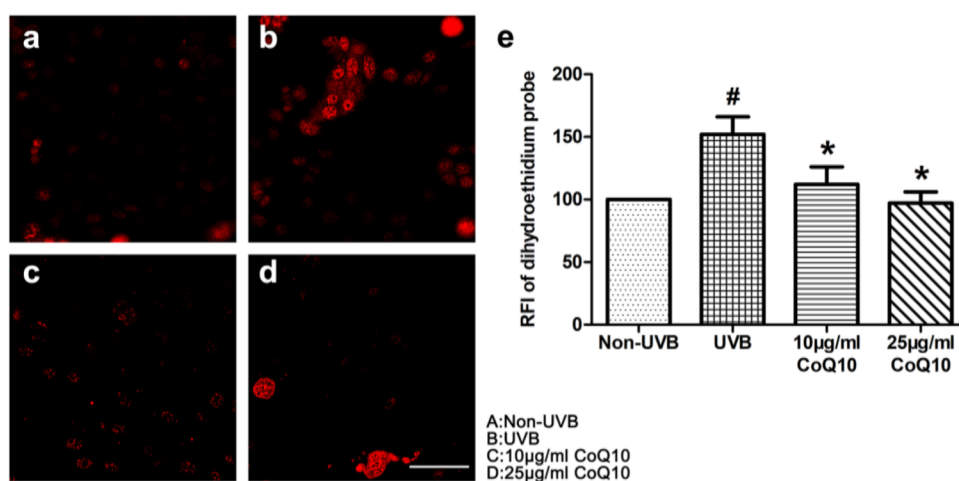
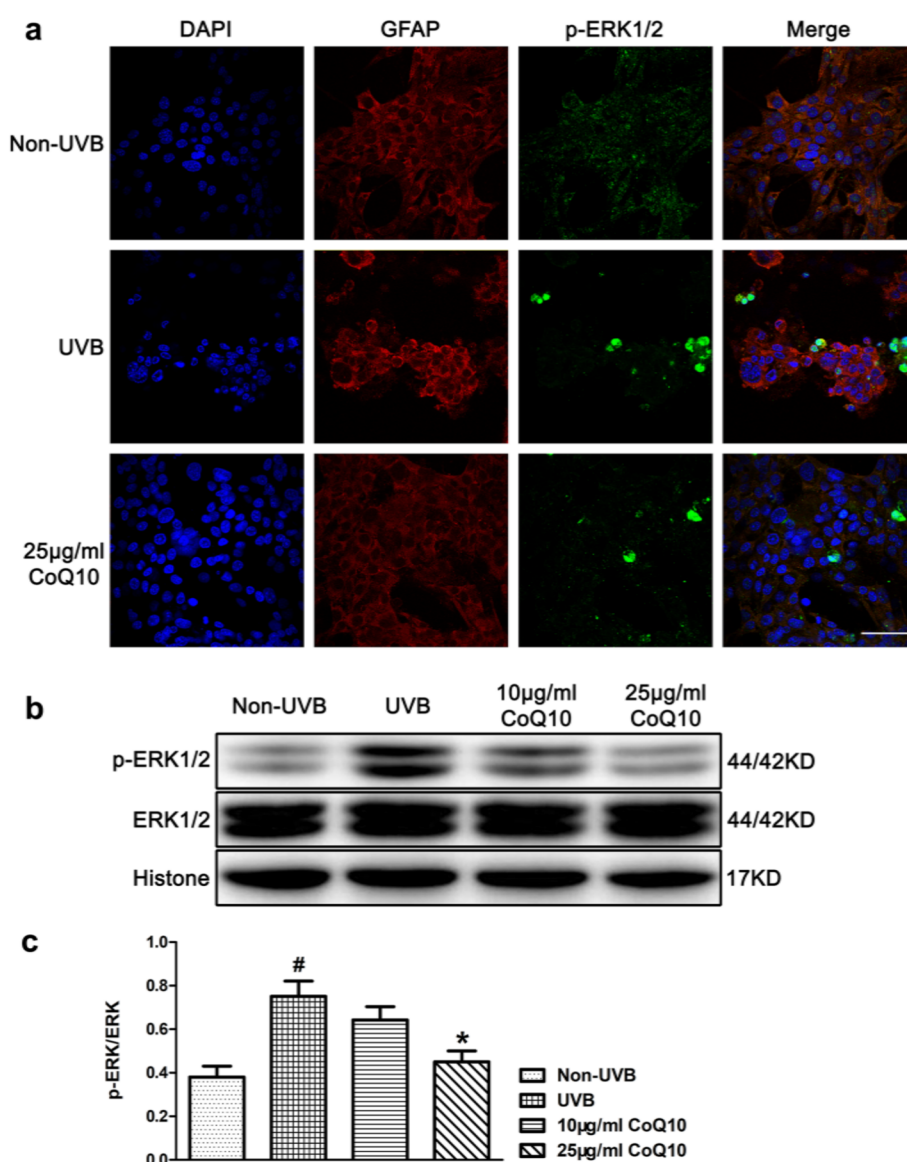


Fig. 4 p-ERK1/2 assay after UVB and CoQ10. **a** The representative photomicrograms of immunofluorescence of p-ERK 1/2 and GFAP. Bar = 50 μm . **b** Representative Western blot showing p-ERK 1/2. **c** Bar graph sums up the ratio of p-ERK 1/2/ERK 1/2. # $p < 0.01$ versus non-UVB and * $p < 0.05$ versus UVB. One-way ANOVA followed by Tukey's test



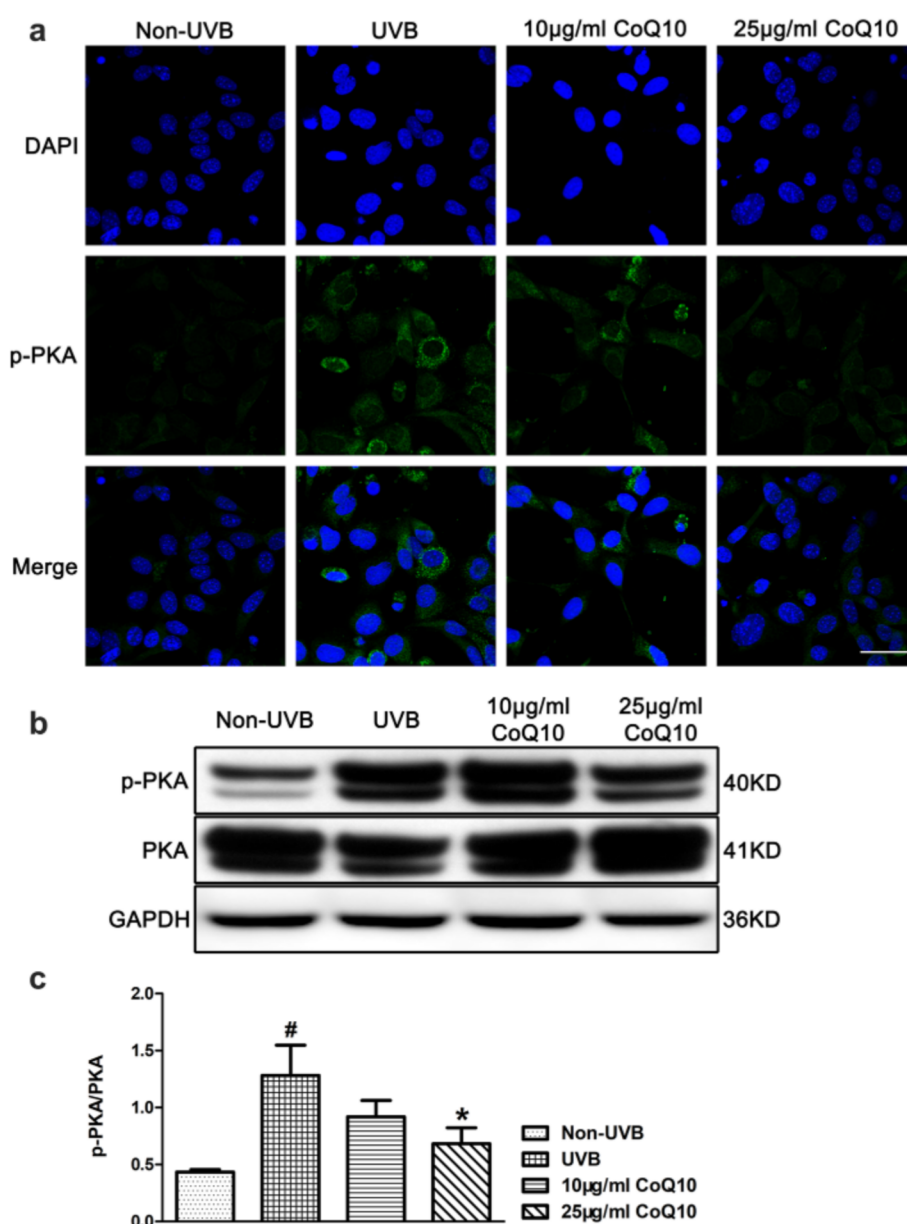
After UVB exposure, p-ERK 1/2 protein expression markedly increased ($p < 0.01$) and 25 $\mu\text{g/ml}$ of CoQ10 treatment significantly reduced the p-ERK 1/2 back to control levels ($p < 0.05$). There was no obvious change in the total amount of ERK 1/2 (Fig. 4b, c).

Effect of CoQ10 on p-PKA After UVB

Because ERK 1/2 can be activated by PKA [3, 10], we decided to study whether CoQ10 blocks the activation of PKA in cells exposed to UVB. PKA and p-PKA were detected in UVB exposed cells treated with or without CoQ10 using immunocytochemistry and Western blotting. As shown in Fig. 5a, a few scattered p-PKA positive

cells were observed in control cells without UVB irradiation (Non-UVB column). Number of p-PKA positive cells which were localized in cytoplasm increased after UVB irradiation (UVB column) and decreased after treating with 10 $\mu\text{g/ml}$ or 25 $\mu\text{g/ml}$ CoQ10 (10- and 25-CoQ10 columns). High dose of CoQ10 (25 $\mu\text{g/ml}$) intend to have better effect than the low dose (10 $\mu\text{g/ml}$) (Fig. 5a). Western blotting using p-PKA antibody that detects 40 KD p-PKA further supports the findings observed in p-PKA immunocytochemistry (Fig. 5b). The protein level of p-PKA was increased markedly after UVB exposure. Pretreatment with 25 $\mu\text{g/ml}$ of CoQ10 suppressed the expression of p-PKA in the cytosolic fraction, but the CoQ10 10 $\mu\text{g/ml}$ group had not statistical significance compared to UVB group (Fig. 5b, c).

Fig. 5 Phospho-PKA assay after UVB and CoQ10. **a** The representative photomicrograms of immunofluorescence of p-PKA, Bar = 25 μm . **b** Representative Western blot showing p-PKA. **c** Bar graph sums up the ratio of p-PKA/PKA. # $p < 0.01$ versus non-UVB and * $p < 0.05$ versus UVB. One-way ANOVA followed by Tukey's test



Discussion

Our results demonstrated that UVB irradiation resulted in astrocyte cell death and CoQ10 with 25 µg/ml significantly increased the cell viability as reflected by trypan blue cell count and MTT assay. These results are consistent with our previous report [16, 26]. It is well known that UVB irradiation causes increased production of ROS [27]. The ROS are mainly produced by the mitochondria, microsomes, and enzyme catalyzed reactions [28]. In normal conditions, the excessive ROS is degraded by intrinsic antioxidants such as superoxide dismutases and glutathione peroxidases [29]. Under pathological conditions, environmental stressors include ischemic glucose/oxygen deprivation, inflammatory, release of excitatory neurotransmitters, and metabolic depression could lead to the accumulation of ROS and induce oxidative stress [30, 31], causing damage to the mitochondria, lipid membranes, proteins and DNA. Mitochondria are considered to be the main source of ROS in activated astrocytes [32]. In the present study, we have found that UVB irradiation significantly increased ROS production as detected by DHE probe, which is consistent to previous reports [33]. CoQ10 treatment successfully blocked the UVB-induced ROS production, almost down to the level of ROS in non-UVB irradiated controls. The effects of CoQ10 on ROS generation have been reported to be involved in pathogenesis of diabetes, myocardial damage, and Glaucoma [34–36]. It has also been reported that the combined application of CoQ10 and vitamin E could protect against UVB radiation-induced skin injury [37].

ERK1/2 are members of the mitogen-activated protein kinases (MAPKs) family. ERK1/2 are normally located in the cytoplasm. When being activated, becoming p-ERK1/2, they translocate to the nucleus, where causing phosphorylation of various transcription factors and nuclear proteins to promote cell proliferation and differentiation [38]. Oxidative stress could activate the MAPK signaling pathways [39]. However, it is still controversial whether such activation is protective or damaging to the cells. In neuronal cells, oxidative stress activates ERK1/2 and inhibition of the ERK1/2 pathway blocked apoptosis [40]. In astrocytes and malignant glioma, oxidative stress activates ERK 1/2 as well [41, 42]. Oxidative stress induced ERK 1/2 activation is associated with astrocyte swelling and cell death [40, 43]. In the present study, it was demonstrated that UVB irradiation induced a significant increase of p-ERK1/2. Treatment with CoQ10 (25 µg/ml) dramatically decreased the protein of p-ERK1/2. These data suggest that activation of ERK1/2 maybe associated with cell death induced by oxidative stress in astrocytes and the protective effect of CoQ10 against UVB-induced

oxidative damage is related to its capacity to suppress ERK 1/2 activation. This notion is supported by the finding that ERK 1/2 promotes caspase-3 activation [44] and ROS-enhanced ERK activation leads to cell death [45].

Protein kinase A (PKA) is a tetramer consisting of two regulatory and two catalytic subunits. cAMP could bind to the regulatory subunits of PKA and induce the phosphorylation of cellular substrates. The cAMP signaling regulates various cellular responses by activating three major cAMP effector molecules: PKA, Epac, and cyclic-nucleotide-gated ion channels [46]. It is known that PKA and MAPK pathways interact with each other [47]. It was reported that the phosphorylation of both PKA and ERK 1/2 was increased in LGALS12 (Galectin-12) knockdown adipocytes [48]. They speculate that phosphorylated ERK 1/2 results from PKA activation [48]. It was found that activation of p-ERK 1/2 was enhanced by pre-treatment with Forskolin, a PKA activator [49]. In this study, p-PKA protein was significantly increased in UVB irradiated cells compared to that of the control cells and treatment with 25 µg/ml of CoQ10 significantly suppressed p-PKA. The result suggests that CoQ10 inhibits the PKA-ERK 1/2 signaling pathway.

The limitation of the present study is that it just provided an association relationship between the UVB damage and extracellular CoQ10 treatment in the astrocytes. In order to clarify the CoQ10 antioxidant role, future studies could use the quantitative analysis of CoQ10 contents before/after supplementation in blood, cells or mitochondria, and more elaborate antioxidation experiments.

In summary, these data have confirmed that UVB irradiation induces astrocytes death, and this detrimental effect is associated with enhanced ROS accumulation and activation of PKA-ERK 1/2 signaling pathway. Suppression of the PKA-ERK 1/2 signaling pathway may be one of important mechanisms by which CoQ10 protects astrocytes from UVB-induced oxidative damage.

Acknowledgements This study was supported by the National Natural Science Foundation of China (Nos. 31780280, 81560208) and West China first-class Disciplines Basic Medical Sciences at Ningxia Medical University (No. NXYLXK2017B07).

Compliance with Ethical Standards

Conflict of interest The authors have declared that no competing interest exists.

References

1. Molofsky AV, Krencik R, Ullian EM, Tsai HH, Deneen B, Richardson WD, Barres BA, Rowitch DH (2012) Astrocytes and disease: a neurodevelopmental perspective. *Genes Dev* 26:891–907

2. Poskanzer KE, Molofsky AV (2018) Dynamism of an astrocyte in vivo: perspectives on identity and function. *Annu Rev Physiol* 80:143–157
3. Thei L, Rocha-Ferreira E, Peebles D, Raivich G, Hristova M (2018) Extracellular signal-regulated kinase 2 has duality in function between neuronal and astrocyte expression following neonatal hypoxic-ischaemic cerebral injury. *J Physiol* 596:6043–6062
4. Xi Y, Wang M, Zhang W, Bai M, Du Y, Zhang Z, Li Z, Miao J (2014) Neuronal damage, central cholinergic dysfunction and oxidative damage correlate with cognitive deficits in rats with chronic cerebral hypoperfusion. *Neurobiol Learn Mem* 109:7–19
5. Bartczek P, Li L, Ernst AS, Bohler LI, Marti HH, Kunze R (2017) Neuronal HIF-1 α and HIF-2 α deficiency improves neuronal survival and sensorimotor function in the early acute phase after ischemic stroke. *J Cereb Blood Flow Metab* 37:291–306
6. Chen X, Zhang X, Liao W, Wan Q (2017) Effect of physical and social components of enriched environment on astrocytes proliferation in rats after cerebral ischemia/reperfusion injury. *Neurochem Res* 42:1308–1316
7. Bylicky MA, Mueller GP, Day RM (2018) Mechanisms of endogenous neuroprotective effects of astrocytes in brain injury. *Oxid Med Cell Longev* 2018:6501031
8. Belanger M, Allaman I, Magistretti PJ (2011) Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metab* 14:724–738
9. Lapp DW, Zhang SS, Barnstable CJ (2014) Stat3 mediates LIF-induced protection of astrocytes against toxic ROS by upregulating the UPC2 mRNA pool. *Glia* 62:159–170
10. Zundorf G, Reiser G (2011) The phosphorylation status of extracellular-regulated kinase 1/2 in astrocytes and neurons from rat hippocampus determines the thrombin-induced calcium release and ROS generation. *J Neurochem* 119:1194–1204
11. Ito JJ, Nagayasu Y, Ogawa T, Okihara H, Michikawa M (2015) Biochemical properties in membrane of rat astrocytes under oxidative stress. *Brain Res* 1615:1–11
12. Ibarretxe G, Sanchez-Gomez MV, Campos-Esparza MR, Alberdi E, Matute C (2006) Differential oxidative stress in oligodendrocytes and neurons after excitotoxic insults and protection by natural polyphenols. *Glia* 53:201–211
13. Zhang M, Gong JX, Wang JL, Jiang MY, Li L, Hu YY, Qi J, Zhang LY, Zhao H, Cui X, Xian XH, Li WB (2017) p38 MAPK participates in the mediation of GLT-1 up-regulation during the induction of brain ischemic tolerance by cerebral ischemic preconditioning. *Mol Neurobiol* 54:58–71
14. Armstead WM, Riley J, Vavilala MS (2016) Norepinephrine protects cerebral autoregulation and reduces hippocampal necrosis after traumatic brain injury via blockade of ERK MAPK and IL-6 in juvenile pigs. *J Neurotrauma* 33:1761–1767
15. Zhu QL, Luo Y, Xue QS, Zhang FJ, Yu BW (2018) Different doses of sevoflurane facilitate and impair learning and memory function through activation of the ERK pathway and synthesis of ARC protein in the rat hippocampus. *Brain Res* 1678:174–179
16. Jing L, Yan R, Cao XM, He QP, Zhang JZ, Li PA (2014) PERK signaling pathway involved in lactic acid induced astrocyte damage. *Int J Sci* 3:29–34
17. Zhang JZ, Jing L, Ma Y, Guo FY, Chang Y, Li PA (2010) Monosialotetrahexosyl-1 ganglioside attenuates diabetes-enhanced brain damage after transient forebrain ischemia and suppresses phosphorylation of ERK1/2 in the rat brain. *Brain Res* 1344:200–208
18. Baillie G, MacKenzie SJ, Houslay MD (2001) Phorbol 12-myristate 13-acetate triggers the protein kinase A-mediated phosphorylation and activation of the PDE4D5 cAMP phosphodiesterase in human aortic smooth muscle cells through a route involving extracellular signal regulated kinase (ERK). *Mol Pharmacol* 60:1100–1111
19. Muthukumaran K, Kanwar A, Vegh C, Marginean A, Elliott A, Guilbeault N, Badour A, Sikorska M, Cohen J, Pandey S (2018) Ubisol-Q10 (a nanomicellar water-soluble formulation of CoQ10) treatment inhibits Alzheimer-type behavioral and pathological symptoms in a double transgenic mouse (TgAPESwe, PSEN1dE9) model of Alzheimer's disease. *J Alzheimers Dis* 61:221–236
20. Sikorska M, Lanthier P, Miller H, Beyers M, Sodja C, Zurakowski B, Gangaraju S, Pandey S, Sandhu JK (2014) Nanomicellar formulation of coenzyme Q10 (Ubisol-Q10) effectively blocks ongoing neurodegeneration in the mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model: potential use as an adjuvant treatment in Parkinson's disease. *Neurobiol Aging* 35:2329–2346
21. Huo J, Xu Z, Hosoe K, Kubo H, Miyahara H, Dai J, Mori M, Sawashita J, Higuchi K (2018) Coenzyme Q10 prevents senescence and dysfunction caused by oxidative stress in vascular endothelial cells. *Oxid Med Cell Longev* 2018:3181759
22. Noh YH, Kim KY, Shim MS, Choi SH, Choi S, Ellisman MH, Weinreb RN, Perkins GA, Ju WK (2013) Inhibition of oxidative stress by coenzyme Q10 increases mitochondrial mass and improves bioenergetic function in optic nerve head astrocytes. *Cell Death Dis* 4:e820
23. Jing L, Kumari S, Mendelev N, Li PA (2011) Coenzyme q10 ameliorates ultraviolet B irradiation induced cell death through inhibition of mitochondrial intrinsic cell death pathway. *Int J Mol Sci* 12:8302–8315
24. Choi JS, Park SY, Yi EY, Kim YJ, Jeong JW (2011) Coenzyme Q10 decreases basic fibroblast growth factor (bFGF)-induced angiogenesis by blocking ERK activation. *Oncol Res* 19:455–461
25. Li X, Guo Y, Huang S, He M, Liu Q, Chen W, Liu M, Xu D, He P (2017) Coenzyme Q10 prevents the interleukin-1 β induced inflammatory response via inhibition of MAPK signaling pathways in rat articular chondrocytes. *Drug Dev Res* 78:403–410
26. Jing L, He MT, Chang Y, Mehta SL, He QP, Zhang JZ, Li PA (2015) Coenzyme Q10 protects astrocytes from ROS-induced damage through inhibition of mitochondria-mediated cell death pathway. *Int J Biol Sci* 11:59–66
27. Oliveira MM, Ratti BA (2019) Dihydrocaffeic acid prevents UVB-induced oxidative stress leading to the inhibition of apoptosis and MMP-1 expression via p38 signaling pathway. *Oxid Med Cell Longev* 2019:2419096
28. Wong HS, Dighe PA, Mezera V, Monternier PA, Brand MD (2017) Production of superoxide and hydrogen peroxide from specific mitochondrial sites under different bioenergetic conditions. *J Biol Chem* 292:16804–16809
29. Trewin AJ, Bahr LL, Almast A, Berry BJ, Wei AY, Foster TH, Wojtovich AP (2019) Mitochondrial ROS generated at the complex-II matrix or intermembrane space microdomain have distinct effects on redox signaling and stress sensitivity in *C. elegans*. *Antioxid Redox Signal*, doi: <https://doi.org/10.1089/ars.2018.7681>
30. Chuang JY, Kao TJ, Lin SH, Wu AC, Lee PT, Su TP, Yeh SH, Lee YC, Wu CC, Chang WC (2017) Specificity protein 1-zinc finger protein 179 pathway is involved in the attenuation of oxidative stress following brain injury. *Redox Biol* 11:135–143
31. Mailloux RJ, Harper ME (2011) Uncoupling proteins and the control of mitochondrial reactive oxygen species production. *Free Radic Biol Med* 51:1106–1115
32. Zhu Z, Li R, Stricker R, Reiser G (2015) Extracellular alpha-crystallin protects astrocytes from cell death through activation of MAPK, PI3K/Akt signaling pathway and blockade of ROS release from mitochondria. *Brain Res* 1620:17–28
33. Ben Jilani KE, Panee J, He Q, Berry MJ, Li PA (2007) Overexpression of selenoprotein H reduces Ht22 neuronal cell death after UVB irradiation by preventing superoxide formation. *Int J Biol Sci* 3:198–204
34. Montano SJ, Grunler J, Nair D, Tekle M, Fernandes AP, Hua X, Holmgren A, Brismar K, Ungerstedt JS (2015) Glutaredoxin

- mediated redox effects of coenzyme Q10 treatment in type 1 and type 2 diabetes patients. *BBA Clin* 4:14–20
35. Emami A, Tofighi A, Asri-Rezaei S, Bazargani-Gilani B (2018) The effect of short-term coenzyme Q10 supplementation and pre-cooling strategy on cardiac damage markers in elite swimmers. *Br J Nutr* 119:381–390
 36. Erb C, Konieczka K (2018) Mitochondrial dysfunctions and role of coenzyme Q10 in patients with glaucoma. *Klin Monbl Augenheilkd* 235:157–162
 37. Pegoraro NS, Barbieri AV, Camponogara C, Mattiazzi J, Brum ES, Marchiori MCL, Oliveira SM, Cruz L (2017) Nanoencapsulation of coenzyme Q10 and vitamin E acetate protects against UVB radiation-induced skin injury in mice. *Colloids Surf B Biointerfaces* 150:32–40
 38. Roskoski R Jr (2012) ERK1/2 MAP kinases: structure, function, and regulation. *Pharmacol Res* 66:105–143
 39. Zhang GY, Lu D, Duan SF, Gao YR, Liu SY, Hong Y, Dong PZ, Chen YG, Li T, Wang DY, Cheng XS, He F, Wei JS, Li GY, Zhang QY, Wu DD (2018) Hydrogen sulfide alleviates lipopolysaccharide-induced diaphragm dysfunction in rats by reducing apoptosis and inflammation through ROS/MAPK and TLR4/NF-kappaB signaling pathways. *Oxid Med Cell Longev* 2018:9647809
 40. Zhuang S, Schnellmann RG (2006) A death-promoting role for extracellular signal-regulated kinase. *J Pharmacol Exp Ther* 319:991–997
 41. Cheng P, Alberts I, Li X (2013) The role of ERK1/2 in the regulation of proliferation and differentiation of astrocytes in developing brain. *Int J Dev Neurosci* 31:783–789
 42. Huang L, Li B, Tang S, Guo H, Li W, Huang X, Yan W, Zou F (2015) Mitochondrial K_{ATP} channels control glioma radioresistance by regulating ROS-induced ERK activation. *Mol Neurobiol* 52:626–637
 43. Dai H, Song D, Xu J, Li B, Hertz L, Peng L (2013) Ammonia-induced Na, K-ATPase/ouabain-mediated EGF receptor transactivation, MAPK/ERK and PI3K/AKT signaling and ROS formation cause astrocyte swelling. *Neurochem Int* 63:610–625
 44. Zhuang S, Yan Y, Daubert RA, Han J, Schnellmann RG (2007) ERK promotes hydrogen peroxide-induced apoptosis through caspase-3 activation and inhibition of Akt in renal epithelial cells. *Am J Physiol Renal Physiol* 292:F440–447
 45. Kano G, Almanan M, Bochner BS, Zimmermann N (2013) Mechanism of siglec-8-mediated cell death in IL-5-activated eosinophils: role for reactive oxygen species-enhanced MEK/ERK activation. *J Allergy Clin Immunol* 132:437–445
 46. Lefkimiatis K, Zaccolo M (2014) cAMP signaling in subcellular compartments. *Pharmacol Ther* 143:295–304
 47. Waltereit R, Weller M (2003) Signaling from cAMP/PKA to MAPK and synaptic plasticity. *Mol Neurobiol* 27:99–106
 48. Wu W, Yin Y, Xu K, Peng Y, Zhang J (2018) Knockdown of LGALS12 inhibits porcine adipocyte adipogenesis via PKA-Erk1/2 signaling pathway. *Acta Biochim Biophys Sin (Shanghai)* 50:960–967
 49. Li L, Ge C, Wang D, Yu L, Zhao J, Ma H (2018) Dehydroepiandrosterone reduces accumulation of lipid droplets in primary chicken hepatocytes by biotransformation mediated via the cAMP/PKA-ERK1/2 signaling pathway. *Biochim Biophys Acta Mol Cell Biol Lipids* 1863:625–638

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.