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Expression of HIF1α, BNIP3, and beclin-1 in the brain of newborn and adult yaks (*Bos grunniens*)

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Abstract

Introduction. As a main consumer of energy, the brain is particularly susceptible to the effects of hypoxia. However, during long-term evolution, the brain of the plateau yak developed adaptive mechanisms enabling it to maintain normal physiological conditions.

Material and methods. A total of 20 male yaks belonging to two age groups [newborns (1–6 days old; n = 10) and adults (3–5 years old; n = 10)] were obtained, and the brain tissue was fixed and processed by standard methods. RT-qPCR, ELISA and IHC assays were used to investigate the expression and localization of HIF1α, BNIP3 and beclin-1 in the hippocampus, cerebral cortex, thalamus, medulla oblongata and cerebellum of newborn and adult yak brains and to explore their potential neuroprotective role.

Results. We found that the expression levels of HIF1α, BNIP3 and beclin-1 at the mRNA and protein levels varied in the different regions of yak brain, with the highest expression observed in the hippocampus, followed by the cerebral cortex, thalamus, medulla oblongata and the cerebellum. Moreover, the HIF1α, BNIP3 and beclin-1 expression were significantly higher in the newborn yaks' brains than in the adult yak. The IHC results showed that HIF1α, BNIP3 and beclin-1 were mainly distributed in the neurons of the cerebral cortex, hippocampus, thalamus, medulla oblongata and cerebellum. In particular, HIF1α accumulated in the nucleus and cytoplasm. Furthermore, the immunoreactivity of BNIP3 and beclin-1 was concentrated in the cytoplasm.

Conclusions. The results indicate that the yak hippocampus and cerebral cortex may be more resistant to hypoxia than thalamus, medulla oblongata and cerebellum, and the expression of BNIP3 and beclin-1 may be regulated by HIF1α to serve a neuroprotective role in the yak's brain to adaptation to hypoxia. Additionally, the brain of adult yaks may have a higher tolerance to hypoxia than the brain of newborn yaks. (*Folia Histochemica et Cytobiologica 2023, Vol. 61, No. 1, 26–33*)

Keywords: yak; brain; HIF1α; BNIP3; beclin-1; qPCR; IHC

Introduction

China is among the countries with the largest plateaus globally. With the steady progress of the "One Belt, One Road" national strategy, the rapid development of the economy and tourism industry in the plateaus has resulted in an increasing number of people going to plateaus for tourism and work. The characteristics of plateaus include low air pressure, hypoxic conditions, cold temperatures and strong radiation [1]. The maintenance of normal brain function depends on a continuous supply of oxygen. Hypoxia can result in comas, seizures, cognitive impairment, neurological disabilities, and in some cases brain death, particularly when acting chronically [2, 3]. Therefore, it is crucial for the brain to be able to detect and respond rapidly to hypoxia. Studies have found that brain damage caused by hypoxia is closely related to neuronal death, and is one of the main factors that cause acute high-altitude brain damage.

Autophagy can clear damaged mitochondria and other cell constituents, reduce the expression of

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pro-apoptotic factors and reactive oxygen species to combat hypoxic stress, and promote cell survival [4, 5]; therefore, it has attracted increasing attention. Autophagy is also activated in neurons exposed to hypoxia [4]. Studies have shown that under hypoxic conditions, autophagy can affect the final outcome of neurons and promote neuronal survival, thereby acting as a protective mechanism [6]. Thus, how is autophagy activated under hypoxic conditions? Accumulated evidence shows that the activation of autophagy is regulated by the HIF1α (Hypoxia-inducible factor 1α and BNIP3 (Bcl-2/adenovirus E1B 19-kDa interacting protein 3) under hypoxia [7, 8]. Additionally, Bellot *et al.* have shown that the HIF1α/BNIP3/beclin-1 axis is an important pathway that induces autophagy under hypoxic conditions [8]. HIF1α, as a major agent, is rapidly activated in the cell survival response when the O_2 tension decreases. Moreover, HIF1α transcription directly regulates BNIP3 which is known to activate autophagy in response to hypoxic stress [8]. Beclin-1 is a key regulator of autophagy and plays an essential role in autophagy activation [9].

The yak (*Bos grunniens*) is among the few species that can adapt to high altitudes and alpine climates. It inhabits plateau areas, such as Tibet, Sichuan, Qinghai, and Gansu, with altitudes in the range of 3,000–6,000 meters. After long-term natural selection, the harsh environment of the high altitude and the hypoxic conditions, have resulted in a unique morphological structure and physiological mechanism in yaks, especially in terms of their respiratory and cardiovascular systems [10, 11]. Hypoxia can cause central nervous system (CNS) damage and ischemic diseases [2]. Interestingly, yaks do not show related symptoms. A recent study confirmed that yak brain tissues have certain hypoxic adaptability [12, 13]. Therefore, in this study, we specifically focused our interest on understanding the expression and localization of HIF1α, BNIP3, and beclin-1 and speculated that they play key roles in the hypoxic adaptation mechanism of yak brain. In the present study RT-qPCR, ELISA, and immunohistochemical analysis were carried out to determine the mRNA and protein expression of HIF1α, BNIP3, and beclin-1 in various brain regions of newborn and adult yaks. The successful implementation of this project will provide an important scientific basis for further exploring the hypoxic adaptive mechanism of the plateau yak brain at the molecular and genetic levels.

Material and methods

Animals. The yaks were obtained from Xining, Qinghai province, China Agricultural Department. A total of 20 male yaks belonging to two age groups [newborns (1–6 days old; n = 10) and adults (3–5 years old; n = 10)] were included in this study. All animals were kept under the same natural conditions at the farm located at the altitude of 3500–4000 m. The yaks were considered clinically healthy based on the results of a physical examination and serum biochemical analysis. All experimental animals were handled according to the Animal Ethics Procedures and Guidelines of the People's Republic of China, and the study was approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine of Gansu Agricultural University (No. GAU-LC-2020-32). Each yak was euthanized with sodium pentobarbital (200 mg/kg, intravenously). The cerebral cortex, thalamus, medulla oblongata, and cerebellum were collected immediately after euthanasia. For immunohistochemistry of paraffin sections, all specimens were fixed in 4% neutral paraformaldehyde phosphate buffer (pH 7.3), and snap-frozen samples taken from all groups were stored in liquid nitrogen until further processing.

Relative RT-qPCR. Total brain tissue RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA was reverse transcribed to single-stranded cDNA using a Reverse Transcription kit (MBI Fermentas, Burlington, ON, Canada) according to the manufacturer's instructions. The RT-qPCR primers were designed according to the *Bos taurus* HIF1α, BNIP3, beclin-1, and *β-actin* gene sequences (GenBank accession numbers: NC037337.1, NM001076366.1, NM001033627.2, and DQ838049, respectively) using Primer 5 software and synthesized by the Beijing Genomics Institute BGI company (Beijing, China). The RT-qPCR primer sequences are shown in Table 1. RT-qPCR was conducted with a Light-Cycler480 thermocycler (Roche, Manheim, Germany) and a 20-μL reaction volume consisting of 1 μL cDNA, 1 μL forward primer, 1 μL reverse primer, 10 μL 2×SYBR Green II PCR mix (TaKaRa, Shiga, Japan), 0.4 μL Rox, and 6.4 μL nuclease-free H_2O. Four replicates were set for each sample to ensure the accuracy of the relative expression of the target gene in the sample. After amplification, according to the system-generated Ct value, the $2^{-\Delta\Delta Ct}$ method was used with *β-actin* as an internal standard to obtain the relative expression of HIF1α, BNIP3, and beclin-1 mRNAs.

Detection of HIF1α, BNIP3, and beclin-1 by enzyme-linked immunosorbent assay (ELISA). The expression of HIF1α, BNIP3, and beclin-1 in yak brain tissues was directly measured using ELISA kits according to the manufacturer's instructions (Bovine HIF1α ELISA kit, JYM0277Bo; Bovine BNIP3 ELISA kit, JYM0237Bo; Bovine beclin-1 ELISA kit, JYM0272Bo; Wuhan ELISA Lab Biotech Co., LTD, Wuhan, China). Tissue samples were frozen in nitrogen and homogenized in ice-cold (4°C) phosphate-buffered saline (PBS, pH 7.4; 0.1 g/mL). We adjusted the aliquots of the supernatant for the ELISA assay (10 μL/well). A well was left empty as a blank control (without tissue sample and HRP conjugate reagent). The optical density (OD) was measured spectrophotometrically at 450 nm. The OD value was proportional to the concentrations of HIF1α, BNIP3, and beclin-1. The concentrations of HIF1α, BNIP3, and bec-

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Table 1. Primers used in this study

Genes	Primer names	Primer sequences (5'-3')	Length (bp)	Annealing (°C)
HIF 1α	P1 P2	F: GGCGCGAACGACAAGAAAAA R: GTGGCAACTGATGAGCAAGC	121	60
BNIP3	P3 P4	F: AGACCCCACAGGACACTAAC R: GACTGGACCAATCCCAAAT	121	60
Beclin-1	P5 P6	F: GAAACCAGGAGAGACCCAGG R: GTGGACATCATCCTGGCTGG	114	57
β-actin	P7 P8	F: AGGCTGTGCTGTCCCTGTATG R: GCTCGGCTGTGGTGGTAAA	207	60

lin-1 in the samples were determined by comparing the OD of the samples to the HIF1α, BNIP3, and beclin-1 standard curves.

Immunohistochemical (IHC) examination. The spatial distribution of HIF1α, BNIP3, and beclin-1 positive cells in yak brains was evaluated by immunohistochemical staining. Paraffin blocks were made and the sections of 4 μm thick were obtained. Fixed tissue specimens were mounted on microscope slides in a routine manner, and exposed to primary antibodies against HIF1α (rabbit anti-HIF1α, bs-0737R; Bioss, Beijing, China, 1:100 dilution), BNIP3 (rabbit anti-BNIP3 antibody, bs-4239R; Bioss, 1:100 dilution), and beclin-1 (rabbit anti-beclin-1, bs-1353R, Bioss, 1:100 dilution) and incubated for 2 h at 37°C in a moist chamber. A biotinylated anti-rabbit secondary antibody (SP-0023, Bioss) was applied for 10 min. Then, streptavidin-conjugated peroxidase was applied to the slide for 10 min. Reaction products were formed with 3,3-diaminobenzidine tetrahydrochloride (c-0010, Bioss). The sections were lightly counterstained with hematoxylin. The negative control had the primary antibody replaced with rabbit serum albumin with all other steps and conditions remaining the same.

The intensity in the immunohistochemical assays was measured using integrated optical density (IOD) and Image–Pro plus 6.0 (Media Cybernetics, Rockville, MD). The IOD of immunohistochemical staining for HIF1α, BNIP3, and beclin-1 were counted in the randomly selected five fields of vision (original magnification 400×) in the section. Five sections were randomly chosen for each brain tissue [14].

Statistical analysis. All statistical analyses were performed using IBM SPSS (version 21.0; SPSS Inc., Chicago, IL, USA). The relative mRNA and protein levels among the study groups were expressed as the mean ± standard error. The data were analyzed by one-way ANOVA. P-values less than 0.05 between groups were considered statistically significant.

Results

Immunohistochemical staining of HIF1α, BNIP3, and beclin-1 in yak brain tissues

The distributions of HIF1α (Fig. 1), BNIP3 (Fig. 2), and beclin-1 (Fig. 3) in the different regions of the yak brain were detected by immunohistochemistry. The distribution patterns of HIF1α-, BNIP3-, and beclin-1-positive cells were similar in the same brain regions of the newborn and adult yaks. In the cerebral cortex, immunoreactive (Ir) products were mostly concentrated in pyramidal neurons in the second and third layers. In the hippocampus, in almost all pyramidal cell layer staining was strong. Additionally, Ir-products were concentrated in the neurons of the thalamus and medulla oblongata. Moreover, the immunoreactivities of HIF 1α, BNIP3, and beclin-1 were distributed in the cerebellar Purkinje cells and were present in a small number of cells in the granular and molecular layers. In particular, HIF1α immunostaining accumulated in the neuronal nucleus and cytoplasm. Furthermore, BNIP3 and beclin-1 immunostainings were mainly concentrated in the neuronal cytoplasm. These proteins were not expressed in the negative control.

Expression of HIF1α mRNA and protein in yak brain tissues

The expression of HIF1α mRNA and protein was determined by RT-qPCR (Fig. 4a), ELISA analysis (Fig. 4b), and IOD analysis (Fig. 4c), respectively. Within the same age group, the HIF1α mRNA and protein expressions differed in different regions of the yak brain. The expression of HIF1α mRNA was higher in the yak hippocampus and cerebral cortex, followed by the thalamus, medulla oblongata, and cerebellum ($P < 0.05$). The HIF1α protein expression was the highest in the yak hippocampus, followed by the cerebral cortex, thalamus, medulla oblongata, and cerebellum ($P < 0.05$). Additionally, we found that the HIF1α mRNA and protein expressions were significantly lower in the brain of adult yaks as compared with newborn ones ($P < 0.05$).

BNIP3 mRNA and protein expression in yak brain

The BNIP3 mRNA and protein expression was determined by RT-qPCR (Fig. 5a), ELISA analysis (Fig. 5b), and IOD analysis (Fig. 5c). In the same

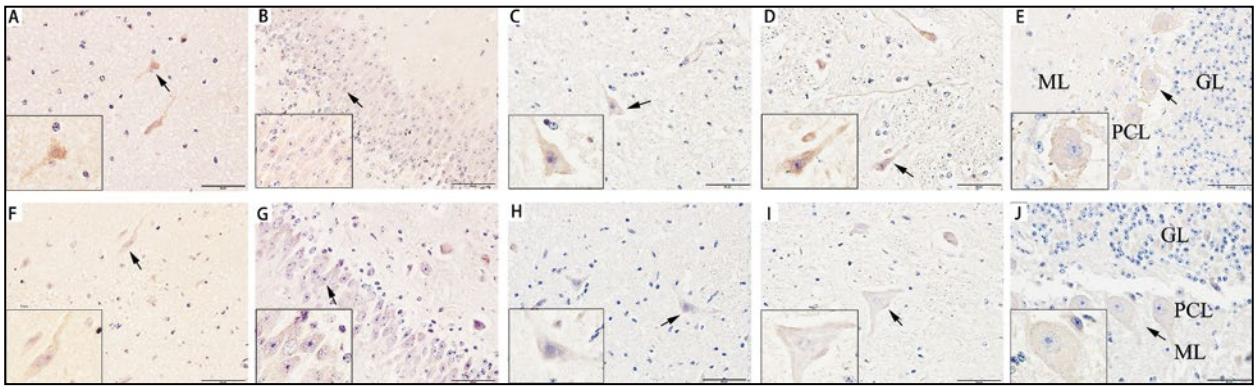

Figure 1. Immunohistochemical staining of HIF1α in the brain of newborn and adult male yaks. **A–E.** Localization of HIF1α in cerebral cortex (A), hippocampus (B), thalamus (C), medulla oblongata (D) and cerebellum (E) of the newborn yak brain. **F-J.** Localization of HIF1α in cerebral cortex (A), hippocampus (B), thalamus (C), medulla oblongata (D) and cerebellum (E) of the adult yak brain. The outlined area is shown at higher magnification (1000×) in the box. Dotted box: the immunoreactivity was greatest in the cytoplasm of neurons. Abbreviations: GL — granular layer; ML — molecular layer; PCL — Purkinje cell layer. Arrows show the immunoreactive cells (brown). Bar = 50 μm (low-power lens), bar = 20 μm (high-power lens on the lower left).

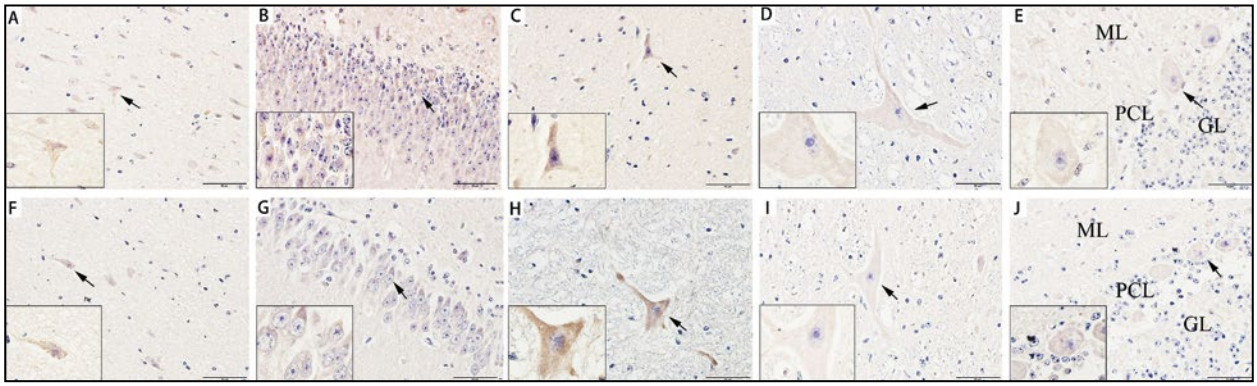

Figure 2. Immunohistochemical staining of BNIP3 in the brain of newborn and adult male yaks (400×). **A–E.** Localization of BNIP3 in cerebral cortex (A), hippocampus (B), thalamus (C), medulla oblongata (D) and cerebellum (E) of the newborn yak brain. **F–J.** Localization of BNIP3 in cerebral cortex (A), hippocampus (B), thalamus (C), medulla oblongata (D) and cerebellum (E) of the adult yak brain. The outlined area is shown at higher magnification (1000×) in the box. Dotted box: the immunoreactivity was greatest in the cytoplasm of neurons. Abbreviations: GL — granular layer; ML — molecular layer; PCL — Purkinje cell layer. Arrows show the immunoreactive cells (brown). Bar = 50 μm (low-power lens), bar = 20 μm (high-power lens on the lower left).

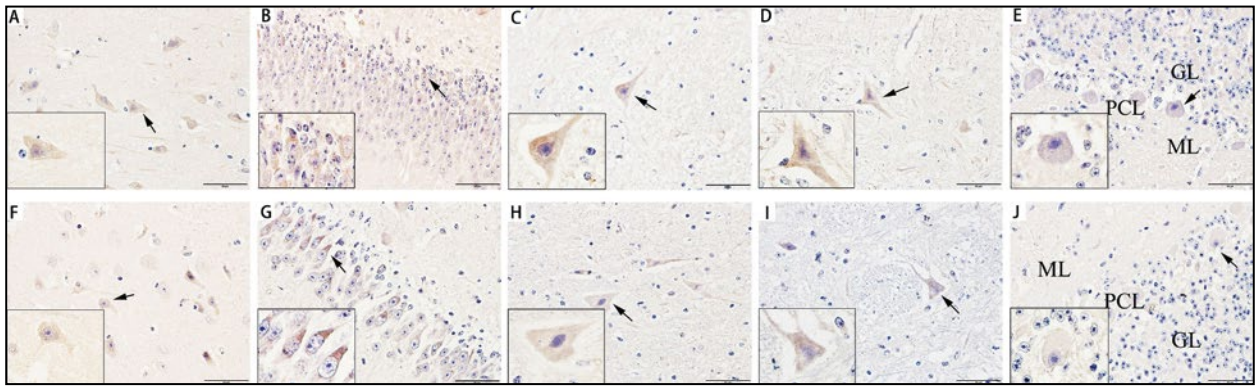

Figure 3. Immunohistochemical staining of beclin-1 in the brain of newborn and adult male yaks (400×). **A–E.** Localization of beclin-1 in cerebral cortex (A), hippocampus (B), thalamus (C), medulla oblongata (D) and cerebellum (E) of the newborn yak brain. **F–J.** Localization of beclin- 1 in cerebral cortex (A), hippocampus (B), thalamus (C), medulla oblongata (D) and cerebellum (E) of the adult yak brain.The outlined area is shown at higher magnification (1000×) in the box. Dotted box: the immunoreactivity was greatest in the cytoplasm of neurons. Arrows show the immunoreactive cells (brown). Bar = 50 μm (low-power lens), bar = 20 μm (high-power lens on the lower left). Abbreviations: GL — granular layer; ML — molecular layer; PCL — Purkinje cell layer.

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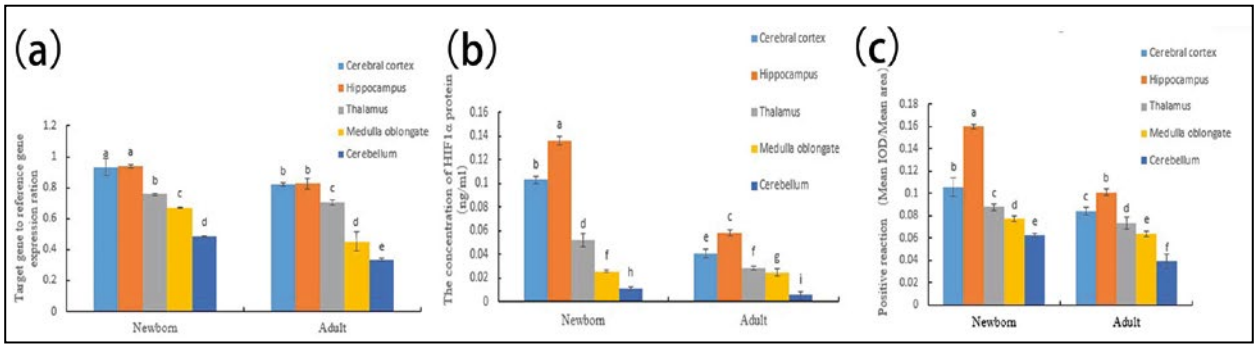

Figure 4. The expression of HIF1α mRNA and protein in various brain regions of newborn and adult yaks. **A.** RT-qPCR analysis of HIF1α mRNA expression. Gene expression levels represent the mRNA levels in relation to the control gene (β-actin) mRNA expression. Values indicate the mean ± SE of $2^{-\Delta\Delta Ct}$. **B.** ELISA analysis of HIF1α protein content in the brain homogenates of newborn and adult yaks brain. Values indicate the mean ± SE. **C.** The immunohistochemical optical density analysis of HIF1α protein expression. Values indicate the mean ± SE. Different letters represent statistically significant differences ($P < 0.05$).

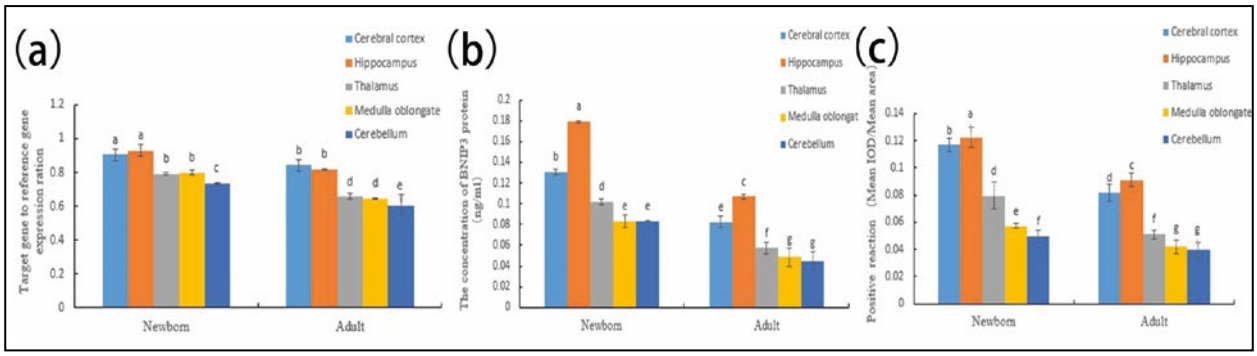

Figure 5. The expression of BNIP3 mRNA and protein in various brain regions of newborn and adult yaks. **A.** RT-qPCR analysis of BNIP3 mRNA expression. Gene expression levels represent the mRNA levels in relation to the control gene (β-actin) mRNA expression. Values indicate the mean ± SE of $2^{-\Delta\Delta Ct}$. **B.** ELISA analysis of BNIP3 protein content in the brain homogenates of newborn and adult yaks brain. Values indicate the mean ± SE. **C.** The immunohistochemical optical density analysis of BNIP3 protein expression. Values indicate the mean ± SE. Different letters represent statistically significant differences ($P < 0.05$).

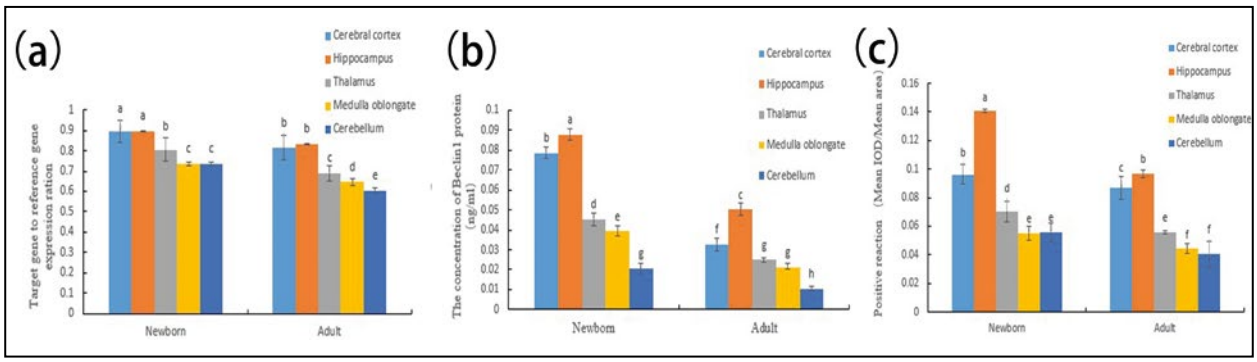

Figure 6. The expression of beclin-1 mRNA and protein in various brain regions of newborn and adult yaks. **A.** RT-qPCR analysis of beclin-1 mRNA expression. Gene expression levels represent the mRNA levels in relation to the control gene (β-actin) mRNA expression. Values indicate the mean ± SE of $2^{-\Delta\Delta Ct}$. **B.** ELISA analysis of beclin-1 protein content in the brain homogenates of newborn and adult yaks brain. Values indicate the mean ± SE. **C.** The immunohistochemical optical density analysis of beclin-1 protein expression. Values indicate the mean ± SE. Different letters represent statistically significant differences ($P < 0.05$).

age group, the expression of BNIP3 mRNA and protein differed in yaks brain regions. Moreover, the BNIP3 mRNA expression was significantly higher in the hippocampus and cerebral cortex than in the thalamus, medulla oblongata, and cerebellum ($P < 0.05$). The BNIP3 protein expression was the highest in the yak hippocampus, followed by the cerebral cortex, thalamus, medulla oblongata, and cerebellum ($P < 0.05$). In particular, the BNIP3 mRNA and protein expression were reduced in adult yaks' brains as compared to newborn ones ($P < 0.05$).

Beclin-1 mRNA and protein expression in yak brain tissues

Beclin-1 mRNA and protein expression was determined by RT-qPCR (Fig. 6a), ELISA analysis (Fig. 6b), and IOD analysis (Fig. 6c). In the same age group, beclin-1 mRNA expression was significantly higher in the hippocampus and cerebral cortex than in the thalamus, medulla oblongata and cerebellum ($P < 0.05$). The beclin-1 protein expression was significantly higher in the hippocampus than in the cerebral cortex, thalamus, medulla oblongata, and cerebellum ($P < 0.05$). In each brain region, the mRNA and protein expression level of beclin-1 decreased with age and was significantly higher in the newborn yaks than in the adult ones ($P < 0.05$).

Discussion

HIF1α is an important component of the brain's adaptive response to hypoxia and is associated with the modulation of developmental cellular processes and cellular oxygen homeostasis regulation [1]. Bergeron *et al.* showed that hypoxia (6% O_2 for 4.5 h) increased the HIF1α levels in the adult rat brain [15]. Furthermore, the present study showed that the HIF1α mRNA and protein were expressed in all studied brain regions of newborn and adult yaks brains. Our results were consistent with those of previous studies that showed HIF1a expression was abundant in the brain of yaks and plateaus pika and may play an important role in the adaptation to a hypoxia environment [13, 16]. Interestingly the expression levels of HIF1α mRNA and protein varied in different brain regions. Specifically, within the same age group HIF1α expression in the hippocampus and cerebral cortex was higher than that in the other brain regions. Moreover, Görlach *et al.* reported that in mice HIF1α was expressed continuously and accumulated in hypoxic cells, but degraded rapidly in normoxic cells [17]. We hypothesized that the hippocampus and cerebral cortex may be more sensitive to hypoxia in the yak brain. Immunohistochemical analysis showed that HIF1α protein was concentrated both in the nucleus and cytoplasm of the brain neurons in newborn and juvenile yaks. It was consistent with a previous study that HIF1α was mainly expressed in the neurons of rat brains under hypoxic conditions (8% O_2) [18, 19]. It was indicated neurons were more susceptible to hypoxia than glial cells. Moreover, Chertok *et al.* and Hashemi *et al.* pointed out that activation of HIF1α may regulate its target genes to promote a widespread adaptive response by increasing glucose transport, glycolysis, vascular growth, and cell survival [20, 21]. Notably, the nuclear location of HIF1α in the yak brain confirmed that HIF1α may serve as a transcription factor and may regulate the expression of several genes that facilitate acclimatization to low oxygen conditions.

BNIP3 is required to initiate the autophagy process in response to hypoxic stress [22]. Zhang *et al.* reported that HIF1α activation in the hypoxic mouse brain may directly activate or induce the expression of BNIP3 and that this regulation may be associated with decreased neuronal death rate [23]. In the present study, we found that within the same age group BNIP3 mRNA and protein expression levels were higher in the hippocampus and cerebral cortex than that in the thalamus, medulla oblongata, and cerebellum of yaks being consistent with the expression pattern of HIF1α mRNA and protein in yak's brain. Chinnadurai *et al.* suggested that autophagy induction by hypoxia is mediated by the increased expression of HIF1α and BNIP3 to promote cell survival [24]. Taken together, the results indicate that greater co-expression of HIF1α and BNIP3 in the yak hippocampus and cerebral cortex may activate autophagy to protect neurons from death in hypoxic conditions. Additionally, in contrast to HIF1α, BNIP3 immunoreactivity was observed mainly in the cytoplasm of neurons. Schmidt-Kastner *et al.* reported that HIF1α controlled the expression of BNIP3 in rat hippocampus to delay neuronal death [25]. The result indicated that HIF1α might modulate BNIP3 to promote neuronal cell survival in the yak's brain.

Beclin-1 expression has been used as a marker of autophagy in the brain under hypoxic conditions [26–28]. Moreover, Balduini *et al.* showed that 24 h after hypoxic brain injury beclin-1 was increased significantly both in the hippocampus and the cerebral cortex in rats [26]. Consistent with the above findings, our study showed that beclin-1 mRNA and protein expression were relatively greater in the hippocampus and cerebral cortex of newborn and adult yaks. Remarkably, the expression pattern of beclin-1 was similar to HIF1α and BNIP3 in the yak brain. Zhu *et al.* reported that hypoxia-induced autophagy was part of a general mechanism of cell survival that is controlled by HIF1α and BNIP3 [29]. It was also shown in the rat that HIF1α can contribute to hypoxia-induced autophagy by increasing the expression of BNIP3 whereas the activation of BNIP3 led to the accumulation of beclin-1 under hypoxic conditions (5% O_2) [30, 31]. Fekadu and Rami's observations on HT22-beclin-1-knockdown cells have confirmed that the enhancement of autophagy has a neuroprotective effect against brain damage by removing amyloid precursor protein metabolites, alpha-synuclein, mutated ataxin, and other disease-related proteins in the nervous system [32]. We hypothesized that the synergistic expression of HIF1α,

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BNIP3, and beclin-1 may promote the adaptation of yak's hippocampus and cerebral cortex to hypoxia. Moreover, the immunohistochemical results of the present study showed beclin-1 immunoreactivity was mainly concentrated in the cytoplasm of neurons, consistent with BNIP3. Erlich *et al.* reported that beclin-1 was expressed in neurons after exposure to hypoxia 4 and 24 h in mice, which was similar to our results [33]. Additionally, it was mentioned before that nuclear HIF1α might may regulate its target genes to promote a widespread adaptive response [20]. Guo reported that HIF1α may ameliorate brain damage during hypoxia through BNIP3-dependent augmentation of autophagic cell survival and reduction in cell apoptosis in rats [34]. Thus, we speculated the cytoplasmic expression of BNIP3 and beclin-1 may be regulated by HIF1α, and these factors may be associated with neuroprotection *via* hypoxia-induced autophagy in the yak brain.

Moreover, we found HIF1α, BNIP3, and beclin-1 were distributed in the yak's brain. The HIF1α signaling can contribute to hypoxia-induced autophagy and increase the expression of BNIP3 under hypoxic conditions [35]. In addition, BNIP3 is closely related to autophagy, and the significance of BNIP3–dependent autophagy not only prevents the accumulation of damaged organelles but also protects the cell from death [8]. Hence, it was suggested the expression of these three factors might promote cell survival to adapt to hypoxia in the yak brain. Additionally, the present study showed a downregulation of HIF1α, BNIP3, and beclin-1 mRNA and protein with age in the yak brain. Taken together, the results indicate that lower expression of these factors in the adult yak brain suggests a role for hypoxia adaptive response.

In summary, this study increases the knowledge of the mRNA and protein expression of HIF1α, BNIP3, and beclin-1 in the newborn and adult yak brains. The high expression of HIF1α, BNIP3, and beclin-1 in the hippocampus and cerebral cortex neurons in the yak brain might indicate that neurons in the two regions are more sensitive to hypoxia. And the low expression HIF1α, BNIP3 and beclin-1 in the adult yaks brain might suggest a role in brain's adaptation to hypoxia.

Conflict of interest

Authors declare that they have no competing interest.

Acknowledgements

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Authors contributions

All authors contributed to experimental design, data collection, data interpretation, and preparation of the manuscript. The authors disclose no conflicts of interest.

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