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Research Paper

The plateau zokors' learning and memory ability is related to the high expression levels of *foxP2* in the brain

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Abstract: Plateau zokor (*Myospalax baileyi*) is a subterranean mammal. Plateau zokor has high learning and memory ability, and can determine the location of blocking obstacles in their tunnels. Forkhead box p2 (FOXP2) is a transcription factor implicated in the neural control of orofacial coordination and sensory-motor integration, particularly with respect to learning, memory and vocalization. To explore the association of *foxP2* with the high learning and memory ability of plateau zokor, the cDNA of *foxP2* of plateau zokor was sequenced; by using plateau pika as control, the expression levels of *foxP2* mRNA and FOXP2 protein in brain of plateau zokor were determined by real-time PCR and Western blot, respectively; and the location of FOXP2 protein in the brain of plateau zokor was determined by immunohistochemistry. The result showed that the cDNA sequence of plateau zokor *foxP2* was similar to that of other mammals and the amino acid sequences showed a relatively high degree of conservation, with the exception of two particular amino acid substitutions [a Gln (Q)-to-His (H) change at position 231 and a Ser (S)-to-Ile (I) change at position 235]. Higher expression levels of *foxP2* mRNA (3-fold higher) and FOXP2 protein (>2-fold higher) were detected in plateau zokor brain relative to plateau pika brain. In plateau zokor brain, FOXP2 protein was highly expressed in the cerebral cortex, thalamus and the striatum (a basal ganglia brain region). The results suggest that the high learning and memory ability of plateau zokor is related to the high expression levels of *foxP2* in the brain.

Key words: plateau zokor (*Myospalax baileyi*); *foxP2*; learning and memory

高原鼢鼠的学习记忆能力与其脑组织中*foxP2*基因的高表达有关

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摘 要：高原鼢鼠(*Myospalax baileyi*)终生营地下洞道生活，具有较强的学习记忆能力和定位洞道中障碍物的能力。叉头框蛋白P2 (forkhead box p2, FOXP2)是一种与口面部协调、运动协调功能有关，并且与学习记忆、以及定位功能有密切联系的转录因子。为了研究和探讨*foxP2*基因与高原鼢鼠发达的学习记忆能力之间的关系，我们克隆了高原鼢鼠*foxP2*基因的编码区序列；以地面动物高原鼠兔为对照，应用real-time PCR和Western blot分别测定高原鼢鼠脑组织中*foxP2* mRNA和FOXP2蛋白的表达水平；应用免疫组织化学法检测FOXP2蛋白在高原鼢鼠脑组织不同区域中的表达。结果显示：(1)与其它哺乳类动物相比，高原鼢鼠*foxP2*编码区序列相对保守，但其编码的氨基酸序列显示存在两个特殊的氨基酸残基突变；(2)高原鼢鼠*foxP2* mRNA的表达量极显著高于高原鼠兔($P < 0.01$)；(3)高原鼢鼠FOXP2蛋白的表达量极显著高于高原鼠兔($P < 0.01$)；(4) FOXP2蛋白在高原鼢鼠脑组织不同区域均有表达，其中以大脑皮层、丘脑和纹状体区域表达量较高。本研究结果提示，高原鼢鼠发达的学习记忆能力与*foxp2*基因在脑组织中的高表达有着密切的联系。

关键词：高原鼢鼠；*foxP2*基因；学习记忆
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The plateau zokor (*Myospalax baileyi*) belongs to the order Rodentia, in the Cricetidae family, genus *Mospalax* [1]. It is a solitary fossorial rodent that excavates and inhabits a sealed system of branching underground tunnels with no external above-ground exits. These animals spend their entire lives in this tunnel system and never leave unless forced to do so. In contrast, the plateau pika (*Ochotona curzniae*), which belongs to the order Lagomorpha, in the Ochotonidae family, genus *Ochotona* [2–4], is a surface-dwelling mammal. Both species are endemic to the Qinghai-Tibetan Plateau [4].

Subterranean mammals dig complex underground tunnel systems comprising networks of shallow feeding tunnels and deeper passages connecting the nest, food and sanitation chambers [3]. In their natural habitat, subterranean mammals encounter different types of obstacles that block and disconnect sections of the tunnel system [3, 5, 6]. Subterranean mammals are functionally blind and possess poor auditory sensitivity, which is limited to low frequency sounds [7, 8]; therefore, a highly developed directional sense and the enhanced abilities of learning and memory would seem to be essential for navigation. These attributes are the most fundamental cognitive processes required for survival, being essential for finding food, locating potential mates, defending territory, escaping predators and bypassing obstacles.

For an animal to possess efficient spatial orientation abilities, it must also possess spatial memory. Previous field studies have revealed that subterranean mammals remember the location of established tunnels, enabling safe return to their nest. Studies have also shown that the blind mole-rats learned significantly faster than rats and voles. Furthermore, their ability to remember the maze was significantly better than that of the rats [9–11]. Our field observations showed that plateau zokors remember their tunnels. Following changes to the original shape of the tunnel and trap-setting, we found that most plateau zokor distinguished the changes and escaped capture by digging bypass tunnels. We also found that plateau zokor could determine the location of blocking obstacles. These observations suggest that the plateau zokor has high learning ability and long-term memory retention of complex routes. Forkhead box p2 (*foxP2*) is the first gene to be identified that is specifically involved in speech and language development in humans [12]. This gene is expressed in several structures including the cortical plate, basal ganglia, thalamus, inferior olives and cerebellum [13]. Studies on avian, rat and crocodilian forebrain show that *foxP2* is expressed predominantly in the striatum, which is a basal ganglia brain region [14]. The *foxP2* expressed in these structures belongs to distributed circuits involved in motor coordination, learning, memory and acquisition of motor skills [15–21].

Based on these observations, we hypothesized that *foxP2* is involved in the high capacity of plateau zokor for learning and long-term memory retention of complex routes. In order to test these hypotheses, we sequenced the cDNA of *foxP2* in plateau zokor and compared the *foxP2* mRNA and protein expression levels between plateau zokor and plateau pika by real-time PCR and Western blot, respectively. Furthermore, the location of FOXP2 expression within the brain of plateau zokor was determined by immunohistochemistry.

1 MATERIALS AND METHODS

1.1 *Animal procedures*

Plateau zokors and plateau pikas were live-trapped from Huangzhong County and Guoluo Dawu County, respectively, in Qinghai Province, China. All animals were anesthetized with sodium pentobarbital (5%) and then sacrificed by cervical dislocation immediately before dissection. Tissues were rapidly removed and frozen in liquid nitrogen for storage. All procedures involving the handling and care of animals were in accordance with the Practice for the Care and Use of Laboratory Animals (China) and were approved by the China Zoological Society (permission number: GB 14923-2010).

1.2 *Cloning and sequencing of plateau zokor foxP2*

Specific primers for amplification of plateau zokor *foxP2* expressed sequence tags (ESTs) were designed from the alignment of highly conserved coding sequence regions of the *foxP2* gene of *Homo sapiens* (NM_001172766.2), *Rattus norvegicus* (XM002729286.1), *Mus musculus* (NM_053242.4) and *Rousettus* (EU076407.1). The EST primers, annealing temperature and the amplified fragment length are listed in Table 1. Total cellular RNA was extracted from frozen brain tissue using the EZ Spin Column Total RNA Isolation Kit (Sangon, Shanghai, China). Total RNA (4 μg) was subjected to reverse transcription and PCR using the AMV one-step RT-PCR Kit (Sangon). The PCR products were sent to the Beijing Genomics Institute for sequencing.

1.3 *Quantification of foxp2 mRNA by real-time PCR*

Comparative quantification of *foxP2* mRNA levels of

Table 1. Primers for amplification and real-time PCR of *foxP2* and *GAPDH* in plateau zokor (*Myospalax baileyi*) and plateau pika (*Ochotona curzoniae*)

Gene	Species	Primer	Sequence	Annealing temperature	Fragment length
foxP2	Zokor	EST primer (1)	F: 5'- ATGTGGGAGCCATACGA -3'	53.8 °C	623 bp
			R: 5'- CACGGGTTCTTCCTTGA -3'		
		EST primer (2)	F: 5'- ACAGTTTTTGAAGCACC -3'	48.6 °C	447 bp
			R: 5'- GCATAAGTAAAGGGAGG -3'		
		EST primer (3)	F: 5'- TCAGCCTTCAGCGTCAG -3'	53.8 °C	562 bp
			R: 5'- TTGGGAGATGGTTTGG -3'		
		EST primer (4)	F: 5'- CAGAAGCGAAGGTCACAAAAGAT-3'	58.4 °C	414 bp
			R: 5'- CCGCTCTCATTCCARRTCYTCAG -3'		
		EST primer (5)	F: 5'- TCCTATGAAGTTGAAACCG-3'	51.8 °C	833 bp
			R: 5'- CAGTCACTTCTTTCCATAGC-3'		
foxP2	Zokor	RT-PCR primer	F: 5'-CCTCCCTTTACTTATGC-3'	50.2 °C	249 bp
			R: 5'-CTTTTGTGACCTTCGCT-3'		
	Pika	RT-PCR primer	F: 5'-CCTCCCTTTACTTATGC-3'	52 °C	249 bp
			R: 5'-CTTTTGTGACCTTCGCT-3'		
GAPDH		RT-PCR primer	F: 5'-AAGAAGGTGGTGAAGCAGGC-3'	60 °C	203 bp
			R: 5'-TCCACCACCCTGTTGCTGTA-3'		

different species was performed using real-time PCR. Specific primer sets for amplification of fragments of *foxP2* and *GAPDH* were designed (Table 1) and evaluated in PCR reactions using non-reverse-transcribed RNA as negative controls.

Standard curves were generated by amplification of 1 μL *foxP2* and *GAPDH* first-strand cDNA with the Premix Ex Taq Version Kit (TaKaRa, Dalian, China). The initial product concentration was set at 1, and standard curves were generated by using 8-fold serial dilutions ranging from 1 to 10^{-8}. Quantitative real-time analysis of *foxP2* and *GAPDH* expression was performed by using the Bio-Rad IQ™5 instrument and software (IQ5 Optical System Software; version 2.1). Real-time PCR was performed with SYBR®Premix Ex Taq™ II (TaKaRa) using 1 μL cDNA and gene specific forward and reverse primers (400 nmol/L). Fluorescence signals were read and collected at 72 °C for each cycle. The amplification program was then followed by a melting cycle of 95 °C for 1 min, annealing temperature for 1 min, and slow heating from annealing temperature to 95 °C. A template-free negative control was included in each experiment.

The *foxP2* mRNA levels were normalized with *GAPDH* mRNA to compensate for variations in input RNA amounts. Normalization was carried out by dividing the logarithmic value of *foxP2* by the logarithmic value of *GAPDH*.

1.4 *Plasmid construction and preparation of recombinant FOXP2 protein*

A 660 bp *Eco*RI/*Xho*I fragment representing the entire *foxP2* coding sequence was amplified by gene synthesis. The *Eco*RI/*Xho*I fragment was subsequently cloned into the pET30a-GST expression vector (Novagen). The recombinant expression shuttle (pET30a-GST-*foxP2*) was transformed into *E. coli* BL21 cells and cultured in LB media.

The recombinant FOXP2 protein was expressed in *E. coli* BL21 cells by induction with 0.5 mmol/L isopropyl-β-*D*-thiogalactopyranoside (IPTG) at 37 °C for 2 h. Purification of the recombinant protein was performed using a GST-trap^R chelating column (Pharmacia Biotech Inc.). SDS-PAGE was performed in Tris/Glycine buffer, pH 8.3, on a 12% (*W*/*V*) separating gel with a 4% (*W*/*V*) stacking gel, and then electroblotted onto a nitrocellulose membrane at a constant current of 80 mA at 4 °C for 1 h. After blocking in TBS (150 mmol/L NaCl, 20 mmol/L Tris-base, pH 7.4) with 5% (*W*/*V*) skimmed milk, the membrane was incubated with an anti-GST monoclonal antibody (1 : 2 000 diluted in TBS) at 37 °C for 2 h. After washing three times, goat anti-rabbit IgG conjugated with HRP was added and incubated for 1 h at room temperature. The membrane was visualized with TMB membrane substrate (Amresco).

1.5 *Production and purification of polyclonal antibody against FOXP2*

Antibodies against recombinant FOXP2 were raised in a male New Zealand white rabbit. The rabbit was injected subcutaneously with 0.2 mg of highly purified recombinant FOXP2 protein dissolved in 0.2 mol/L NaCl and emulsified in 0.5 mL of Freund's complete adjuvant to enhance the response to the immunogen. Two booster injections of 0.2 mg recombinant protein each in incomplete Freund's adjuvant were given at 2-week intervals to obtain a prolonged persistence of the immunogen in tissues and a continuous stimulation of the immune system. Ten days after the final injection, 50 mL of blood was collected and stored for 30–120 min at room temperature to allow clotting. The crude antiserum was collected by centrifugation (5 000 r/min for 10 min), and purification of the anti-FOXP2 was performed using an immunoaffinity chromatography.

1.6 *Western blotting*

The brain tissues of plateau zokor and plateau pika were homogenized in extraction buffer (RIPA with 0.2 mmol/L phenylmethanesulfonyl fluoride) and centrifuged for 10 min at 4 °C and 15 000 *g*. The supernatant was recovered, and protein concentration was measured with the Pierce protein assay kit. Proteins were separated on a 12% SDS-PAGE gel, transferred to PVDF membrane, blocked by 5% milk, and incubated with anti-FOXP2 antibody at 4 °C overnight. The blots were incubated with secondary antibody at room temperature for 2 h and washed by TBS-T (Tween). Antibody binding was visualized with an ECL kit (Pierce Biotechnology).

1.7 *Immunohistochemistry*

The brain tissues of plateau zokor were placed in 4% paraformaldehyde (Sigma) fixative at 4 °C overnight. The tissues were dehydrated and paraffin-embedded. Microtome sections (3 μm) were obtained and mounted on glass slides. For immunohistochemistry, the slides were deparaffinized in xylene and then rehydrated for 5 min each in a graded series of ethyl alcohol (100%, 95%, 85%, 75% and 70%) with a final wash in ddH_2O. Antigen retrieval was accomplished by incubating slides in 10 mmol/L sodium citrate and heating in a microwave oven on high for 25 min. The slides were cooled in sodium citrate solution for 20 min, washed in PBS, and then incubated in 3% hydrogen peroxide, before being washed in PBS for 15 min. The slides were incubated overnight in primary antibody (anti-FOXP2) diluted 1 : 250 in the blocking solution. A ChemMate™ Envision™ Detection kit was used for immunohistochemistry staining according to the manufacturer's instructions and visualized with DAB substrate. Immunohistochemical images were acquired on an OLYMPUS DP71 microscope using DP CONTROLLER software.

1.8 *Data analysis*

All values were expressed as the mean ± standard deviation (SD). Statistical analysis was performed by one-way analysis of variance (ANOVA) and Duncan's test using SPSS. 17.0 (SPSS Inc., Chicago, IL, USA). A *P*-value < 0.05 was considered to reflect a statistically significant difference between groups.

2 RESULTS

2.1 Cloning and sequence analysis of plateau zokor *foxP2*

Plateau zokor *foxP2* was cloned, and the sequence was deposited in GenBank (accession number JQ929758.1). The cDNA sequence of *foxP2* was 2 148 bp, with an open reading frame (ORF) of 2 148 bp, encoding 715 amino acids, with predicted molecular weight and pI values of 79 968.28 Da and 6.09, respectively. The cDNA sequence of plateau zokor *foxP2* shares 90.04%, 87.48%, 86.46% and 89.86% nucleotide sequence homology with that of *Homo sapiens, Rattus norvegicus, Hipposideros Armiger* and *Rousettus*, respectively (Fig. 1*A*),

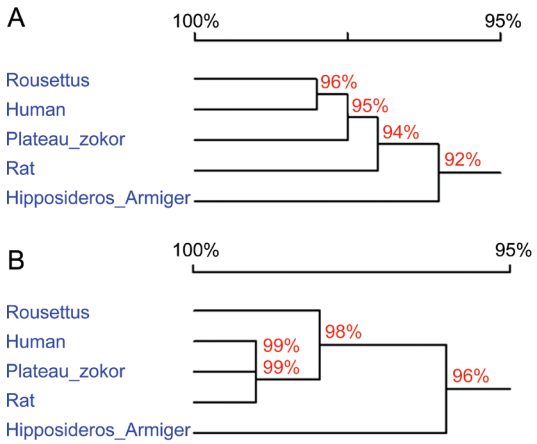

Fig. 1. Homologous tree of *foxp2*. *A*: The cDNA sequence of plateau zokor *foxp2* shares 90.04%, 87.48%, 86.46% and 89.86% nucleotide sequence homology with that of *Homo sapiens, Rattus norvegicus, Hipposideros Armiger* and *Rousettus*, respectively. *B*: The amino acid sequence of plateau zokor inferred from the *foxp2* cDNA shares 94.02%, 92.16%, 89.64% and 93.49% homology with that of *Homo sapiens, Rattus norvegicus, Hipposideros Armiger* and *Rousettus*, respectively.

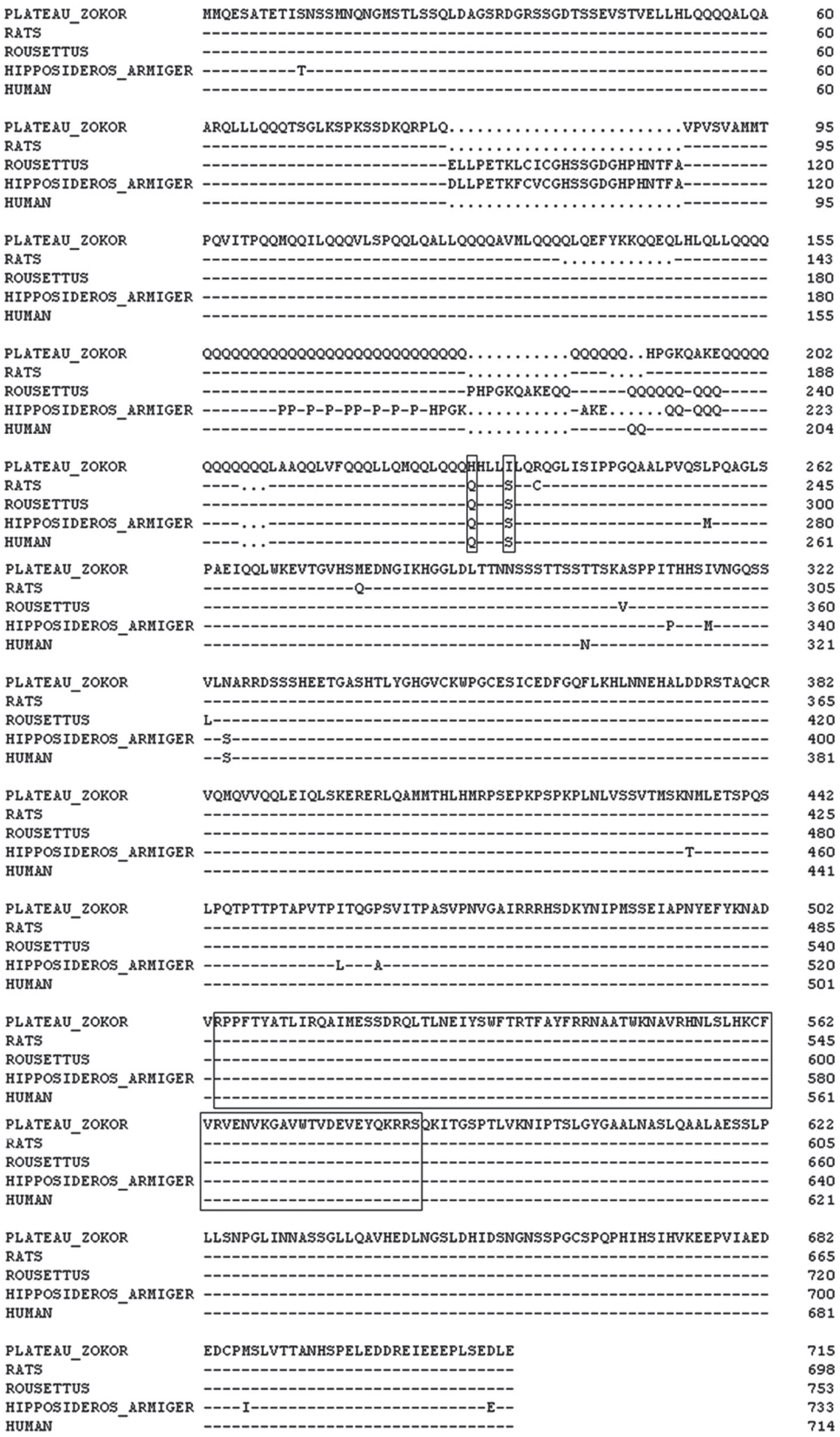

Fig. 2. Alignment of the amino acid sequences inferred from the *foxP2* cDNA sequences. The two particular amino acid substitutions and the forkhead domain are boxed. Dashes represent identical amino acids to the plateau zokor sequence, and dots represent alignment gaps.

and the amino acid sequence of plateau zokor *foxP2* shares 94.02%, 92.16%, 89.64% and 93.49% homology with that of *Homo sapiens, Rattus norvegicus, Hipposideros Armiger* and *Rousettus*, respectively (Fig. 1*B*). Plateau zokor *foxP2* is relatively conserved, although it has some specialized amino acid mutations. Compared with the other mammals, there were two amino acid substitutions, a Gln (Q)-to-His (H) change at position 231 and a Ser (S)-to-Ile (I) change at position 235 (Fig. 2).

2.2 Quantification of *foxP2* mRNA in plateau zokor and plateau pika brain

The levels of mRNA levels in brain tissues of plateau zokor and plateau pika were analyzed by quantitative real-time PCR analysis using primers designed to amplify a 249 bp fragment of the transcript according to the sequence of *foxP2*. RT-PCR showed a single band as the amplification product from the brain tissues (Fig. 3*A*). The sequence of the 249 bp transcripts was identical to the cloned *foxP2* (JQ929758.1), indicating that the primers specifically amplified *foxP2* in brain tissues. By quantitative real-time PCR analysis with *GAPDH* as a reference, the relative expression levels of *foxP2* were (0.836 ± 0.043) arbitrary units (AU) and (0.362 ± 0.067) AU in brain of plateau zokor and plateau pika, respectively. Statistical analysis showed that the *foxP2* mRNA expression level in plateau zokor was significantly higher than that of plateau pika ($P < 0.01$; Fig. 3*B*).

2.3 Quantification and identification of FOXP2 in plateau zokor and plateau pika brain

A recombinant FOXP2 protein was produced for the production of a specific polyclonal antibody. SDS-PAGE (Fig. 4*A*) and Western blot (Fig. 4*B*) using an anti-GST monoclonal antibody indicated that the purified recombinant protein migrated with an apparent molecular mass of 55–60 kDa. This suggested that the purified recombinant protein was plateau zokor FOXP2. Recombinant FOXP2-specific antibodies were produced and used to probe extracts of cytoplasm in brain tissues by Western blotting. A positive signal was obtained for the plateau zokor (Fig. 5*A*) and plateau pika (Fig. 5*B*) brain tissues. According to semi-quantitative analysis with GAPDH as a reference, the relative expression level of FOXP2 was (2.15 ± 0.45) units and (0.94 ± 0.15) units in brain tissues of plateau zokor and plateau pika, respectively. Statistical analysis showed that the FOXP2 expression level in plateau zokor was

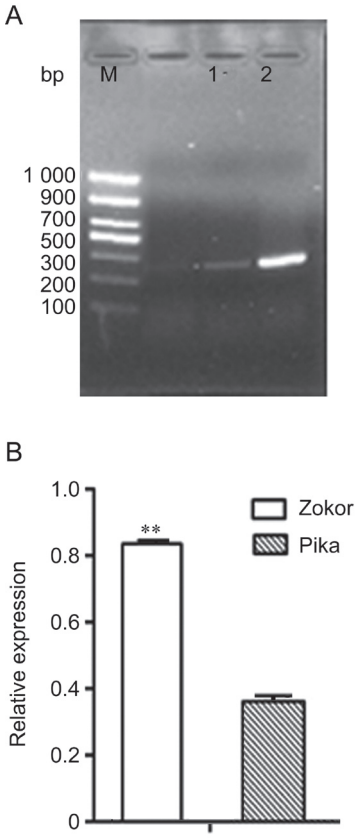

Fig. 3. Quantification of *foxP2* mRNA levels in plateau zokor and plateau pika brain. *A*: Specific *foxP2* primers for real-time PCR. M: marker; Lanes 1, 2: PCR products from brain of plateau pika and plateau zokor, respectively. *B*: Quantification of *foxP2* mRNA levels in plateau zokor and plateau pika brain. The *foxP2* mRNA levels were quantified against a standard curve and expressed as means ± SD ($n = 16$) in arbitrary units after normalization with *GAPDH*. $^{**}P < 0.01$ *vs* Pika.

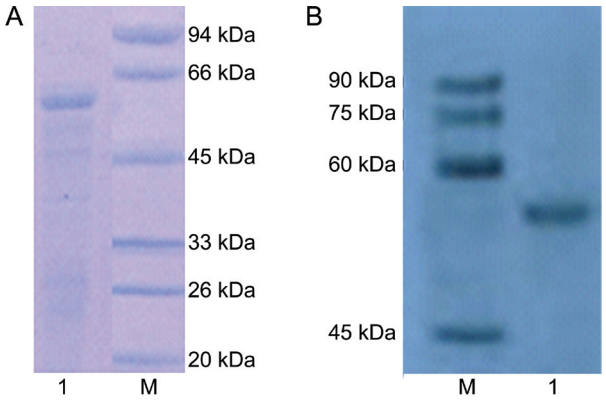

Fig. 4. Identification of the purified recombinant FOXP2 protein. *A*: SDS-PAGE analysis of the purified recombinant protein. *B*: Western blot analysis of the recombinant protein. M: Marker; Lane 1: purified recombinant protein.

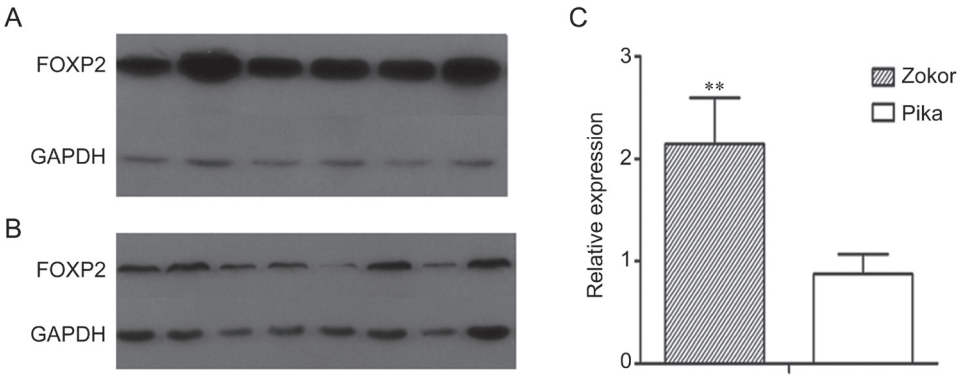

Fig. 5. Western blot of brain tissue extracts probed with anti-FOXP2. *A*: Western blotting for identification of FOXP2 in plateau zokor brain. *B*: Western blotting for identification of FOXP2 in plateau pika brain. *C*: Quantification of FOXP2 protein levels in plateau zokor and plateau pika brain. FOXP2 protein levels were expressed as means ± SD ($n = 6$) in arbitrary units after normalization with GAPDH. $^{**}P < 0.01$ *vs* Pika.

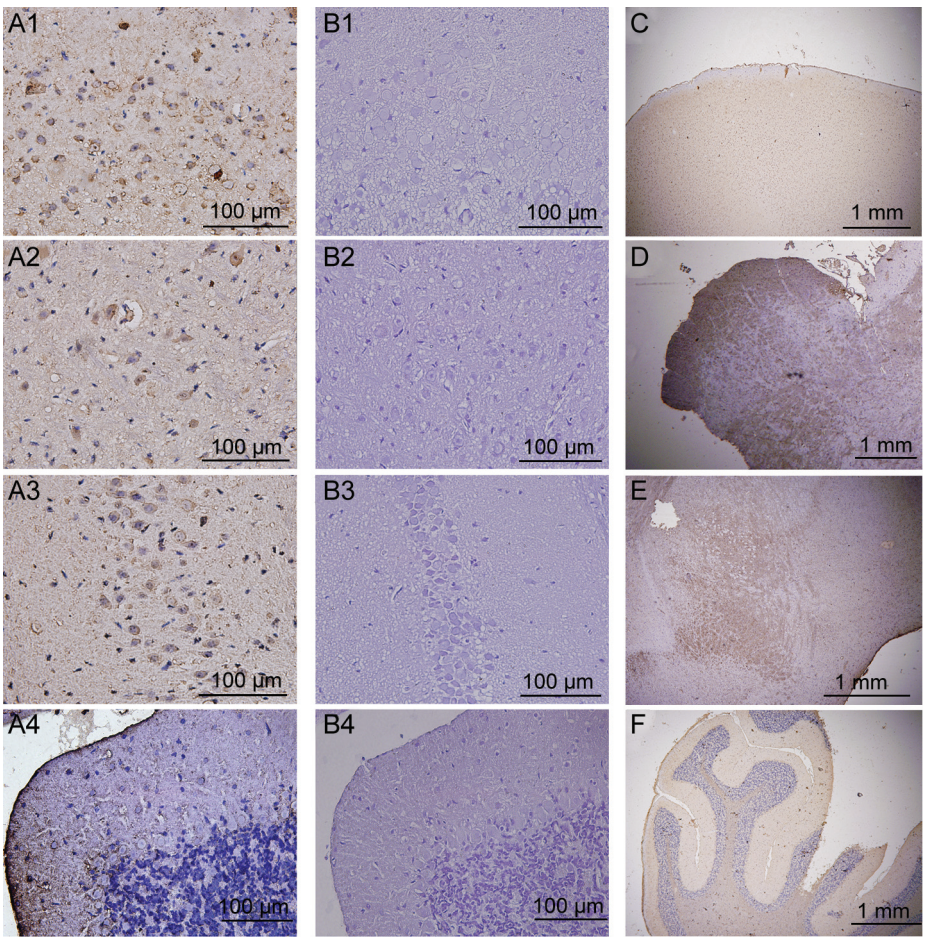

Fig. 6. Immunohistochemical analyses of FOXP2 in plateau zokor brain. Immunohistochemical analyses showing the wide distribution of FOXP2 in plateau zokor whole brain, with predominant expression in the basal ganglion and cortical plate. FOXP2 protein is stained yellow-brown, and nuclei are stained purple. *A*1–*A*4: Sections incubated with antibody to FOXP2. *B*1–*B*4: Sections incubated with PBS as a control. Numbers 1–4: cerebral cortex, thalamus, basal ganglion and cerebellum in plateau zokor. Scale bar, 100 μm. *C–F:* FOXP2 expression in cerebral cortex, thalamus, basal ganglion and cerebellum in plateau zokor. Scale bar, 1 mm.

significantly higher than that of plateau pika ($P < 0.01$; Fig. 5*C*). Immunohistochemistry showed that FOXP2 was widely distributed in the whole brain in plateau zokor, with predominant expression in striatum, thalamus and cerebral cortex (Fig. 6).

3 DISCUSSION

Subterranean mammals have high learning ability and long-term memory retention of complex routes [11, 15, 16, 18]. During the wet season subterranean mammals collect and store food in its food storage areas, exploiting the resource several months later during the dry season when the soil is hard and food is scarce. During the dry season subterranean mammals excavate along a moisture gradient from drier soil towards moist soil, where digging requires less energy and food is more abundant, and the subterranean mammals always start to dig towards more moist soils before a moisture gradient is detectable [21–23]. In order to investigate the learning ability of plateau zokor, we changed the original shape of the tunnel and set traps. We found that most plateau zokors distinguished the change of their tunnel and escaped capture by digging bypass passages.

The *foxP2* gene is the first to be linked to human speech and has been the target of positive selection during recent primate evolution [14]. Recent research showed that this gene was involved in learning, memory, vocalization, motor coordination and the acquisition of motor skills [15–20]. Studies on zebra finches showed that the expression levels of *foxP2* changed during the seasons and that increased *foxP2* expression is associated with the process of vocal-learning. Male zebra finches expressed increased levels *foxP2* mRNA in the striatum, which is necessary for vocal-learning, whereas the female zebra finches, which do not learn song did not show any differential *foxP2* expression[14]. Disruption of the *Foxp2* gene in mice showed cerebellar abnormalities and caused the decrease of learning and memory ability [25]. In this study, we compared the *foxP2* expression levels of plateau zokor and plateau pika. The results showed that the expression levels of *foxP2* were 3-fold higher, and the FOXP2 were more than 2-fold higher in plateau zokor relative to plateau pika. Furthermore, immunohistochemical analysis showed that FOXP2 was expressed at higher levels in the cerebral cortex, thalamus and the basal ganglion, which are areas of the brain involved in learning, memory and motor coordination [13, 23, 25, 26]. Above results suggest that the high learning and memory ability of plateau zokor might be related to high expression levels of *foxP2* in the brain.

Few studies of spatial orientation have been conducted on subterranean mammals, due to the inherent difficulties of observation in both laboratory and field situations. Of these studies, all indicate that subterranean mammals can assess the location, size and density of an underground obstacle, and accordingly excavate the most efficient bypass tunnel to detour around the obstacles [6, 9, 10, 27–30]. Studies on the blind mole-rat showed that they produce low frequency (150–250 Hz) seismic signals by rapidly striking the flattened anterodorsal surface of its head against the tunnel roof [7, 8, 27–29]. However, these seismic signals used by the blind mole-rat have only been demonstrated with relation to long-distance intraspecific communication [7, 29]. Other subterranean mammals apparently use the seismic channel for communication as well as for prey detection [9, 10, 30]. Previous studies indicated that subterranean mammals also use seismic signals in some kind of 'echolocation' mechanism when orienting underground[6]. In this study, we also found that plateau zokor could determine the location of blocking obstacles set within their tunnels. Studies on mice showed that disruption of the *Foxp2* gene cause an absence of ultrasonic vocalization in response to stressors [24]. Therefore, the ability of locating obstacles might be also related to the *foxP2* gene expression level in brain of plateau zokor.

The *foxP2* gene shows almost no variation across vertebrates, yet it differs by two amino acids between humans and chimpanzees [31, 32]. The complete FOXP2 protein sequences of human, mouse, chicken, zebra finch, and budgerigar show no uniquely shared substitutions between the vocal-learning animals or between the two vocal-learning birds [12]. Studies have shown greater variation in *foxP2* among echolocating bats, indicating that this accelerated evolution is associated with the development of echolocation mechanisms [33, 34]. In this study, we found that the *foxP2* of plateau zokor was similar to that of other mammals and the amino acid sequences were relatively conserved, with the exception of two particular amino acid substitutions: a Gln (Q)-to-His (H) change at position 231 and a Ser (S)-to-Ile (I) change at position 235. It can be speculated that these two changes in plateau zokor are associated with its sensitivity to low frequency seismic signals.

In conclusion, the high learning and memory ability of plateau zokor might be related to the high *foxP2* expression levels in the brain.

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