Brain Derived Neurotrophic Factor Pathway is Involved in Adult Xenopus Tropicalis Heart Regeneration

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Abstract

Brain-derived neurotrophic factor (BDNF) and its receptor Tyrosine kinase B (TrkB) have been shown to play a critical role in physiopathology of cardiac vascular system. Xenopus tropicalis (X. tropicalis) have become emerging model animals in recent years. However, there are no reports on the temporal and spatial expression patterns of BDNF and TrkB during myocardial injury and regeneration in adult X. tropicalis. In this study, using an adult X. tropicalis heart apical resection model, we showed that compared with non-amputation control, mRNA expression of BDNF in the heart tissues dramatically decreased within a short period of 4 days after apical amputation, while in 14-day after amputation, the expression of BDNF was around 3-fold increase. The peak level of increasing expression (arround 4-fold increase) was in 30-day after amputation. Upon in 42-day after amputation, the expression level of BDNF was turned back to nearly normal. Parallel, the temporal and spatial expression pattern of TrKB was well matched dynamic changes of BDNF expression pattern. The results of this study documented that BDNF-TrkB pathway was involved in the regeneration of X. tropicalis heart.

Keywords

BDNF; TrkB; Heart regeneration; Xenopus tropicalis.

1. Introduction

Recent study showed that BDNF and its receptor TrkB are expressed in the endothelial cells of the coronary arteries [1], and are known to be associated with the development of capillaries and cardiac endothelium formation [2]. While BDNF overexpression in the midgestational mouse heart results in an increase in capillary density [3]. Parallel, $TrkB^{-/-}$ mice show a marked reduction in blood vessel density and an increased number of apoptotic endothelial cell, predominantly in the subpericardial region of the developing heart [4]. In addition, BDNF is able to promote young CMECs to migrate via activation of the BDNF-TrkB-FL-PI3K/Akt pathway [5]. More recent progresses demonstrated that cardiomyocytes express BDNF and its receptor, TrkB.T1 [6-9]. BDNF/TrkB signaling is required for the heart to fully contract and relax. BDNF-induced enhancement of myocardial performance occurs via direct modulation of Ca^{2+} cycling in a calmodulin-dependent protein kinase II-dependent manner

[8]. In addition, BDNF in regulating the cardiac contraction force independent of the nervous system innervation. This function is mediated by the truncated TrkB.T1 receptor expressed in cardiomyocytes. Loss of TrkB.T1 in these cells impairs calcium signaling and causes cardiomyopathy [9]. The above studies proposed that BDNF-TrkB pathway play a critical role in physiopathology of cardiac vascular system and its regeneration.

X. tropicalis have become emerging model animals in recent years due to their short maturity cycle, large amount of eggs, simple culture, easy observation, high synteny with the human genome and regenerative capacity of organs [10-14]. Recently, we reported that adult *X. tropicalis* heart is able to be regenerated in a scar-free manner after the amputated injury [15]. At present, there are no reports on the temporal and spatial expression patterns of BDNF and TrkB during myocardial injury and regeneration in adult *X. tropicalis*. Whether BDNF-TrkB pathway is involved in regeneration of adult injured *X. tropicalis* myocardium is still unknown. This study is therefore designed to address this intriguing issue.

2. Methods

2.1 Experimental Animals

X. tropicalis (Nigerian strain) were purchased from NASCO (USA), and maintained in a freshwater tank at 26°C under a 12/12 light cycle. All the experimental protocol related with *X. tropicalis* was approved by the Jinan University Animal Care Committee.

2.2 Apical Resection of the X. Tropicalis Heart

The model of adult *X. tropicalis* heart injury and regeneration was constructed by resection of 10% of the apex of the heart in 12-month-old *X. tropicalis*. The detail protocol is followed our previous report [15]. Briefly, *X.* tropicalis were randomly divided into 2 groups: 3 in the sham non-amputation control group and 18 in the apical amputation group. For the sham non-amputation control group, the heart tissues (around 10% heart) which are matched to collected tissue of amputation group were collected at 0-days (Fig.1-2). For the amputation groups 10% of heart tissue included the wound zone were collected at 0.25 days, 4 days, 14 days, 21 days, 30 days, and 42 days after heart apical amputation (Fig.1-1). All the collected tissues were applied to total RNA extraction and Real-time PCR analysis.

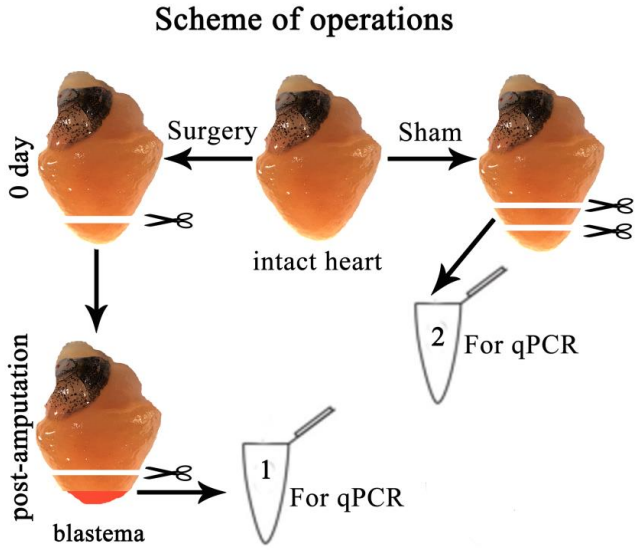

Figure 1. Schematic diagram for preparation of analyzed tissues. 1: Around 10% of heart tissue is collected in area of apex in 0.25 days, 4 days, 14 days, 21 days, 30 days, and 42 days after apical amputation. 2: Similar volume of heart tissue is collected from non-amputation control heart in parallel position.

2.3 Real-time PCR

Total RNA of the collected heart tissues was extracted using Trizol kit (Invitrogen, Cat. 15596018, USA), followed by measurement of RNA concentration using a micro-ultraviolet spectrophotometer (Nano Drop 2000 spectrophotometer Thermo). After the RNA integrity was confirmed by agarose gel electrophoresis, the RNA was reverse transcribed into cDNA using a reverse transcription kit (TOYOBO, FSQ-301, Japan). The primers which are applied for BDNF and TrkB genes were designed and synthesized as shown in Table 1. Amplification and detection of the target product was carried out using a Bio-Rad real-time PCR instrument. The composition of the reaction system was as follows: 2×SYBR Green Realtime PCR Master Mix 10 μl, qPCR-specific primer F 0.8 μl, qPCR-specific primer R 0.8 μl, cDNA template 1 μl, and the remaining volume was made up to 20 μl with mini-Q water. The reaction conditions were set to a cap temperature of 108°C, pre-denaturation at 95°C for 4 min, and then 40 cycles of the following three steps: the program was denatured at 95°C for 30 s, annealed at 60°C for 30 s, and extended at 72°C for 30 s. At the end of the amplification, the PCR product melting curve was analyzed at 65°C - 95°C. The corresponding Ct value of all samples were analyzed by Opticon Monitor 2.2 (Bio-Rad). The quantity of each gene expression was analyzed using following quantification protocol: Odc (ornithine decarboxylase) was selected as a reference gene. The difference in gene expression levels between samples was analyzed by $2^{-\Delta\Delta Ct}$ method. All samples were repeated 3 times, samples with Ct value < 40, and their melting curves were analyzed to determine their specific amplification.

2.4 Statistical Analysis

All data are mean ± standard deviation (SD). The significant difference between the experimental groups and the control group was tested by SPSS one-way ANOVA. The *p* value of less than 0.05 was defined as the difference.

Table 1. qPCR primer sequence

Primer name	Sequence	Length(bp)
BDNF qPCR probe-F	TGGAGCCACCACTGCTTTTC	189 bp
BDNF qPCR probe-R	TAACCGTTTGCCCCGACAT	
TrkB qPCR probe-F	AATCCTTTCCAGTGCTCGTGC	245 bp
TrkB qPCR probe-R	GAAACATTTGGGTCTGGGAGTC	
Odc qPCR probe-F	TGTTCTGCGCATAGCAACTG	199 bp
Odc qPCR probe-R	ACATCGTGCATCTGAGACAGC	

3. Result

3.1 The Protein Encoding Sequence and Functional Domain of BDNF is Highly Conserved During Evolution

To reveal function of BDNF pathway, we first compared the protein encoding sequence of BDNF in different species. The BDNF protein encoding sequences of *X. tropicalis*, *X. laevis*, human, chicken, rat, mouse, zebrafish, pig and ape were compared. The phylogenetic tree analysis shown that identity of BDNF protein sequence between *X. tropicalis* and *X. laevis* is 98% and the sequence of functional domain is basically the same. BDNF protein encoding sequence of *X. tropicalis* is 87% consistent with chicken, 86% consistent with human, rat, mouse and ape, 85% consistent with pig, and 70% consistent with zebrafish. The 133-240 segment of the BDNF protein sequence is belonged to functional domain,

It can be seen that the sequence of functional domain is largely the same among species (Fig.1A and B). The results of the homology and phylogenetic tree analysis doucmented that BDNF protein encoding sequence as well as it's functional domain is highly conserved during evolution. It suggested that physiopathological function of BDNF pathway is similar among species.

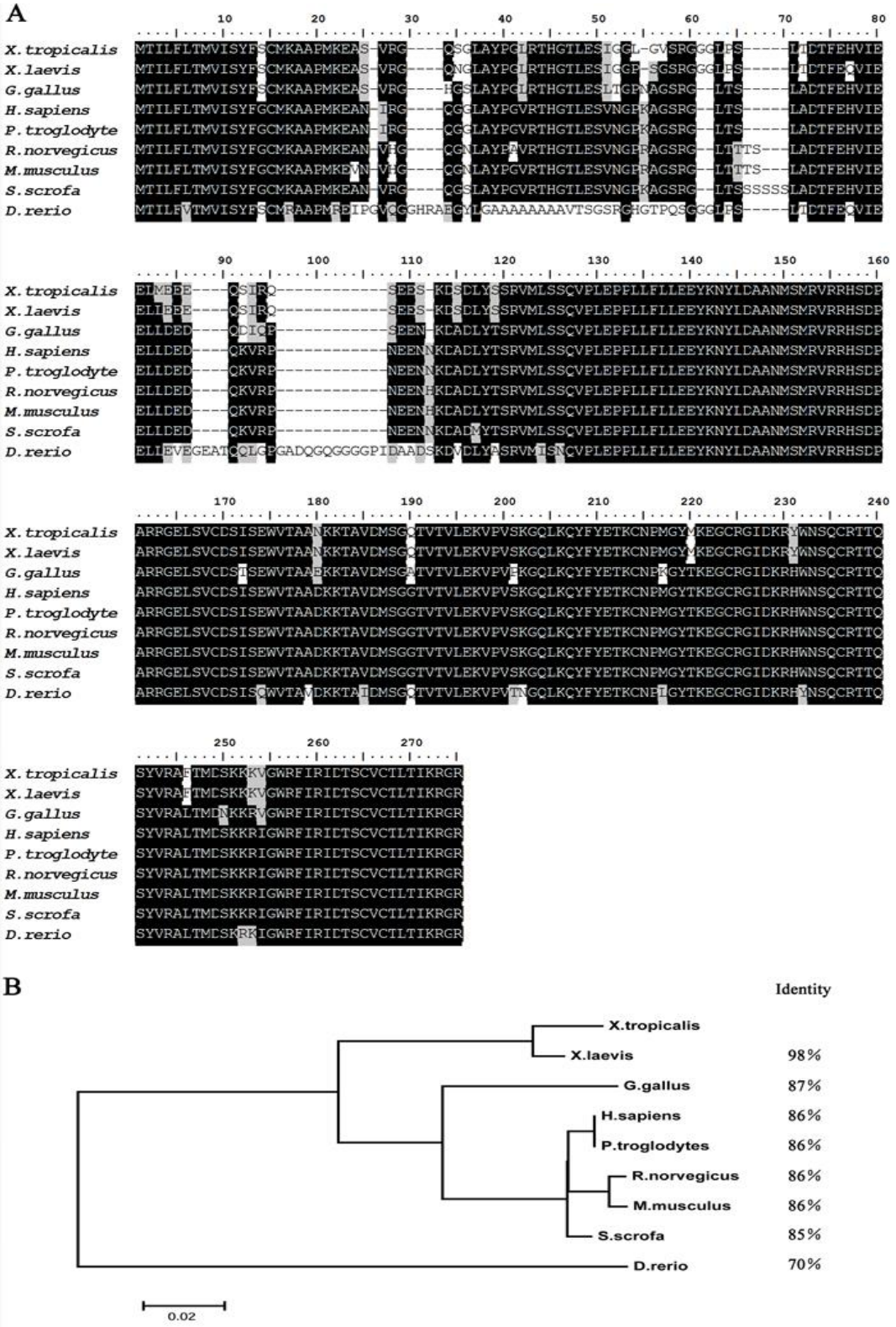

Figure 2. Homology analysis of the BDNF gene among species. A: BDNF protein sequence alignment among of X. tropicalis, X. laevis, human, chicken, rat, mouse, zebrafish, pig and ape. The identical amino acid sequences are marked with black shading, similarly shaded with gray. B: The phylogenetic

tree analysis of BDNF proteins among X. tropicalis, X. laevis, human, chicken, rat, mouse, zebrafish, pig and ape. The right is the percentage of BDNF protein homologous sequences of individual species.

3.2 BDNF-TrkB Pathway was Involved in the Regeneration of Xenopus Tropicalis Heart Regeneration

The results of RT-PCR analysis revealed that comparing with non-amputation control, BDNF expression in 0.25- to 4-day after apical amputation was significantly decreased and the lowest level was in 4-day after amputation, while in 14-day after amputation, the expression of BDNF was around 3 fold increased compared with non-amputation control. The peak level of increasing expression of BDNF (around 4 fold increased compared with non-amputation control) was in 30-day after amputation. While, upon in 42-day after amputation, the expression level of BDNF was turned back to nearly normal (Fig.3). Parallel, the results of RT-PCR analysis documented that the expression pattern of TrkB was similar to the temporal and spatial pattern of BDNF. The expression of TrkB was decreased in 0.25- to 4-day after apical amputation, the lowest level was in 4-day after amputation, while after that, the expression of TrkB was turned to increase and the peak level of increasing expression of TrkB was in 30-day after amputation. While, upon in 42-day after amputation, the expression level of TrkB was turned back to nearly normal (Fig.4).

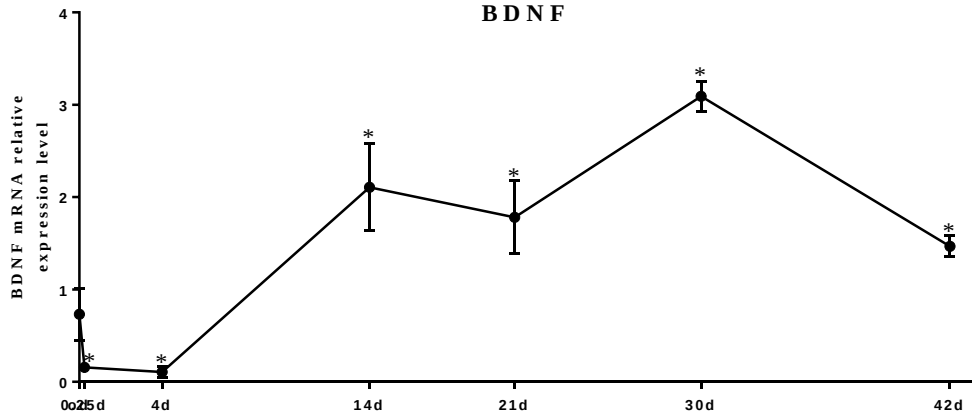

Figure 3. BDNF expression pattern during Xenopus tropicalis heart regeneration. n=3. * $p < 0.05$ vs. non-amputation control.

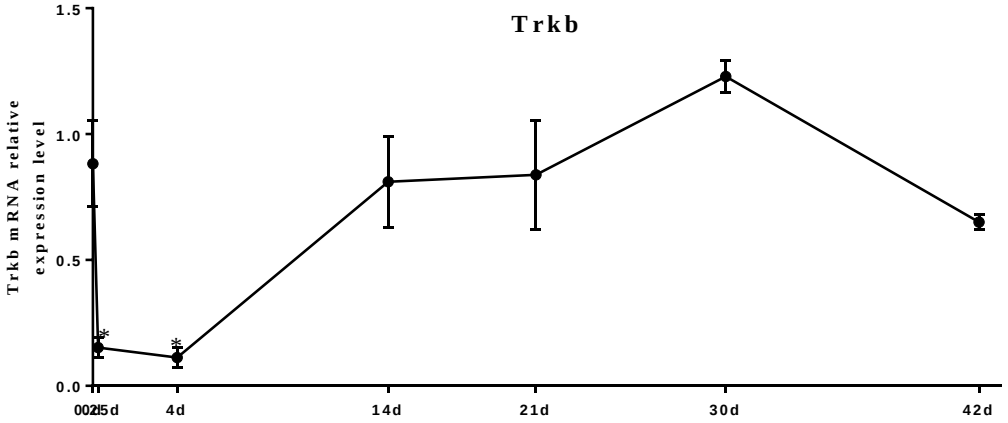

Figure 4. TrkB expression pattern during Xenopus tropicalis heart regeneration. n=3. *$p < 0.05$ vs. non-amputation control.

4. Discussion

In present study, using the homology and phylogenetic tree analysis, we showed that BDNF protein encoding sequence as well as it's functional domain is highly conserved in amoug *X. tropicalis*, *X.*

laevis, human, chicken, rat, mouse, zebrafish, pig and ape. These suggested us that physiopathological function of BDNF pathway is similar among species. Previous studies shows that after sciatic nerve transection in adult rats, BDNF mRNA levels in the gastrocnemius muscle was decreased slightly at 6 hr and 12 hr postoperatively (approximately 40% decrease vs. the control group), and then increased to around 2-fold expression level compared with control group at 1 week and the level of BDNF mRNA in the spine was decreased slightly at 6 hr and 12 hr after injury, while 30% increased expression level at 3 days, and then returned to the basic level at 1 week [16]. Take the finding which the physiopathological function of BDNF pathway might be highly conserved during evolution into consideration, it suggests that BDNF-TrkB pathway in *X. tropicalis* might be similar to mammals, and play an important role in physiopathology and regeneration of cardiac vascular system.

Indeed, our temporal and spatial expression pattern study demonstrated that BDNF expression in 0.25- to 4-day after apical amputation was significantly decreased, while in 14-day after amputation, the expression of BDNF was around 3 fold increased compared with non-amputation control. The peak level of increasing expression was in 30-day after amputation. Upon in 42-day after amputation, the expression level of BDNF was turned back to nearly normal. Parallel, the temporal and spatial expression pattern of BDNF receptor, TrkB, was well matched dynamic changes of BDNF expression pattern. It suggested that BDNF-TrkB pathway was involved in the regeneration of *X. tropicalis* heart regeneration. Recently, using same model, we have reported that adult injured *X. tropicalis* myocardium is able to be regenerated in a scar-free manner in around 30 day after damage [15]. For the finding which BDNF and TrkB expressions were initiated to increase in around 5-day, and reached at the peak level in 30-day after apical amputation in present study might further suggested that BDNF-TrkB signaling might be necessary to maintain the regeneration of injured myocardium. The further functional analysis and mechanism study for the BDNF-TrkB pathway in myocardium might benefit us to tailor novel strategy to regenerate mammalian damaged myocardium.

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