trim46 is associated with the Zebrafish Neurogenesis via Foxa2

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Abstract

Trim46 is a RING finger E3 ligase which belongs to TRIM (tripartite motif-containing) protein family. TRIM46 is required for neuronal polarity and axon specification by driving the formation of parallel microtubule arrays, whereas its embryological functions remain to be determined yet. As an initial attempt to study biological functions of Trim46 in vertebrate embryogenesis, spatiotemporal expression patterns of *trim46* were examined in zebrafish embryos. Maternal transcripts of *trim46* were present in 1 cell stage embryos, and zygotic messages were localized in the eyes and throughout the brain region, predominantly in the midbrain-hindbrain boundary (MHB) and hindbrain at 24 hpf. Bioinformatic studies found that the promoter regions of *trim46* contain *cis*-acting elements binding to Foxa2. Cyclopamine, an inhibitor of SHH, a transcriptional regulator of *foxa2*, was treated to zebrafish embryos at 4 hpf through 24 hpf to repress the transcription of *foxa2*. The treatment caused not only the severe defects in the midbrain patterning but also significant reduction in the transcripts level of *foxa2*, *trim46*, and *shha* at 24 hpf. It is thus conceivable that Trim46 contributes to development of midbrain and MHB in response to Shh signaling via Foxa2.

Keywords: RING finger E3 ligase, Trim46, Neurogenesis, Foxa2, Shh signaling

Introduction

The tripartite motif-containing (TRIM) proteins are a family of proteins that have been involved in many biological processes including cell differentiation, apoptosis, transcriptional regulation and signaling pathways[1]. TRIM46 (tripartite motif-containing 46) is a member of the C-Ⅰ TRIM family, a subfamily of the RBCC (N-terminal RING finger/B-box/coiled coil)/TRIM superfamily[2].
van Beuningen et al. (2015) showed that TRIM46 specifically localizes to the proximal axon, where it forms microtubule bundles oriented with their plus-end pointing outward[3]. By forming uniform microtubule bundles in the axon, TRIM46 is required for neuronal polarity and axon specification *in vitro* and *in vivo*, defining a unique axonal cytoskeletal compartment for regulating microtubule organization during neuronal development[4]. Recent studies revealed that TRIM46 organizes microtubule fasciculation in the axon initial segment (AIS), and microtubule (MT)-based motor KIF3A/B/KAP3 transports TRIM46 influenced by a specific MARK2 phosphorylation cascade[5][6]. It was also demonstrated that TRIM46 is a rescue factor that forms stable parallel microtubule bundles and TRIM46-bound microtubules direct Neurofascin-186 trafficking to the proximal axon[7].

Materials and Methods

Whole-mount in situ hybridization (WISH)
Nature protocol described by Thisse(2008) was used. The digoxigenin *trim46a*, *foxa2*, and *shha* sense and antisense RNA probes were synthesized.
Bioinformatic analysis of putative promoter regions
The upstream 3000 bp DNA sequences of *trim46a* were obtained from Ensembl database (http://www.ensembl.org/) and searched for the occurrences of binding motifs for transcription factors (TF) as defined by Transfac database[8].
Cyclopamine treatment
Embryos were incubated beginning at 4 hpf until 24 hpf in embryo medium containing 20 μM cyclopamine (LC Laboratories), diluted from a 10 mM stock in ethanol, at 28.5 °C. Control embryos were treated with an equivalent concentration of ethanol.

Results

1. Expression patterns of *trim46a* in zebrafish embryos

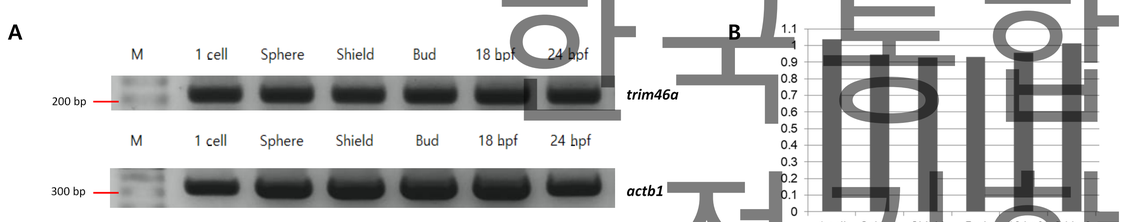
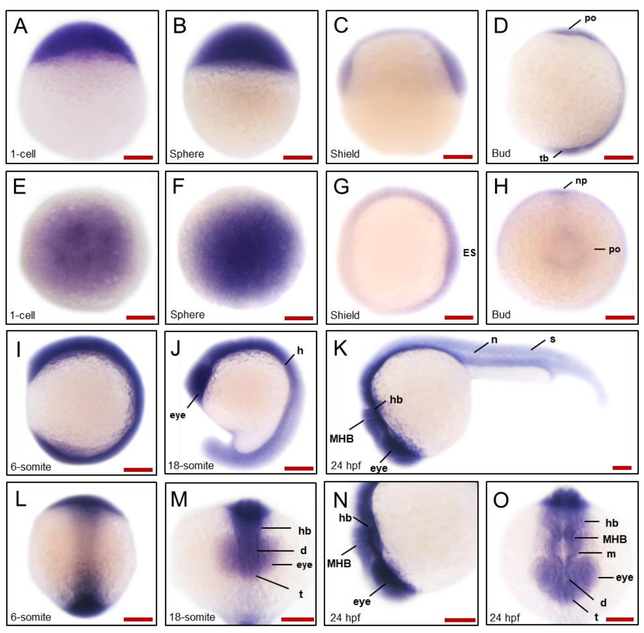


Fig.1. Reverse Transcription PCR (RT-PCR) of total RNAs from *trim46a* at 6 embryonic stages; 1-cell (0.2 hpf), sphere (4 hpf), shield (6 hpf), bud (10 hpf), 18 hpf, and 24 hpf. (A) *actb1* was used as an internal control. *trim46a* transcripts were detected at 1-cell through 24 hpf along zebrafish embryonic development. (B) Image Normalization of (A) through ImageJ software (version 1.52p; Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). *trim46a* transcripts were highly detected from the 1-cell stage to 24 hpf, indicating *trim46a* is expressed both maternally and zygotically.



Abbreviations: po, polster; tb, tail bud; ES, Embryonic Shield; np, neural plate; h, hypochord; MHB, midbrain-hindbrain boundary; hb, hindbrain; n, notochord; s, skeletal muscles; t, telencephalon; d, diencephalon; hb, hindbrain; m, midbrain;. Scale bars A-O: 50 μm.

Fig.2. Spatiotemporal expression patterns of zebrafish *trim46a.* Whole-mount *in situ* hybridization (WISH) was conducted to identify the spatiotemporal expression pattern of *trim46a* using digoxygenin-labeled RNA probe at 1 cell(0.2 hpf), sphere (4 hpf), shield(6 hpf), bud(10 hpf), 6-somite(12 hpf), 18-somite(18 hpf) and 24 hpf. (A,E) *trim46a* was expressed in the blastodisc at 1 cell stage, indicating it is maternally expressed. (B,F) After Mid-Blastula Transition (MBT, 512-cell), *trim46a* was expressed in deep cell layer (DEL), enveloping layer (EVL), and yolk syncytial layer (YSL). (C,G) *trim46a* was expressed in both ventral & dorsal region at shield stage. (D,H,I,L) The transcripts were abundant in the central nervous system at bud stage through 6-somite stage. (J,M) At 18-somite stage, *trim46a* transcripts were distributed in the precursor region of brain along the AP axis. (K,N,O) *trim46a* was predominantly expressed in the eyes and throughout the brain region, especially in the midbrain-hindbrain boundary (MHB) and hindbrain at 24 hpf.

2. Bioinformatic analysis of putative *trim46a* promoter region found that it contains *cis*-acting elements binding to Foxa2.

Fig.3. Putative *cis*-acting elements residing upstream 3000 bp of *trim46a.* There were ten putative *cis*-acting elements binding to nine different *trans*-acting elements defined by the Transfac database[8]. Foxa2 was considered as a potential regulator of the transcription of *trim46a* since its known molecular functions in zebrafish were similar to those of TRIM46. The winged-helix transcription factor, Foxa2 was previously identified to be involved in development of myelinated axons and ventral CNS development in zebrafish[9][10].

3. Cyclopamine, a SHH antagonist, caused severe defects in the midbrain patterning and significant reduction in the transcripts level of *foxa2* and *trim46a* at 24 hpf.

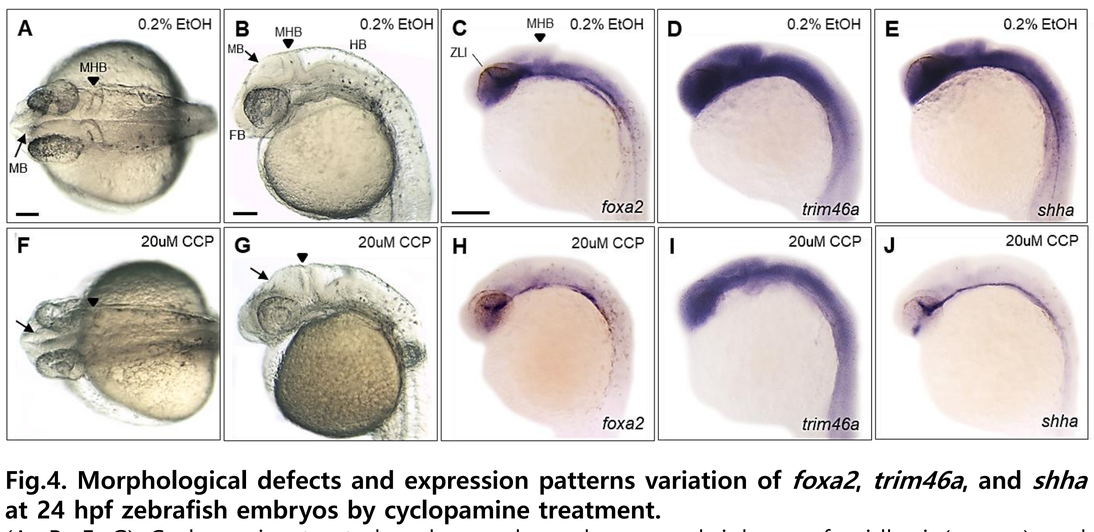


Fig.4. Morphological defects and expression patterns variation of *foxa2*, *trim46a*, and *shha* at 24 hpf zebrafish embryos by cyclopamine treatment.
(A, B, F, G) Cyclopamine-treated embryos showed severe shrinkage of midbrain(arrows) and abnormal midbrain-hindbrain boundary (MHB) (arrowheads) at 24 hpf. (C-E, H-J) The transcripts level of *foxa2*, *trim46a* and *shha* of cyclopamine-treated embryos remarkably decreased at 24 hpf.

Abbreviations: CCP, Cyclopamine; MB, midbrain; FB, Forebrain, HB, hindbrain, MHB, midbrain-hindbrain boundary; ZLI, Zona Limitans Intrathalamica. Scale bars A, B, F, G: 400 μm, C-E, H-J: 50 μm.

Conclusion

1. The spatiotemporal expression patterns indicate that *trim46a* transcripts are of maternal origin and its zygotic transcripts were concentrated in the eyes and throughout the brain region, predominantly in the midbrain-hindbrain boundary (MHB) and hindbrain at 24 hpf.
2. Bioinformatic analysis of putative *trim46a* promoter region found that it contains *cis*-acting elements binding to Foxa2, which might regulates *trim46a* expression.
3. Cyclopamine caused severe defects in the midbrain patterning and significant reduction in the transcripts level of *foxa2* and *trim46a* at 24 hpf, which may indicates that Trim46 contributes to zebrafish neurogenesis in response to Shh signaling via Foxa2.

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