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NEURAL EXPRESSION OF THE TRANSCRIPTION FACTOR THAP1 DURING DEVELOPMENT IN RAT

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Abstract—Loss of function mutations in *THAP1* has been associated with primary generalized and focal dystonia in children and adults. *THAP1* encodes a transcription factor (THAP1) that harbors an atypical zinc finger domain and plays a critical role in G_1-S cell cycle control. Current thinking suggests that dystonia may be a neurodevelopmental circuit disorder. Hence, THAP1 may participate in the development of the nervous system. Herein, we report the neurodevelopmental expression patterns of *Thap1* transcript and THAP1 protein from the early postnatal period through adulthood in the rat brain, spinal cord and dorsal root ganglia (DRG). We detected *Thap1* transcript and THAP1-immunoreactivity (IR) in the cerebral cortex, cerebellum, striatum, substantia nigra, thalamus, spinal cord and DRG. *Thap1* transcript expression was higher in the brain than in spinal cord and DRG at P1 and P7 and declined to similar levels at P14 and later time points in all regions except the cerebellum, where it remained high through adulthood. In the brain, THAP1 expression was highest in early development, particularly in the cerebellum at P7. In addition to Purkinje cells in the cerebellum, THAP1-IR was also localized to pyramidal neurons in the cerebral cortex, relay neurons in the thalamus, medium spiny and cholinergic neurons in the striatum, dopaminergic neurons in the substantia nigra, and pyramidal and interneurons in the hippocampus. In the cerebellar cortex, THAP1-IR was prominently distributed in the perikarya and proximal dendrites of Purkinje cells at early time-points. In contrast, it was more diffusely distributed throughout the dendritic arbor of adult Purkinje cells producing a moderate diffuse staining pattern in the molecular layer. At all time points, nuclear IR was weaker than cytoplasmic IR. The prominent cytoplasmic and developmentally regulated expression of THAP1 suggests that THAP1 may function as part of a cell surface-nucleus signaling cascade involved in terminal neural differentiation.

Key words: THAP1, Purkinje cells, cerebellum, dystonia, DYT6, transcription factor.

INTRODUCTION

Over 60 distinct mutations in human *THAP1* have been associated with varied anatomical distributions of primary dystonia (Fuchs et al., 2009; Xiao et al., 2010; Blanchard et al., 2011; LeDoux et al., 2012). Mean age of onset is 16.8 years and ranges from 3 years to the seventh decade of life (LeDoux et al., 2012). Human *THAP1* maps to Chr. 8p11.21 and encodes a transcription factor which contains a sequence-specific zinc-dependent DNA-binding THAP domain (1–81aa), a proline-rich region, a nuclear localization signal (146–162aa) and a coiled-coil domain (Bessiere et al., 2008). THAP1 plays an important role in transcriptional regulation in the context of cell proliferation and the pRb/E2F G_1-S cell cycle pathway (Cayrol et al., 2007; Bessiere et al., 2008). Most THAP1 mutations appear to manifest via loss of function (LeDoux et al., 2012). The roles of THAP1 in motor system molecular biology and the pathophysiology of THAP1 dystonia are unidentified.

Suggestions regarding the function of THAP1 in neural tissues can be derived from studies of angiogenesis. In particular, THAP1 expression is central to endothelial cell proliferation (Cayrol et al., 2007). The anti-proliferative effects of THAP1 on endothelial cells are not dependent on apoptosis but progression from the G_1 to S phases of the cell cycle. Overexpression of THAP1 in human endothelial cells inhibits cell-cycle progression at the G_1-S transition due to repression of pRb/E2F cell-cycle target genes (Cayrol et al., 2007). THAP1 co-localizes with the proapoptotic leucine-zipper protein Par-4 and potentiates TNFα-induced apoptosis (Roussigne et al., 2003). These data suggest that neuronal THAP1 may function in concert with other proteins during neurodevelopment through regulation of the G_1-S transition and apoptosis.

There are 12 THAP genes in human genome (THAP0-11). THAP family members harbor similar N-terminal zinc finger domains, but recognize different DNA target sequences (Bessiere et al., 2008). The THAP family appears to function as sequence-specific DNA-binding factors with roles in proliferation, apoptosis, G1-S

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Abbreviations: ChAT, choline acetyl-transferase; cRNA, complementary RNA; DRG, dorsal root ganglia; IR, immunoreactivity; PB, phosphate buffer; PBS, phosphate-buffered saline; QRT-PCR, quantitative real-time RT-PCR; ROI, region of interest; TH, tyrosin hydroxylase; THAP, THAP domain-containing, apoptosis-associated protein; $\varepsilon_{H\text{-}F}$, Huyn–Feldt epsilon.

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cell-cycle control, chromatin modification, and transcriptional regulation (Gau et al., 2008; Balakrishnan et al., 2009; Mazars et al., 2010; Trollmann et al., 2010; Cilenti et al., 2011).

At present, little is known regarding the temporal and spatial distribution of THAP1 in the brain. Understanding the development expression of THAP1 in sensorimotor regions of the brain is of utmost importance given that primary dystonia may be a neurodevelopment network disorder (Carbon and Eidelberg, 2009; Niethammer et al., 2011; Zhao et al., 2011; LeDoux et al., 2012). Other dystonia-related proteins have shown distinct expression patterns and neurodevelopmental profiles in the central nervous system. TorsinA, for instance, it expressed at high levels in striatal cholinergic neurons and cerebellar Purkinje cells at P14 (Xiao et al., 2004), and TAF1, a transcription factor associated with X-linked dystonia-Parkinsonism, shows preferential expression in striatal medium spiny neurons located in the striosomal compartments of the striatum (Sako et al., 2011). Herein, we investigated the expression of rat *Thap1* transcript and THAP1 protein from the early postnatal period through adulthood in the rat brain, spinal cord and dorsal root ganglia (DRG). These data should form a platform for an analysis of corresponding animal models and facilitate studies of THAP1 neurodevelopmental biology.

EXPERIMENTAL PROCEDURES

Experimental animals

All experiments were performed in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and approved by our Institutional Animal Care and Use Committee. Male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) were used for all experiments described herein.

Tissue collection

Rats were rapidly and deeply anesthetized with 5% isoflurane in a bell-jar prior to decapitation and tissue harvesting for RNA and protein extraction. Using a three-person technique, tissue samples were harvested from all regions within 3 min after decapitation. For *in situ* hybridization, rats were rapidly and deeply anesthetized with 5% isoflurane prior to decapitation, brain removal and freezing in isopentane chilled to −40 °C. For immunohistochemistry, rats were overdosed with pentobarbital (100 mg/kg, i.p.) prior to transcardiac perfusion with heparinized saline and then 4% paraformaldehyde/0.1 M phosphate buffer (PB). Brains were post-fixed for 2 h, blocked and incubated in a cryoprotectant solution (30% sucrose/0.1 M PB, pH 7.4) for at least 48 h. Blocks for *in situ* hybridization were sectioned at 16 μm on a cryostat and sections were collected onto SuperFrost®-Plus glass slides (Fisher Scientific, Pittsburgh, PA, USA). Slides were stored at −80 °C in sealed slide boxes with desiccant capsules. Blocks for immunohistochemistry were sectioned at 20 μm, collected onto SuperFrost®-Plus glass slides and allowed to dry on a slide warmer for 10 min prior to placement in phosphate-buffered saline (PBS). At least three rats were used for tissue collection at each developmental time point for each analysis.

Relative quantitative reverse transcription PCR (QRT-PCR)

Total RNA was extracted with TRI reagent® (Ambion, Austin, TX, USA). Genomic DNA was removed with DNA-free (Ambion, Austin, TX, USA) prior to reverse transcription of RNA. Total RNA quality was examined with agarose gel electrophoresis and a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Reverse transcription was performed with Ambion's RETROscript kit using 500-ng total RNA as template. The reaction mixture was incubated at 44 °C for 1 h and then at 92 °C for 10 min. QRT-PCR was performed using the Roche LightCycler 480 with gene specific primers and Universal Taqman® probes for both the target genes (*Thap1* isoform 1 and *Thap1* isoform 2) and 18S endogenous control (Table 1). The chosen control (18S) showed the least temporal and spatial variability among a panel of six endogenous control genes. The specificity of *Thap1* primers was established with agarose gel electrophoresis and Sanger sequencing of PCR products.

Radioactive *in situ* hybridization

Radiolabeled (^{35}S-UTP) complementary RNA (cRNA) probes were used to localize *Thap1* in cryostat sections of the rat brain. To begin with, a PCR strategy was used to add a T7 RNA polymerase promoter site to DNA templates for *in vitro* transcription of cRNA. Rat cerebellar cortex cDNA derived from reverse transcription served as PCR template. For confirmation, double-stranded cDNA was gel purified and Sanger sequenced in the forward and reverse directions. Both the sense and antisense radiolabeled (^{35}S-UTP) cRNA probes were synthesized with Ambion's MEGAscript® T7 Kit. The probes were then column purified with ProbeQuant G-50 Micro Columns (Amersham Biosciences, Piscataway, NJ, USA) and quantified with a liquid scintillation counter. A detailed hybridization protocol from our laboratory was previously

Table 1. Probe and primers used for QRT-PCR and primers used for the generation of *in situ* hybridization probes

Name	Sequence (5′ → 3′)	Locus (NM_001008340)	Usage	Product (bp)
Thap1_Is1F	ccggtacgataaggacaagc	269–288nt	QRT-PCR	
Thap1_Is1R	agactgggacgagtaagaggaa	328–307nt	QRT-PCR	60 (with IS1F)
Thap1_Is1P	ctccttcc	293–300nt	QRT-PCR probe	
Thap1_Is2F	cccgtctccttccacaaaag	288–304nt + 505–507nt	QRT-PCR	
Thap1_Is2R	tggtcacagaaaactgagaggtt	628–606nt	QRT-PCR	141 (with IS2F)
Thap1_Is2P	tcctctcc	587–594nt	QRT-PCR probe	
Thap1_AF	atggtgcagtcctgttccg	234–252nt	*In situ* probe	
Thap1_AR	**gataatacgactcactataggg**tcatgctggcacttcaacaat*	866–846nt	*In situ* probe	633 (with AF)
Thap1_SF	**gataatacgactcactataggg**atggtgcagtcctgttccg*	234–252nt	*In situ* sense probe	
Thap1_SR	tcatgctggcacttcaacaat	866–846nt	*In situ* sense probe	633 (with SF)

* The promoter sequence for T7 RNA polymerase is indicated with bold text.