The International Journal of Biochemistry & Cell Biology 42 (2010) 573–575

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Molecules in focus

Neuropathy target esterase: An essential enzyme for neural development and axonal maintenance

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ARTICLE INFO

Article history:
Received 31 October 2009
Received in revised form 5 December 2009
Accepted 7 December 2009
Available online 16 December 2009

Keywords:
Neuropathy target esterase
Phospholipids
Embryonic survival
Neural development
Motor neuron disease

ABSTRACT

Neuropathy target esterase (NTE) is an endoplasmic reticulum-anchored protein conserved across species. The N-terminal regulatory region of NTE contains three cyclic nucleotide binding domains while the C-terminal catalytic domain has a patatin domain. The NTE gene is expressed in mouse early at embryonic day 7 and its expression is maintained throughout embryonic development. NTE protein is mainly distributed in the nervous system with a pattern that is more restricted to large neurons in older animals. NTE regulates phospholipid metabolism and is known to be a phospholipase B. Knockout of NTE is embryonic lethal in mice, indicating that NTE is essential for embryonic survival. Neuronal specific NTE knockouts survive to adulthood, but show vacuolation and neuronal loss characteristic of neurodegenerative diseases. Recently, mutations in human NTE have been shown to cause a hereditary spastic paraplegia called NTE-related motor neuron disorder, suggesting a critical role for NTE in the nervous system.

1. Introduction

Neuropathy target esterase (NTE), previously called neurotoxic target esterase, was originally discovered 40 years ago as the primary target of organophosphorus compounds (OPs) that cause a delayed paralyzing syndrome with degeneration of nerve axons called OP-induced delayed neuropathy (OPIDN) (Johnson, 1969). However, the human NTE gene was cloned only 10 years ago (Lush et al., 1998). NTE is highly conserved across species including mammals, insects, nematodes, and yeast (Moser et al., 2000). It is a member of the patatin-like phospholipase (PNPLA) family with critical roles in diverse aspects of lipid metabolism and signaling (Wilson et al., 2006).

2. Structure

The human NTE gene, found on chromosome 19p13.3, is over 27 kb and consists of 35 exons and 34 introns (Winrow et al., 2003). The transcript is about 4.3 kb encoding a protein with 1327 amino acids. NTE has two distinct functional domains, an N-terminal regulatory domain of about 700 residues and a C-terminal catalytic domain (Fig. 1) (Lush et al., 1998). At the end of the N-termini, there is a transmembrane segment (residues 10–31) required for its association with endoplasmic reticulum (ER) (Li et al., 2003). Three putative cyclic nucleotide binding domains (residues 163–262, 480–573 and 597–689) are situated in the regulatory domain, which implies that NTE may be regulated directly by the binding of cyclic AMP (Glynn, 2005). In addition, there is a similar sequence to the destruction-box (D-box) of cyclin B3 within the regulatory domain (RVTFLALHN, residues 276–284), suggesting that NTE could undergo degradation by the ubiquitin–proteasome pathway. The catalytic domain spans the region of 727–1216 residues. This domain alone shows the same esterase activity as the full length NTE, so it was named the NTE esterase domain (NEST) (Atkins and Glynn, 2000). A patatin-like domain (residues 933–1024) in NEST contains the serine hydrolase signature motif GXSXG (residues 964–967, GTSIG). Within the human NEST domain, Ser^{966} and two aspartate residues, Asp^{960} and Asp^{1086}, are critical for NTE activity (Atkins and Glynn, 2000). However, the modelled active sites only consist of Asp^{960} and Asp^{1086} catalytic dyad as other PNPLAs (Wijeyesakere et al., 2007). Moreover, there is a glycosaminoglycan attachment site (residues 735–738), a tyrosine kinase phosphorylation site (residues 403–410), and a cell attachment sequence (residues 663–665) predicted for NTE in silico (Wilson et al., 2006).

3. Expression, cellular location and regulation

NTE is usually detected in tissue homogenates by colorimetric assay of its esterase activity with phenyl valerate, a non-physiological substrate. In mice, NTE is expressed as early as embryonic day 7 (E7) and throughout embryonic development

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1357-2725/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biocel.2009.12.007

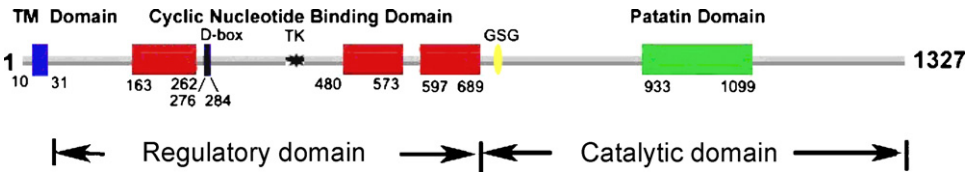

Fig. 1. Human NTE protein structure. NTE has a transmembrane (TM) domain (blue rectangle), a regulatory domain containing three cyclic nucleotide binding domains (red rectangle) and a destruction-box (D-box; black vertical line); and a carboxyl-terminal catalytic region with a patatin domain (green rectangle). Numbering corresponds to amino acid residues at the N- and C-termini of each domain. In addition, NTE contains a tyrosine kinase (TK) phosphorylation site (residues 403–410) and a glycosaminoglycan (GSG) attachment site (residues 735–738) as shown with black star and orange ellipse respectively.
This figure was adapted from Wijeyesakere et al. (2007) and Wilson et al. (2006).

(Winrow et al., 2003). At E13.5, strong expression is shown in the cells of the developing lens and along the developing spinal cord. In the adult, NTE is observed throughout the brain, particularly in the cortex, in the Purkinje cells of the cerebellum and in the hippocampus (Moser et al., 2000; Winrow et al., 2003). In addition, the expression of NTE is also detectable in non-neural tissues, such as kidneys, liver and testes in mouse (Winrow et al., 2003). Similarly, there is a broad tissue distribution of NTE mRNA with high levels in brain (Wilson et al., 2006).

NTE is anchored in ER via its TM domain (Li et al., 2003; Akassoglou et al., 2004). Most of the NTE molecule is exposed on the cytoplasmic face of ER membranes and the catalytic domain interacts with the cytoplasmic face of the ER. Moreover, over-expression of NTE induces ER aggregation through intermolecular association of its catalytic domain, which may be hindered by its regulatory domain to some degree (Li et al., 2003).

Although there are three cyclic nucleotide binding domains in the regulatory region of NTE, neither direct binding of cAMP to NTE nor regulation of NTE activity by cGMP in brain homogenates have been observed (Glynn, 2005; Casida et al., 2008). However, cAMP can affect NTE protein levels and activity via the regulation of NTE mRNA levels in a protein kinase A (PKA)-dependent manner (our unpublished data). In addition, our previous study showed that NTE may be also controlled by protein kinase C (PKC) as mRNA levels of NTE are downregulated by the PKC activator phorbol 12-myristate 13-acetate (PMA) in a manner blocked by PKC inhibitor staurosporine (Chen et al., 2007b). Moreover, another report demonstrated that high NaCl induces increased NTE expression in mouse renal cells, which is mediated by the transcription factor TonEBP/OREBP (Gallazzini et al., 2006). Yeast two hybrid screening revealed that G protein beta-2 was associated with the catalytic domain of NTE and regulated NTE activity without any effect on NTE protein expression level (Chen et al., 2007a). Additionally, NTE is demonstrated to be post-translationally regulated by the macroautophagic lysosomal pathway (Long et al., 2009). Because there is sequence similarity to the destruction-box (D-box) of cyclin B3 within the regulatory domain, human NTE may also be degraded by the ubiquitin–proteasome pathway. In fact, the same sequence also exists in the regulatory domain of chicken NTE, which is cleared by the proteasome (Chang et al., 2009).

4. Biological function

Recombinant human NEST is able to catalyze hydrolysis of naturally membrane-associated lipids; lysophospholipids are the most avidly hydrolyzed substrates, indicating that membrane lipids are the putative cellular substrates (van Tienhoven et al., 2002). Furthermore, NTE has been demonstrated to have potent lysophospholipase activity in mice (Quistad et al., 2003) and deacylate phosphatidylcholine (PC) to glycerophosphocholine (GPC) as a phospholipase B in mammalian cells (Zaccheo et al., 2004). Indeed, NTE deficiency induced increased levels of neural PC in mouse and *Drosophila* (Read et al., 2009; Mühlig-Versen et al., 2005). Therefore, NTE regulates phospholipids, which accounts for a significant portion of total brain lysophosphatidylcholine (LPC) hydrolysis and may also act on PC with lower affinity (Casida et al., 2008).

Complete inactivation of the NTE gene results in embryonic lethality at E9 (Winrow et al., 2003; Moser et al., 2004). As early as E7.5, mutant embryos show growth retardation and no embryo can be found after E11, showing that NTE is essential for embryonic survival beyond E8. Failed placental development causes massive apoptosis within the developing embryo preceding its resorption and results in growth retardation (Moser et al., 2004). Histological analysis indicates that NTE is essential for the formation of the labyrinth layer and survival and differentiation of secondary giant cells (Moser et al., 2004). Additionally, NTE deficiency impairs vasculogenesis in the yolk sac. Although NTE is not required for cell division in the early embryo or neurite elongation in mouse embryonic stem cells, it is involved in the optimal rate of neurite initiation (Li et al., 2005).

In order to study the role of NTE in the adult brain, a conditional mutant *NTE* strain has been constructed that specifically deletes NTE in mouse neuronal tissues (Akassoglou et al., 2004). Absence of NTE results in disruption of the ER, vacuolization of nerve cell bodies, and abnormal reticular aggregation. The prominent neuronal pathology is mainly shown in the hippocampus and thalamus. Defects also exist in the cerebellum, which include a partial loss of Purkinje cells as well as thinner Purkinje dendritic trees in the molecular layer. Thus, these data indicate that NTE plays a role in normal neural development and brain specific deletion of NTE contributes to neurodegenerative diseases characterized by vacuolization and neuronal loss (Akassoglou et al., 2004). A strikingly similar phenotype is also shown in *Swiss-cheese* (*sws*), the ortholog of NTE, mutant flies and the phenotype can be rescued by mouse NTE (Kretzschmar et al., 1997; Mühlig-Versen et al., 2005). Furthermore, distal degeneration of the longest spinal axons has been observed in adult mice with NTE-deficient neural tissue. Cultured NTE-deficient neurons display modestly impaired secretion (Read et al., 2009). Thus, NTE is required for axonal maintenance. In addition, SWS acts as a noncanonical regulatory subunit to regulate the localization and kinase activity of the C3 catalytic subunit of PKA (PKA-C3), and disruption of this regulation can induce neurodegeneration (Bettencourt da Cruz et al., 2008).

In addition to a critical role for normal nervous system development, NTE catalyzes the production of GPC to maintain osmolyte levels in renal cells in response to high NaCl levels (Gallazzini et al., 2006), which acts synergistically with glycerophosphodiester phosphodiesterase domain containing 5 (GDPD5) to adapt cells to stress of the normally high salt and urea in the renal medulla (Gallazzini et al., 2008). The biological function of NTE in embryo and adult is summarized in Fig. 2.

5. Medical applications: link between NTE and motor neuron disease

NTE was firstly identified in the process of OPIDN pathogenesis and the inhibition and subsequent aging of NTE has been proposed to be the initiating event (Casida et al., 2008). OPIDN is

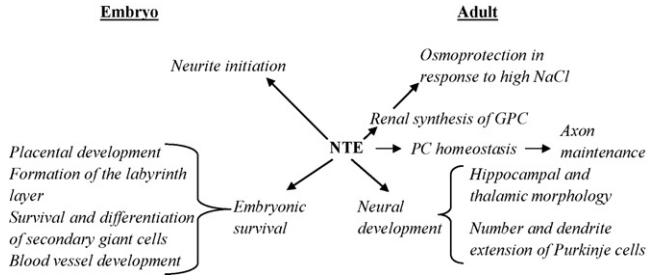

Fig. 2. Biological functions of NTE. As an ER-anchored phospholipase B, NTE regulates phospholipids homeostasis and plays potential roles in both embryogenesis and the adult.

characterized by distal degeneration of long axons in the spinal cord and peripheral nerves. Distal axon degeneration is also the primary neuropathologic feature of hereditary spastic paraplegia (HSP). Rainier et al. (2008) identified several NTE mutations in patients from a consanguineous family and a nonconsanguineous family by genome-wide linkage analysis. The affected phenotype in each family conformed to both OPIDN and "Troyer syndrome", the latter of which was an autosomal-recessive form of HSP. This disorder is referred as NTE-related motor neuron disease (MND). NTE mutations in NTE-MND subjects disturb NTE's catalytic domain, which could result in altered NTE activity *in vivo*. Disease-specific, nonconserved NTE mutations in unrelated MND patients indicate the importance of NTE in maintaining axonal integrity and support the role of NTE abnormalities in OPIDN. Thus these NTE mutations may be sufficient to cause the autosomal-recessive MND even in the absence of apparent exposure to neurotoxic OPs and raise the possibility that NTE pathway disturbances contribute to other MNDs (Rainier et al., 2008).

Classic OPIDN syndrome has not been observed in mice, but loss of 90% brain NTE activity leads to axonal degeneration and hind limb ataxia (Read et al., 2009). However, NTE heterozygote mice ($NTE^{+/-}$) showed 40% decrease in esterase activity and did not develop neurodegeneration (Akassoglou et al., 2004). The relationship between NTE activity and neurodegeneration seems to be in accordance with the established threshold to the occurrence of OPIDN in adult hens: over 70% inhibition of NTE by OPs (Casida et al., 2008). In addition, mice treated with tri-*o*-cresyl phosphate (TOCP) daily for 9 months also showed axonal degeneration and hind-limb paralysis. The same neuropathic syndrome induced by genetic knockout and chronic chemical dosing indicates that the OP is indeed acting by inactivating NTE. The relative distribution of axonal lesions in the motor corticospinal and sensory dorsal tracts in upper and lower spinal cord of the adult mice with NTE-deficient neural tissue closely mirrors that reported in human HSP (Read et al., 2009). NTE deficiency induces minor increased neural PC levels and impairs neuronal secretion (Read et al., 2009). The damage *in vivo* is initially confined to distal regions of long axons, and axon transportation may be further affected. Therefore, NTE-deficient mice comprise a useful new model for HSP.

Acknowledgments

The work was partially supported by grants from the National Natural Science Foundation of China (Nos. 30600329, 30870298, and 30870537). Authors would like to thank Ms. J. Wentzell and Dr. M. Li for their help in polishing the English of the revised manuscript.

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