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Molecular cloning and expression analysis of cDNA ends of chicken neuropathy target esterase

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Abstract

Neuropathy target esterase (NTE) was proposed as the initial target during the process of organophosphate-induced delayed neuropathy (OPIDN) in human and some sensitive animals. Adult hens are usually the animal model for experimental studies of OPIDN. However, little is known about the sequence and characteristics of chicken NTE. We report here the cloning of the 5′ and 3′ cDNA ends of chicken NTE through rapid amplification of cDNA ends (RACE) and their expression profiles in different tissues with northern blotting. The cloned 3′ cDNA end of chicken *NTE* is 801 base pair (bp) in length with an open reading frame (ORF) of 379 bp. It contains a termination codon (TAG) and a 422-nucleotide noncoding sequence with the polyA sequence (GenBank accession no. DQ126678). The chicken NTE 5′ cDNA end is 665 bp in length with an ORF of 552 bp. It contains an iniation codon (ATG) and a 113-bp untranslated region (GenBank accession no. DQ126677). The deduced proteins from 5′ and 3′ cDNA ends have a high degree of homology to humans and mouse NTE at the amino acid level. Chicken NTE is suggested to be a transmembrane protein by the transmembrane helix prediction of the deduced N-terminal sequence. The chicken NTE gene is expressed as a 4.5 kb transcript in different tissues, including brain, kidney, liver and testis. Moreover, the mRNA expression of chicken *NTE* is highest in brain, and the mRNA levels of chicken *NTE* in testis, kidney and liver are about 75%, 47% and 24% of that in brain, respectively. These results should be helpful in cloning chicken full-length *NTE* gene.

Keywords: Chicken (*Gallus gallus*); Neuropathy target esterase; cDNA ends; Gene expression

1. Introduction

Organophosphorus compounds are a diverse group of chemicals used primarily as pesticides, plasticizers, plastic softeners, flame-retardants, antioxidants, and hydraulic fluids. Exposure to almost all organophosphorus pesticides (OPs) can induce acute toxicity in human and animals due to inhibition of acetyl-

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cholinesterase (AChE). Single or multiple doses of some OPs, such as mipafox and diisopropyl phosphorofluoridate (DFP) can additionally induce delayed effects; so-called organophosphate-induced delayed neuropathy (OPIDN), which is characterized by distal axonal degeneration and secondary demyelination of central and peripheral axons [1]. Although neuropathy target esterase (NTE) was discovered over 30 years ago and the inhibition and subsequent aging of NTE has been proposed to be the initiating event in OPIDN, the events that occur between NTE inhibition and the appearance of clinical effects are not completely understood [2]. Elucidation of the molecular and cellular functions of NTE is a priority in understanding the pathogenesis of OPIDN.

Human NTE gene was first cloned in 1998 and it encodes a polypeptide of 1327 amino acids [3]. NTE was anchored to the cytoplasmic side of endoplasmic reticulum by an amino terminal transmembrane segment in mammalian cells and neurons [4,5]. The cDNA sequence of mouse *NTE* was then cloned and is highly identical to human *NTE* [6]. NTE is a novel serine esterase protein that is highly conserved among various species including insects, nematodes, yeast, and bacteria [3]. Previous observations indicated that NTE displayed potent lysophospholipase activity in mouse brain [7]. NTE was further found to be responsible for converting phosphatidylcholine to glycerophosphocholine in mammalian cells and to regulate phosphatidylcholine homeostasis in *Drosophila* [8,9]. In mice, complete inactivation of the NTE gene resulted in embryonic lethality due to placental failure and impaired vasculogenesis [10,11], while mice with a brain-specific deletion of NTE exhibited neurodegeneration [5]. In adult *Drosophila*, loss of the swiss cheese/NTE activity causes neuronal and glial death [9]. Together, these data suggest that NTE is essential for embryonic and nervous development.

NTE was proposed to be largely redundant in adulthood and inhibition of this enzyme after exposure to OP compounds was suggested to result in a novel and toxic gain of function rather than the abolishment of a vital property of the protein [12]. In contrast, $nte^{+/-}$ mice appear hyperactive and are more sensitive than wild-type mice to a fatal form of octylphosphonofluoridate (EOPF) toxicity, which suggests that OP toxicity occurs directly through inhibition of NTE without the requirement for NTE gain of function or aging [10]. Species differences occur after exposure to neuropathy-inducing OPs, as OPIDN in susceptible species differs from OP-induced toxicity in mice [13].

Although adult hens are usually the animal model for experimental studies of OPIDN and the inhibition and subsequent aging of NTE have been proposed to be the initiating event in OPIDN [1], the molecular cloning and characteristics of chicken NTE are unknown. A large portion of chicken NTE was predicted (GenBank accession no. XM_423161) after the chicken genome sequence project was primarily achieved. However, the predicted chicken NTE cDNA lacks ends sequence. In this report, we present the cDNA ends sequences of chicken NTE cloned by rapid amplification of cDNA ends (RACE) and their expression profiles in different tissues analyzed by northern blotting.

2. Materials and methods

2.1. Materials

The domestic adult Sanhuang chicken (*Gallus gallus*) strain was purchased from the Nanshan Poultry Farm (Chongqing, China). pMD18-T vector, T4 DNA ligase, DNA gel extraction kit, TaKaRa Ex Taq™ PCR (polymerase chain reaction) kit (Hot start version), X-gal and IPTG were from Takara (Dalian, China). Trizol and SuperScript™ III First-Strand Synthesis System for RT-PCR (reverse transcription–PCR) were purchased from Invitrogen (Groningen, The Netherlands). DEPC was obtained from Sigma (St. Louis, MO, USA). Terminal deoxynucleotidyl transferase and Prime-a-Gene Labeling System were from Promega (Madison, WI, USA). (α-32^P) dCTP (specific activity 3000 Ci/mmol) was purchase from the Beijing Furui Company of Biotechnology (Beijing, China).

2.2. Total RNA isolation

The chicken was sacrificed by decapitation and tissues, including brain, liver, kidney and testes, were immediately isolated. Total RNA extraction was performed with Trizol according to supplier's instruction. Total RNA was quantified with ultraviolet absorption assay and electrophoresed on a denatured agarose gel to investigate its integrality. Total RNA was preserved in DEPC-treated water.

2.3. Molecular cloning of 3′ cDNA end of NTE gene

2.3.1. Primer design

According to the putative chicken NTE cDNA fragment in GenBank (XM_423161), two gene-specific primers, 5′TGCGTGCGGCAGCTGGAGGT3′ and 5′CGACCGCTTCAAGACGATGG3′, were designed and named as GSP1 (gene-specific primer 1) and GSP2, respectively. At the same time, adapter primer (AP), 5′GGCCACGCGTCGACTAGTACTTTTTTTTTTTT-