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IDENTIFICATION AND LOCALIZATION OF A NEURON-SPECIFIC ISOFORM OF TAF1 IN RAT BRAIN: IMPLICATIONS FOR NEUROPATHOLOGY OF DYT3 DYSTONIA

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Abstract—The neuron-specific isoform of the *TAF1* gene (*N-TAF1*) is thought to be involved in the pathogenesis of DYT3 dystonia, which leads to progressive neurodegeneration in the striatum. To determine the expression pattern of *N-TAF1* transcripts, we developed a specific monoclonal antibody against the N-TAF1 protein. Here we show that in the rat brain, N-TAF1 protein appears as a nuclear protein within subsets of neurons in multiple brain regions. Of particular interest is that in the striatum, the nuclei possessing N-TAF1 protein are largely within medium spiny neurons, and they are distributed preferentially, though not exclusively, in the striosome compartment. The compartmental preference and cell type-selective distribution of N-TAF1 protein in the striatum are strikingly similar to the patterns of neuronal loss in the striatum of DYT3 patients. Our findings suggest that the distribution of N-TAF1 protein could represent a key molecular characteristic contributing to the pattern of striatal degeneration in DYT3 dystonia.

Key words: TAF1, DYT3 dystonia, striosome, neurodegeneration, transcription dysregulation syndrome.

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Abbreviations: BSA, bovine serum albumin; *C-TAF1*, common form of the *TAF1* gene; DARPP-32, the dopamine and cAMP-regulated phosphoprotein of 32 kDa; GCL, granule cell layer; H, hilus dentata; HD, Huntington's disease; KLH, keyhole limpet hemocyanin; mAb, monoclonal antibody; ML, molecular layer; MOR, μ-opiate receptor; MSNs, medium spiny neurons; *N-TAF1*, neuron-specific isoform of the *TAF1* gene; PCL, Purkinje cell layer; RNAPII, RNA polymerase II; SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum; TBP, TATA box-binding protein; TFIID, transcription factor IID complex.

Among the monogenic primary dystonias (Müller, 2009), DYT3 dystonia, also named X-linked dystonia-parkinsonism (XDP/DYT3, MIM314250), is the result of disrupted alternative splicing regulation. A series of linkage analyses (Haberhausen et al., 1995; Nolte et al., 2003) identified the disease locus of the *DYT3* gene as Xq13.1, including TAF1 [TATA box-binding protein (TBP) associated factor 1], formerly called $TAF_{II}250$. TAF1 is the largest subunit of the transcription factor IID complex (TFIID), which is composed of TBP and 13 different TAFs. TAF1 appears to function as a major scaffold by which TBP and other TAFs interact in the assembly of TFIID. TAF1 is an essential component of the transcription machinery and is known to be a key regulator for RNA polymerase II (RNAPII)-dependent gene transcription that involves conversion of cellular signals provided by gene-specific activator proteins into the synthesis of mRNA (Wassarman and Sauer, 2001). Makino et al. (2007) recently reported that the *TAF1* gene is the causative gene of DYT3 dystonia and showed that there is a specific reduction of the neuron-specific isoform of the *TAF1* gene (*N-TAF1*) in DYT3 patients. DYT3 dystonia thus can be classified as one of the neurodegenerative disorders associated with transcriptional dysregulation.

DYT3 dystonia is clinically characterized by an adult-onset movement disorder that manifests progressive and severe dystonia followed by overt parkinsonism in the later years of life (Lee et al., 2002). The neuropathology of DYT3 (Goto et al., 2005) involves a primary and progressive degeneration of medium spiny neurons (MSNs) in the striatum, with sparing of large cholinergic neurons. There is also differential neurodegeneration in the striatal compartments, judging by immunostaining, with a predominant loss of neurons in striosomes relative to the surrounding matrix compartment in the DYT3 striatum. How such impaired transcription is related to the neurodegeneration and symptoms characteristic of DYT3 dystonia is still not well understood.

As a first step to addressing this issue, we generated a monoclonal antibody (mAb) that specifically recognizes N-TAF1 protein for the purpose of studying the spatial distribution of N-TAF1 protein in the brain. The antibody successfully stained neurons expressing N-TAF1 in rat brains, and here we show that N-TAF1 is located exclusively in the nuclei of neurons in multiple brain regions. Notably, within the striatum, N-TAF1-positive ($N\text{-}TAF1^{+}$) nuclei were largely within neurons of the medium spiny type, and there was a marked tendency of these N-TAF1-positive neurons to lie in the striosomal compartment in the striatum. As striosomes may be a primary site of neurode-

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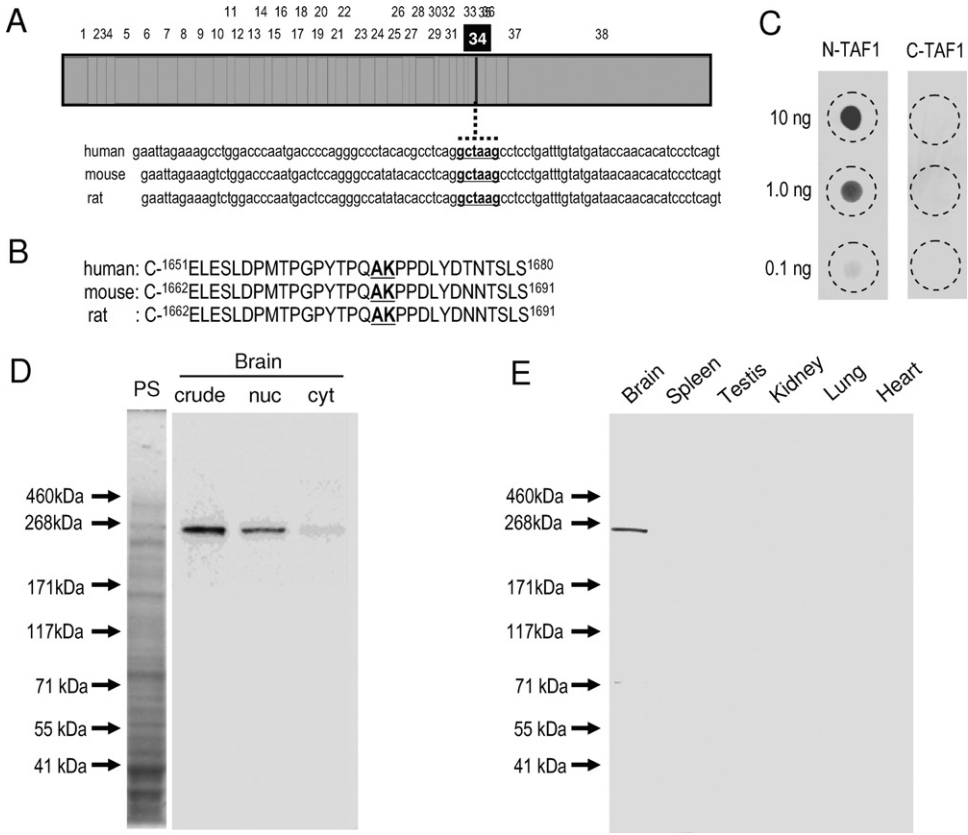

Fig. 1. Production and characterization of monoclonal antibody against N-TAF1 protein. (A) Nucleotide sequence neighboring exon 34' in a full-length cDNA for N-TAF1. (B) The amino acid residues neighboring exon 34' of human, mouse, and rat. (C) Dot blot assay. Indicated amounts of KLH-conjugated N-TAF1-peptide (N-TAF1) or BSA-conjugated C-TAF1-peptide (C-TAF1) were spotted onto a nitrocellulose membrane and detected by dot immunobinding assay with anti-N-TAF1 antibody mAb-3A11F. (D) Western blot assay on the brain extracts. Crude homogenates (20 μg of protein) extracted from a rat brain (crude) were loaded onto 10% SDS-PAGE and then processed for the transimmunoblot technique using mAb-3A11F. Nuclear (nuc) and cytosol (cyt) fractions (20 μg of protein) of a rat brain were also processed for the transimmunoblot. PS, protein staining. (E) Western blot assay on the multiple organ extracts. Crude homogenates (20 μg of protein) extracted from a rat brain, spleen, testis, kidney, lung, and heart were loaded onto 10% SDS-PAGE and then processed for the transimmunoblot technique using mAb-3A11F.

generation early in the progression of neuropathology in DYT3 patients (Goto et al., 2005), these data suggest a link between N-TAF1 expression and specific patterns of neuropathology in DYT3 dystonia.

EXPERIMENTAL PROCEDURES

All procedures involving the use of animals and analysis of brain anatomy were approved by the Institutional Care and Use Committees of the University of Tokushima and Shiga University of Medicines.

Production of antibodies

Sequence data were used for antibody production. Fig. 1A illustrates the nucleotide sequence of a full-length cDNA for N-TAF1 and that of neighboring exon 34′. Based on a comparison of the amino acid sequence of N-TAF1 (GenBank, accession number AB300418) to that of the common form of TAF1 (C-TAF1) (GenBank, accession number NM_004606), the N-TAF1-peptide for immunization was chosen and synthesized as *C*-^{1659}TPGPYTPQAKPPDLY^{1673}, for which the residues between E-1651 and S-1680 of humans are highly conservative across species (Fig. 1B). This epitope peptide corresponds to the peptide for C-TAF1 (*C*-^{1659}TPGPYTPQPPDLY^{1671}) with an insertion of two amino acids (Ala–Lys) between Q-1666 and P-1667. N-TAF1- and C-TAF1-synthetic peptides were chemically conjugated to the carrier protein keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA), respectively. The KLH-conjugated N-TAF1-peptide was used to produce mouse mAbs against N-TAF1 by hybridoma technology. Among four positive hybridoma cell lines identified by screening and purified by limiting dilution and single-cell cloning, we chose mAb-3A11F for this study.

Dot blot assay

Peptide samples were spotted onto nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA). The air-dried membranes were then blocked with 5% skim milk in 0.01 M Tris-buffered saline containing 0.1% Tween 20 and were incubated overnight at room temperature with anti-N-TAF1 antibody mAb-3A11F (0.2 μg/ml). We detected the bound antibodies using the Elite ABC kit (Vector Laboratory, Burlingame, CA, USA) with diaminobenzidine (0.6 mg/ml), H_2O_2 (0.001%), and $(NH_4)_2Ni(SO4)_2 \cdot 6H_2O$ (2.5 mg/ml).

Western blot assay

Adult Sprague-Dawley rats (250–300 g, 63–70 days old; Nihon SLC Co., Shizuoka, Japan) were used. The animals were deeply anaesthetized with a lethal i.p. dose of pentobarbital (Sigma-