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Research article

Prion-like transmission of α-synuclein pathology in the context of an NFL null background

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ABSTRACT

Neurofilaments are a major component of the axonal cytoskeleton in neurons and have been implicated in a number of neurodegenerative diseases due to their presence within characteristic pathological inclusions. Their contributions to these diseases are not yet fully understood, but previous studies investigated the effects of ablating the obligate subunit of neurofilaments, low molecular mass neurofilament subunit (NFL), on disease phenotypes in transgenic mouse models of Alzheimer's disease and tauopathy. Here, we tested the effects of ablating NFL in α-synuclein M83 transgenic mice expressing the human pathogenic A53T mutation, by breeding them onto an NFL null background. The induction and spread of α-synuclein inclusion pathology was triggered by the injection of preformed α-synuclein fibrils into the gastrocnemius muscle or hippocampus in M83 versus M83/NFL null mice. We observed no difference in the post-injection time to motor-impairment and paralysis endpoint or amount and distribution of α-synuclein inclusion pathology in the muscle injected M83 and M83/NFL null mice. Hippocampal injected M83/NFL null mice displayed subtle region-specific differences in the amount of α-synuclein inclusions however, pathology was observed in the same regions as the M83 mice. Overall, we observed only minor differences in the induction and transmission of α-synuclein pathology in these induced models of synucleinopathy in the presence or absence of NFL. This suggests that NFL and neurofilaments do not play a major role in influencing the induction and transmission of α-synuclein aggregation.

1. Introduction

Neurofilaments (NFs) are the major type of intermediate filament that constitute the axonal cytoskeleton in neurons of both the central and peripheral nervous systems [1,2]. NFs provide structural support and scaffolding for organelle organization in addition to maintaining axon caliber [1–3]. NFs are composed of three proteins: the light (NFL), medium (NFM) and heavy (NFH) molecular mass subunits [1,2]. NFL is the obligate subunit of NFs forming either homopolymers, or heteropolymers with NFM or NFH, whereas NFM and NFH are not able to self-assemble and polymerize into mature filaments [4,5]. Despite the neuronal cytoskeletal importance of NFs, both the naturally occurring Japanese quail (quiver), which has a nonsense mutation in the NFL gene (*NEFL*), and mice genetically engineered to ablate the *NEFL* gene are viable [6–8]. Quiver quail display reduced axonal diameter and conduction velocity associated with intermittent quivering [7,9]. On the other hand, NFL null mice do not display any overt neurological phenotype, perhaps due to a compensatory increase in tubulin expression however, they display reduced axonal diameter and impaired sprouting following injury [8].

NFs are known to accumulate into inclusions in a number of neurodegenerative diseases including neurofilament inclusion disease (NFID) [10–14], amyotrophic lateral sclerosis (ALS) [15,16], neuroaxonal dystrophy [17–19], Charcot Marie Tooth (CMT) disease [20], and

Abbreviations: Aβ, amyloid β; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; αS, α-synuclein; BCA, bicinchoninic acid; BSA, bovine serum albumin; CMT, Charcot Marie Tooth; DAB, 3,3′ diaminobenzidine; *E. coli*, *Escherichia coli*; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; K114, (trans, trans)-1-bromo-2, 5-bis-(4-hydroxy)styrylbenzene; M83, A53T human αS transgenic mice; *NEFH*, heavy molecular mass neurofilament subunit gene; *NEFL*, low molecular mass neurofilament subunit gene; NF, neurofilament; NFH, heavy molecular mass neurofilament subunit; NFID, neurofilament inclusion disease; NFL, low molecular mass neurofilament subunit; NFM, medium molecular mass neurofilament subunit; NFT, neurofibrillary tangle; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PD, Parkinson's disease; pSer129, phosphorylated serine 129; SDS, sodium dodecyl sulfate; *SNCA*, α-synuclein gene; T44, human tau transgenic mice; TBS, Tris buffered saline

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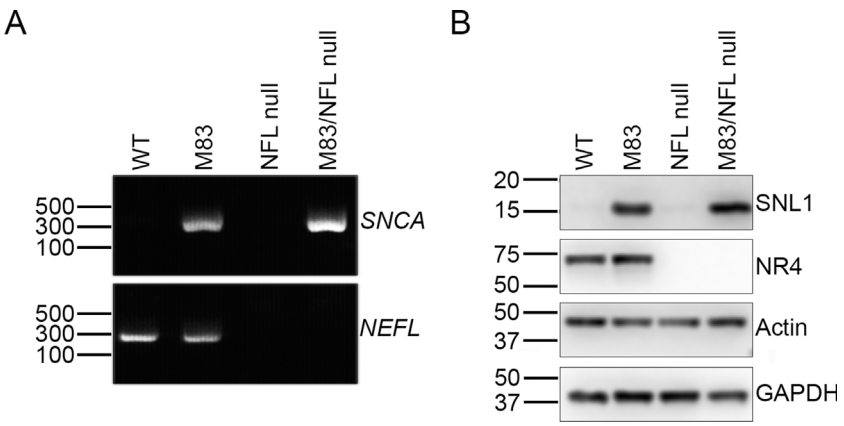

Fig. 1. Generation of M83 transgenic mice on an NFL null background. (A) Genotyping analyses to confirm the presence of the *SNCA* transgene and absence of *NEFL*. Sizes are in base pairs. (B) Western blot analysis of total protein lysates from spinal cord of wild-type (WT), M83, NFL null and M83/NFL null mice. Antibody NR4 shows the presence/absence of NFL. Antibody SNL1 shows the overexpression of αS. GAPDH and β-actin are shown as loading controls. Molecular weights are in kDa.

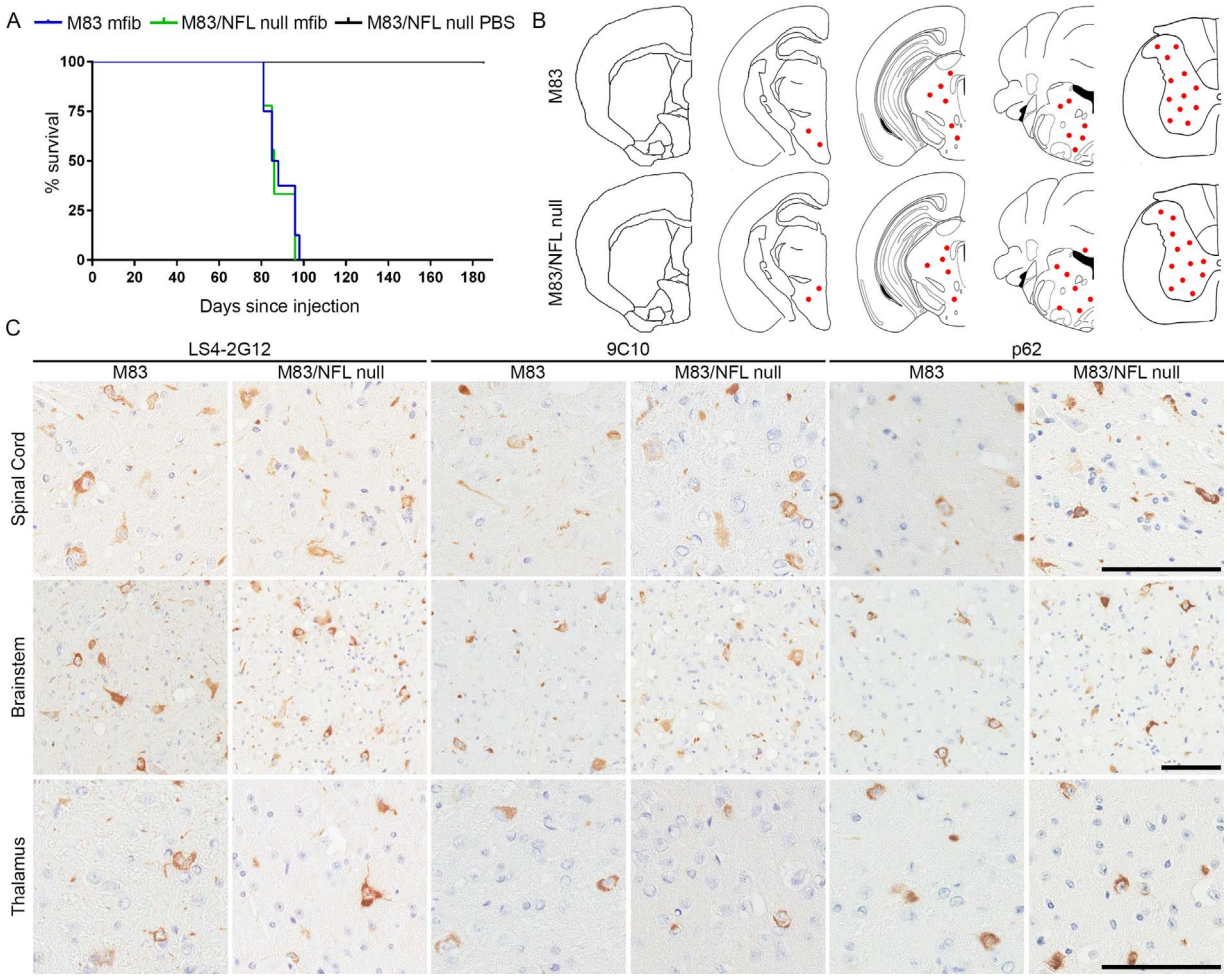

Fig. 2. Assessment of motor impairment and αS inclusion pathology of M83/NFL null mice versus M83 mice following intramuscular injection of αS fibrils. (A) Survival curve of M83 and M83/NFL null mice following intramuscular injection of mouse αS fibrils (n = 7 and 9, respectively) or PBS (n = 10; M83/NFL null mice only). Curves of mouse αS fibril injected mice are not significantly different (Log-rank test; p = 0.662). (B) Distribution maps of LS4-2G12 immunopositive αS pathology in mouse αS fibril muscle injected M83 and M83/NFL null mice. (C) Representative images of immunohistochemistry staining of αS pathology in the spinal cord, brainstem and thalamus of mouse αS fibril muscle injected M83 and M83/NFL null mice. LS4-2G12 detects pSer129 αS, 9C10 detects the N-terminus of αS and p62 is a general inclusion marker. Scale bars = 100 μm.

within Lewy bodies of Parkinson's disease (PD) [21–24] and neurofibrillary tangles (NFTs) of Alzheimer's disease (AD) [25,26]. Whether NFs generally play a causative role in disease, or if they accumulate solely as a result of neuronal impairment or proteostatic imbalance is unknown in most cases. However, in some cases mutations in the *NEFL* and *NEFH* genes can directly cause CMT subtype 2E/1F [2,27–29] or ALS, respectively [2,30,31].

The involvement of NFs in neurodegeneration is an active area of research and has been investigated by breeding transgenic mouse models of AD and tauopathy onto an NFL null background in order to assess the contribution of NFs to the pathogenesis of disease in these particular models [32,33]. Ishihara et al. observed delayed development of the typical behavioral phenotype and reduced pathology in mice transgenic for human tau (T44 mice) [34] when they lacked NFs compared to T44 mice with NFs [33] suggesting that NFs, at least in part, contribute to the onset of tauopathy in these mice. However,