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Protein homeostasis gene dysregulation in pretangle-bearing nucleus basalis neurons during the progression of Alzheimer's disease

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ABSTRACT

Conformational phosphorylation and cleavage events drive the tau protein from a soluble, monomeric state to a relatively insoluble, polymeric state that precipitates the formation of neurofibrillary tangles (NFTs) in projection neurons in Alzheimer's disease (AD), including the magnocellular perikarya located in the nucleus basalis of Meynert (NBM) complex of the basal forebrain. Whether these structural changes in the tau protein are associated with pathogenic changes at the molecular and cellular level remains undetermined during the onset of AD. Here, we examined alterations in gene expression within individual NBM neurons immunostained for pS422, an early tau phosphorylation event, or dual labeled for pS422 and TauC3, a later stage tau neoepitope, from tissue obtained postmortem from subjects who died with an antemortem clinical diagnosis of no cognitive impairment, mild cognitive impairment, or mild/moderate AD. Specifically, pS422-positive pretangles displayed an upregulation of select gene transcripts subserving protein quality control. On the other hand, late-stage TauC3-positive NFTs exhibited upregulation of messenger RNAs involved in protein degradation but also cell survival. Taken together, these results suggest that molecular pathways regulating protein homeostasis are altered during the evolution of NFT pathology in the NBM. These changes likely contribute to the disruption of protein turnover and neuronal survival of these vulnerable NBM neurons during the progression of AD.

1. Introduction

Cholinergic neurons located within the region of the substantia innominata termed the nucleus basalis of Meynert (NBM) degenerate early in Alzheimer's disease (AD) (Giannakopoulos et al., 1995; Mesulam et al., 2004; Mufson et al., 2000, 2002; Price et al., 1991). These neurons provide the primary source of acetylcholine to the entire cortical mantle (Mesulam and Geula, 1988; Mesulam et al., 1983) and their degeneration correlates with deficits of memory and attention in AD patients (Bierer et al., 2002; Pappas et al., 2000). Several lines of evidence suggest that cholinergic NBM cytopathology begins before the onset of mild cognitive impairment (MCI), a putative prodromal stage of AD. For example, stereologic analysis revealed that NBM neurons display a phenotypic downregulation of the trkA cognate nerve growth factor receptor (Ginsberg et al., 2006; Mufson et al., 2000) and the $p75^{NTR}$ pan-neurotrophin receptor (Mufson et al., 2002) in the MCI brain (Counts and Mufson, 2005; Mufson and Kordower, 1997, 1999).

NBM neurons are exquisitely prone to neurofibrillary tangle (NFT) cytopathology (Candy et al., 1983), which correlates with tau pathology within their cortical projection fields in AD (Geula et al., 1998; Giannakopoulos et al., 1995; Mesulam et al., 2004; Mufson et al., 2000, 2002; Price et al., 1991). NFTs are primarily composed

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of abnormally phosphorylated aggregates of the microtubule-associated protein tau (Grundke-Iqbal et al., 2004; Lee et al., 1991; Mesulam and Geula, 1988; Mesulam et al., 1983; Wood et al., 1986). Biochemical and neuropathologic studies indicate that conformational changes in the tau protein drive this molecule from the soluble, microtubule-bound state to the relatively insoluble polymeric form that precipitates NFT formation within NBM neurons in MCI and AD (Bierer et al., 2002; Garcia-Sierra et al., 2003; Guillozet-Bongaarts et al., 2005; Mesulam et al., 2004; Pappas et al., 2000; Vana et al., 2011). Antibodies that label the early stages of NFT formation, such as AT8, Alz-50, and pS422, can be detected in NBM neurons in the MCI brain (Ginsberg et al., 2006; Mesulam et al., 2004; Mufson et al., 2000; Perez et al., 2012; Vana et al., 2011). Interestingly, the appearance of pS422-immunoreactivity in NBM neurons is a stronger correlate of cognitive decline than later stage tau epitopes (Mufson et al., 2002; Vana et al., 2011), suggesting that the appearance of discrete tau epitopes related to NFT evolution paces the cascade of pathophysiological events leading to NBM neuronal and cortical axonal disconnection. However, whether progressive stages of NFT formation are associated with differential gene expression and cellular pathogenic alterations within NBM neurons during the progression of AD remains unknown.

In the present study, we quantified gene expression patterns of individual NBM neurons singly immunostained for the pS422 tau epitope, an early phosphorylation event preceding C-terminal truncation of tau at D421, or dual labeled for pS422 and TauC3, a later stage tau neoepitope revealed by tau truncation at D421 (Counts and Mufson, 2005; Guillozet-Bongaarts et al., 2006; Mufson and Kordower, 1997, 1999). Individual NBM neurons were microdissected from tissue sections obtained postmortem from subjects who died with an antemortem clinical diagnosis of no cognitive impairment (NCI), MCI, or AD followed by custom-designed microarray analysis. Our data show that the dysregulation of select genes regulating protein modification, quality control, and turnover are differentially associated with the appearance of the pS422 and TauC3 epitopes, suggesting that these molecular pathways may be involved in early pathogenic mechanisms underlying selective neuronal vulnerability during the progression of AD.

2. Methods

2.1. Subjects

Custom-designed microarray analysis of single NBM neurons was performed using tissue obtained postmortem from 28 participates in the Rush Religious Orders Study (Bennett et al., 2002; Candy et al., 1983). Demographic, clinical, and neuropathologic characteristics of the subjects are summarized in Table 1. Details of cognitive evaluations and diagnostic criteria have been extensively published (Bennett et al., 2002; Counts et al., 2006; Mufson et al., 1999; Perez et al., 2015). Briefly, a team of investigators performed annual neuropsychological performance testing including the Mini-Mental State Examination and 19 additional neuropsychological tests referable to 5 cognitive domains: orientation, attention, memory, language, and perception. A global cognitive score, consisting of a composite z-score calculated from this test battery, was determined for each

Table 1
Clinical, demographic, and neuropathologic characteristics by diagnosis category

	Clinical diagnosis			*p* value	Pairwise comparison
	NCI (N=10)	MCI (N=10)	AD (N=8)		
Age (y) at death				0.6[a]	–
Mean ± SD	84.6 ± 4.3	85.4 ± 3.9	84.7 ± 5.0		
(Range)	(78–92)	(79–91)	(76–88)		
Number (%) of males	5 (50%)	5 (50%)	3 (38%)	0.5[b]	–
Years of education				0.2[a]	–
Mean ± SD	18.7 ± 1.6	17.5 ± 4.3	19.1 ± 3.5		
(Range)	(16–21)	(15–25)	(16–24)		
Number (%) with ApoE ε4 allele	1 (10%)	3 (33%)	4 (50%)	0.007[b]	NCI < AD
MMSE				<0.001[a]	(NCI, MCI) > AD
Mean ± SD	27.8 ± 1.6	27.4 ± 2.7	22.1 ± 5.3		
(Range)	(26–30)	(22–30)	(15–26)		
Global cognitive score				<0.0001[a]	NCI > MCI > AD
Mean ± SD	0.04 ± 0.3	−0.06 ± 0.3	−1.3 ± 0.4		
(Range)	(−0.4 to 0.4)	(−1.2 to 0.2)	(−2.0 to 0.9)		
Postmortem interval (h)				0.5[a]	–
Mean ± SD	5.9 ± 2.5	6.0 ± 2.6	5.5 ± 4.0		
(Range)	(3.3–9.0)	(2.0–10.0)	(2.5–12.0)		
Distribution of Braak scores				0.004[a]	NCI < (MCI, AD)
0	0	0	0		
I/II	4	2	1		
III/IV	6	5	2		
V/VI	0	3	5		
NIA-Reagan diagnosis (likelihood of AD)				0.002[a]	NCI < (MCI, AD)
No AD	0	0	0		
Low	6	3	1		
Intermediate	3	5	2		
High	1	2	5		
CERAD diagnosis				0.01[a]	(NCI, MCI) < AD
No AD	3	3	0		
Possible	4	3	1		
Probable	3	3	3		
Definite	1	1	4		

Key: AD, Alzheimer's disease; ApoE, apolipoprotein E; CERAD, Consortium to Establish a Registry for Alzheimer's disease; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NCI, no cognitive impairment; SD, standard deviation.
[a] Kruskal-Wallis test, with Bonferroni correction for multiple comparisons.
[b] Fisher's exact test, with Bonferroni correction for multiple comparisons.