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Probing the correlation of neuronal loss, neurofibrillary tangles, and cell death markers across the Alzheimer's disease Braak stages: a quantitative study in humans

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ABSTRACT

Clarifying the mechanisms connecting neurofibrillary tangle (NFT) neurotoxicity to neuronal dysfunction in humans is likely to be pivotal for developing effective treatments for Alzheimer's disease (AD). To model the temporal progression of AD in humans, we used a collection of brains with controls and individuals from each Braak stage to quantitatively investigate the correlation between intraneuronal caspase activation or macroautophagy markers, NFT burden, and neuronal loss, in the dorsal raphe nucleus and locus coeruleus, the earliest vulnerable areas to NFT accumulation. We fit linear regressions with each count as outcomes, with Braak score and age as the predictors. In progressive Braak stages, intraneuronal active caspase-6 positivity increases both alone and overlapping with NFTs. Likewise, the proportion of NFT-bearing neurons showing autophagosomes increases. Overall, caspases may be involved in upstream cascades in AD and are associated with higher NFTs. Macroautophagy changes correlate with increasing NFT burden from early AD stages.

1. Introduction

Alzheimer's disease (AD) features positive (β-amyloid neuritic plaques and phosphorylated-tau neurofibrillary tangles [NFTs]) and negative (neuronal and synaptic loss) lesions (Duyckaerts et al., 2009). Since the late 1980s, when virtually every autosomal dominant AD cases have been linked to mutations in genes involved with amyloid precursor protein processing, the conventional view on AD pathogenesis places β-amyloid deposition as a central etiological event driving a cascade of pathological events resulting in neuronal loss (Hardy, 2017). However, the massive failure of clinical trials focusing on modulating the amyloid cascade put in check the amyloid hypothesis (Korczyn, 2012; Ricciarelli and Fedele, 2017).

Although AD-related neuropathogenic mechanisms remain elusive and disease-modifying treatments are still unavailable, numerous independent studies demonstrated that neuronal loss and synaptic loss are the best predictors of cognitive decline (Andrade-Moraes et al., 2013; Arendt, 2009; Coleman et al., 2004;

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DeKosky and Scheff, 1990; Giannakopoulos et al., 2009; Terry et al., 1991); therefore, interventions targeting pathways involved in AD-related neuronal loss are likely to be critical.

Interestingly, autopsy and molecular imaging studies failed to demonstrate a good correlation between distribution and burden of β-amyloid plaques and cognitive scores. On the other hand, the spread of NFTs through neuronal networks, best represented by Braak staging system (Braak and Braak [BB]) (Braak and Braak, 1991), has a strong correlation to neuronal loss and cognitive decline (Giannakopoulos et al., 2009; Suemoto et al., 2017). The BB system that initially included cortical areas only (BB I–VI), and revisited in 2011 to include brainstem structures in which NFT formation precedes cortical NFTs (BB 0 a–c) (Braak et al., 2011; Grinberg et al., 2009), is highly reproducible both in autopsy and in longitudinal tau positron emission tomography imaging studies (Scholl et al., 2016), suggesting that NFT formation is involved in processes culminating in neuronal death (Gomez-Isla et al., 1997; Iqbal et al., 2009). Thus, clarifying the mechanisms connecting NFT neurotoxicity to neuronal dysfunction is likely to be pivotal for developing effective treatments.

Among the cell death pathways linked to NFT formation, caspase (Casp)-dependent and autophagy pathways have been implicated in AD pathophysiology (Cotman and Su, 1996; Cotman et al., 2005; de Calignon et al., 2010; Dickson, 2004; Guo et al., 2004; LeBlanc et al., 2014; Martinez-Vicente and Cuervo, 2007; Piras et al., 2016; Rohn and Head, 2008). In experimental models of AD, active caspases (aCasp), including active caspase-6 (aCasp-6), are the best inductors of neuronal cell death (Zhang et al., 2000) and are also linked to upstream events leading to the formation of NFTs and β-amyloid plaques (Albrecht et al., 2007; de Calignon et al., 2010; Hyman and Yuan, 2012; Murray and Renslo, 2013). Specifically, by truncating tau, active effector Casp can create cleaved-tau species that are prone to aggregation and toxicity (de Calignon et al., 2010; LeBlanc, 2005). In fact, higher levels of Casp-cleaved tau in the cerebrospinal fluid correlate with AD severity (Ramcharitar et al., 2013). For a thorough review of Casp and their role in neurodegenerative diseases, please see Graham et al. (2011), LeBlanc (2013) and Shalini et al. (2015). Autophagy, a highly regulated process responsible for the breakdown of misfolded or aggregated proteins in healthy cells, becomes dysfunctional in AD models and fails to degrade toxic abnormal tau species potentially contributing to neuronal death (Martinez-Vicente and Cuervo, 2007; Nixon, 2013; Nixon et al., 2005; Piras et al., 2016; Vilchez et al., 2014; Wong and Cuervo, 2010).

However, despite this large body of literature derived from experimental models, attempts to validate these findings in human tissue were mostly restricted to semi-quantitative or qualitative studies comparing controls versus severe AD, and little is known about the co-occurrence of markers of specific cell death pathways with NFTs or with neuronal loss or at which point of AD temporal progression such pathways are activated.

Hypothesizing that changes in Casp-dependent and autophagic pathways occur from early AD stages and correlated with increased tau burden along AD progression in humans, we conducting a study using double-stained immunofluorescence and quantitative methods to investigate how early in AD progression Casp-6 and autophagy are activated, and their relationship to NFT burden and neuronal loss along AD neuropathological progression. This study innovates by focusing on the dorsal raphe nucleus (DRN), a serotonin-producing nucleus in the midbrain, and locus coeruleus (LC), a noradrenergic nucleus located in the pons (Fig. 1), which show the earliest vulnerability to accumulated tau cytoskeletal pathology in AD, before any cortical areas (Ehrenberg et al., 2017; Grinberg et al., 2009; Grudzien et al., 2007; Stratmann et al., 2015; Theofilas et al., 2017; Tomlinson et al., 1981). These nuclei degenerate progressively with AD increasing severity, thus representing a crucial intervention target in early AD stages when the neurons are still viable. This study employs a unique postmortem brainstem collection comprising controls (BB 0), early (BB stages I–II), intermediate (BB III–IV), and advanced (BB V–VI) AD (Braak and Braak, 1991). By comparing controls and individuals at well-defined progressive AD stages, our study design minimizes the inherent cross-sectional and descriptive nature of postmortem human studies by modeling the chain of events associated with AD progression that enables verifying the translational relevance of results derived from experimental models, identification of relevant therapeutic targets, and generation of testable hypothesis.

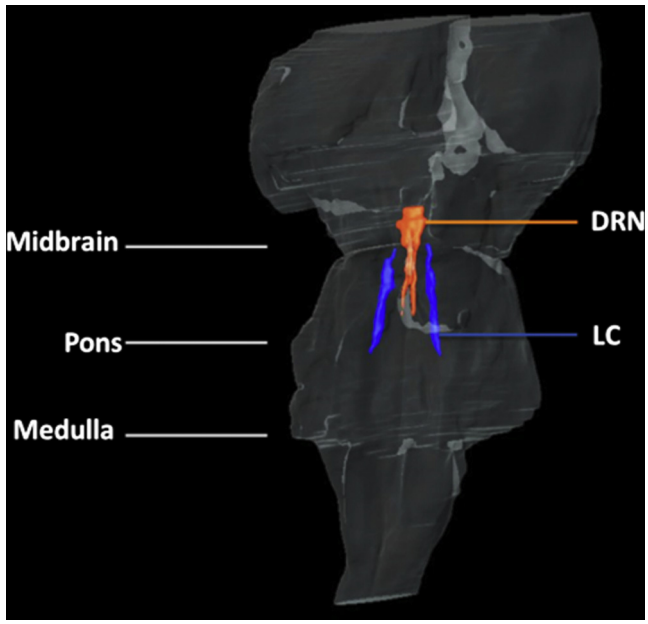

Fig. 1. Three-dimensional volume reconstruction of the LC and DRN of the human brainstem. Serial histological sections of the brainstem were used to reconstruct the volume of LC (blue) and DRN (red) using advanced graphics software. The transparent surface represents the brainstem boundaries. The 2 aminergic nuclei are one of the earliest brain regions affected by tau pathology in individuals with AD. Abbreviations: AD, Alzheimer's disease; DRN, dorsal raphe nucleus; LC, locus coeruleus. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2. Materials and methods

2.1. Participants

The 24 cases included in this study (Table 1) were sourced from the Brain Bank of the Brazilian Aging Brain Study Group (BBBABSG) (Ferretti et al., 2010; Grinberg et al., 2007) and the Neurodegenerative Disease Brain Bank from the University of California, San Francisco (UCSF). All cases represented sporadic AD. The institutional review boards of both participating institutions approved this study. The BBBABSG is supplied by the São Paulo City Autopsy Service that performs approximately 13,000 autopsies per year. The selection criteria for this study included the absence of non–AD-related neurodegenerative pathology or significant cerebrovascular lesions and availability of an intact brainstem. Subjects were excluded if they had a history of seizures, other neurological diseases, a primary Axis I psychiatric diagnosis (major mood disorders, psychotic disorders, and dissociative disorders), or gross