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IMMUNOLOCALIZATION OF HUMAN ALPHA-SYNUCLEIN IN THE THY1-ASYN (''LINE 61'') TRANSGENIC MOUSE LINE

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Abstract—Alpha-synuclein (a-syn) is the major component of the intracytoplasmic inclusions known as Lewy bodies (LB), which constitute the hallmark of Parkinson's disease (PD). Mice overexpressing human a-syn under the Thy-1 promoter (ASO) show slow neurodegeneration and some behavioral deficits similar to those seen in human PD patients. Here, we describe a whole-brain distribution of human a-syn in adult ASO mice. We find that the human a-syn is ubiquitously distributed in the brain including the cerebellar cortex, but the intensity and sub-cellular localization of the staining differed in the various regions of the central nervous system. Among particular CNS areas with human a-syn immunoreactivity, we describe staining patterns in the olfactory bulb, cortex, hippocampus, thalamic region, brainstem nuclei and cerebellar cortex. This immunohistochemical study provides an anatomical map of the human a-syn distribution in ASO mice. Our data show that human a-syn, although not present at levels that were detectable by immunostaining in dopaminergic neurons of substantia nigra or noradrenergic neurons of locus coeruleus, was highly expressed in other PD relevant regions of the brain in different neuronal subtypes. These data will help to relate a-syn expression to the phenotypic manifestations observed in this widely used mouse line.

Key words: alpha-synuclein, transgenic mouse, Thy-1 promoter, immunohistochemistry.

INTRODUCTION

Parkinson's disease (PD) is mainly characterized by the loss of dopaminergic (DAergic) neurons in the substantia nigra (SN) and the appearance of intracellular inclusions throughout the brain known as Lewy bodies (LBs) and Lewy neurites (LNs), which are mainly composed of alpha-synuclein (a-syn) (Spillantini et al., 1997). Though the precise cause of PD is unknown, a large body of evidence implicates a-syn in the

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Abbreviations: 3Cb, 3rd cerebellar lobule; 3N, oculomotor nucleus; 3V, 3rd ventricle; 4V, 4th ventricle; 5HT, 5hydroxytryptamine (serotonin); AOL, anterior olfactory nucleus, lateral part; Aq, aqueduct (Sylvius); ASO, adult Thy-1 mice; a-syn, alpha-synuclein; BLA, basolateral amigdaloid nucleus, anterior part; Cg, cingulate cortex; Cl, claustrum; Cp, cerebral peduncle, basal part; CPu, caudate putamen (striatum); DAB, 3,3-diaminobenzidine-tetrahydrochloride; DG, dentate gyrus; DLG, dorsal lateral geniculate nucleus; DM, dorsomedial hypothalamic nucleus; DR, dorsal raphe nucleus; ECIC, external cortex of inferior colliculus; EPi, external plexiform layer of the olfactory bulb; EPiA, external plexiform layer of the accessory olfactory bulb; fmi, forceps minor of the corpus callosum; fr, fasciculus retroflexus; GI, glomerular layer of the olfactory bulb; Gi, gigantocellular reticular nucleus; Gr, gracile nucleus; GrDG, granular layer of the dentate gyrus; GrO, granular cell layer of the olfactory bulb; Hb, habenular complex; ic, internal capsule; IgG, immunoglobulin G; IO, inferior olive; IPCAL, interstitial nucleus of the posterior limb of the anterior commissure, lateral part; IPCAM, interstitial nucleus of the posterior limb of the anterior commissure, medial part; LB, Lewy bodies; LC, locus coeruleus; LDVL, laterodorsal thalamic nucleus, ventral part; LGP, lateral globus pallidus; LN, Lewy neurite; LS, lateral septal nucleus; M1, primary motor cortex; Me5, mesencephalic trigeminal nucleus; MGV, medial geniculate nucleus, ventral part; Mi, mitral cell layer of the olfactory bulb; mlf, medial longitudinal fasciculus; MM, medial mammillary nucleus, medial part; MO, medial orbital cortex; Mo5, motor trigeminal nucleus; Mol, molecular layer of the dentate gyrus; MS, medial septal nucleus; NaPB, sodium phosphate buffer; NMS, non-motoric symptoms; ON, olfactory nerve layer; Op, optic nerve layer of the superior colliculus; Or, Oriens layer of the hippocampus; PAG, periaqueductal gray; PBS, phosphate buffer; PCRt, parvicellular reticular nucleus; PD, Parkinson's disease; PF, parafascicular thalamic nucleus; Pir, piriform cortex; Pn, pontine nuclei; PnC, pontine reticular nucleus, caudal part; PoDG, polymorph layer of the dentate gyrus; PV, paraventricular thalamic nucleus; Py, pyramidal cell layer of the hippocampus; py, pyramidal tract; Rad, stratum radiatum of the hippocampus; RMC, red nucleus, magnocellular part; Rt, reticular thalamic nucleus; RtTg, reticulotegmental nucleus of the pons; S1HL, primary somatosensory cortex, hindlimb region; S2, secondary somatosensory cortex; SI, substantia innominata; Sim, simple lobule; SN, substantia nigra; SNC, substantia nigra, pars compacta; SNL, substantia nigra, lateral part; SNR, substantia nigra, reticular part; Sp5I, spinal trigeminal nucleus; STh, subthalamic nucleus; SuG, superficial gray layer of the superior colliculus; TH, tyrosine hydroxylase; tu, olfactory tubercule; VeCb, vestibulocerebellar nucleus; VGAT, vesicular GABA transporter; VM, ventromedial thalamic nucleus; VMH, ventromedial hypothalamic nucleus; VMHDM, ventromedial hypothalamic nucleus, ventromedial part; VPM, ventral posteromedial thalamic nucleus; VTA, ventral tegmental area; wt, wild-type.

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pathogenesis of sporadic PD as supported by the multiple genome-wide association studies confirming the risk associated with genetic variability in the a-syn gene, SNCA (Winkler et al., 2007; Simon-Sanchez et al., 2009; Klein and Ziegler, 2011). In addition mutations in SNCA cause rare inherited forms of PD (Polymeropoulos et al., 1997; Kruger et al., 1998; Zarranz et al., 2004) and duplication and triplication of the SNCA gene, and therefore the mere overexpression of a-syn is sufficient to cause PD (Chartier-Harlin et al., 2004; Singleton et al., 2003; Ibanez et al., 2004).

Despite the constant presence of a-syn within LBs and LNs, the exact mechanism leading to neurodegeneration remains poorly understood. However, post translational modifications, oligomerization or aggregation of the protein has been shown to play an important role in the disease (Conway et al., 2000; Volles and Lansbury, 2003; Lee et al., 2011). Although the debate of what are the exact toxic a-syn species (oligomers vs. fibrils; phosphorylated or nitrated) is ongoing, there is a consensus that the pathological accumulation of a-syn in particular brain areas is a sign of neurodegeneration. Most of the studies on PD have specifically focused their attention on the SN and the striatum, but imaging studies have shown other areas affected in PD, and post mortem analysis indeed revealed LBs and LNs in non-dopaminergic neuronal subpopulations (Braak and Braak, 2000; Braak et al., 2004). Certainly cell loss is also found in the locus coeruleus (LC) (catecholaminergic), dorsal nucleus of the vagus, raphe nucleus (serotoninergic) and nucleus basalis of Meynert (cholinergic) (Dauer and Przedborski, 2003). It is therefore likely that the degeneration of these neurons accounts for the non-motoric symptoms (NMS) observed in patients in parallel or preceding the typical movement defects.

Abnormal accumulation of a-syn in the brain is a common feature of PD that has been modeled in mice overexpressing wild-type (wt) or mutated human a-syn under different promoters such as tyrosine hydroxylase (TH), prion, PDGF beta and Thy-1 (Chesselet and Richter, 2011). The majority of a-syn transgenic mouse lines present a progressive neurodegeneration in different brain regions and/or behavioral deficits associated to these affected areas; although no true LB has ever been observed in mice and few lines exhibit loss of nigrostriatal DA neurons (Fleming et al., 2005; Magen and Chesselet, 2010). However, despite the inability of most of these lines to show dopaminergic neurodegeneration, they certainly provide a tool to understand the physiological function of a-syn, its toxicity and the implication of other neuronal populations in PD. Among these lines the a-syn transgenic mice under the Thy-1 promoter, originally generated by Masliah et al. (Rockenstein et al., 2002) has been widely studied and characterized both behaviorally and pharmacologically (Fleming et al., 2004, 2006, 2008; Wang et al., 2008; Lam et al., 2011; Wu et al., 2010). This mouse line is reported to present a high level of a-syn expression in neurons, and pathological changes such as proteinase K-resistant a-syn aggregates (Fernagut et al., 2007) phosphorylated Ser129 a-syn (Chesselet et al., 2012) and signs of neuroinflammation (Watson et al., 2012). They exhibit progressive sensorimotor and cognitive deficits and other anomalies such as colonic problems, anxiety and olfactory defects (Fernagut and Chesselet, 2004; Fleming et al., 2004, 2006; Magen et al., 2012). These characteristics are seen in PD patients and demonstrate the relevance of this mouse model to the human disease. However, although several reports regarding this mouse line have shown human a-syn staining in specific areas of the brain (Rockenstein et al., 2002; Fleming et al., 2008; Magen et al., 2012) so far no studies have shown a full detailed localization of the human a-syn in this transgenic animal. Therefore, it has not been possible to fully acquire a picture of the possible role of the different neuronal populations in the changes observed in these animals. For this reason, using several specific antibodies against human a-syn, we have analyzed via a systematic approach the localization of human a-syn within the brain of adult Thy-1 mice (ASO).

EXPERIMENTAL PROCEDURES

Animals and samples collection

The experiments described below were performed using female and male transgenic mice overexpressing human a-syn under the Thy-1 promoter. The animals were generated in a mixed C57BL/6-DBA/2 background as described previously (Rockenstein et al., 2002). Animals were maintained on the mixed C57BL/6-DBA/2 background by breeding mutant females with wt C57BL/6-DBA/2 males. Offspring was genotyped with PCR analysis of tail DNA around postnatal day 10 (P10). The animals were bred and group housed with littermates of the same sex with *ad libitum* access to food and water and exposed to 12-h light/dark cycle according to the rules set by the Ethics Research Committee at Aarhus University, which adhere to the guidelines of the European Union legislation regarding laboratory animals. Eleven animals including ASO mice ($n = 5$) and their littermate wt control ($n = 6$) of 3–4 months were deeply anaesthetized with pentobarbital and perfused intracardially with cold saline and subsequently with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (NaPB) pH 7.4. Brains were removed and post fixed for 2–4 h in the same solution and later transferred to 25% sucrose in 0.1 M NaPB for cryoprotection. Sectioning was performed on a freezing microtome (Microm HM 450, Brock and Michelsen, Birkerød, Denmark). Coronal sections of 40-μm-thick brain slices were collected in antifreeze solution and stored at −20 °C.

Immunohistochemistry

Peroxidase 3,3-diaminobenzidine-tetrahydrochloride (DAB) immunohistochemistry was performed on free floating coronal sections, representing the whole brain, using antibody raised against human a-syn (ab1904 1:5000, immunogen recombinant human a-syn form *E. Coli*, clone 4B12, mouse monoclonal, Abcam, Cambridge, UK; Sig39730 1:2000, immunogen recombinant human a-syn form *E. Coli*, clone 4B12, mouse monoclonal, Covance; ab138501 1:2000, immunogen synthetic peptide epitope 118–123, rabbit polyclonal, Abcam, Cambridge, UK). Sections were