100 YEARS OF LEWY BODIES – WHERE ARE WE NOW?
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New monoclonal antibody 5G4 suitable for specific detection of disease-associated α-synuclein in the human brain

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» INTRODUCTION

α-Synuclein is present in the brain in physiological conditions as a presynaptic protein and it is the major protein associated with Lewy body dementia, Parkinson's disease and multiple system atrophy. Because of its diseaseassociated modifications the aim of our project was development of specifically monoclonal antibodies (mabs) suitable for detection of pathological changes connected with α-synuclein.

» AIMS

Presentation of monoclonal antibody 5G4 which recognizes pathological related epitope of human α-synuclein.

» METHODS

For generation of mabs high specific and sensitive for disease-associated α-synuclein, synthetic peptides containing different amino acid sequences and full length human α-synuclein were used for immunization of mice.

After generation of α-synuclein aggregates, ELISA and immunoblotting were used to test the specificity of antibodies.

Tissue microarray sections originating from different human α-synucleinopathies were used to perform immunostaining in comparison to other commercially available anti-a-synuclein antibodies (4D6, epitope: full length; Signet; 15G7, aa 1161-31; Alexis; KM51 full length; Novocastra; LB509, aa 115-122; Invitrogen; 42/αS, aa 15-123; Transduction Laboratories.

RESULTS

Immunochemistry
Epitope of 5G4 is amyloidogenic determinant of α-synuclein.

Peptide sequences with reactivity of mab 5G4.

spot	name	MW	sequence
211	H22	1053.2	K-T-K-E-G-V-V-H-G-V
212	H23	996.1	K-E-G-V-V-H-G-V-A-T
213	H24	909	G-V-V-H-G-V-A-T-V-A

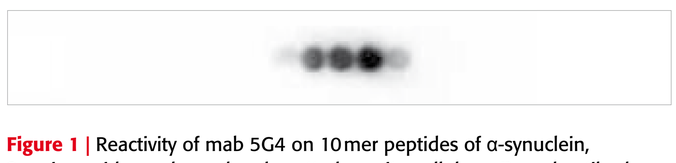

Figure 1 | Reactivity of mab 5G4 on 10mer peptides of α-synuclein, 2 amino acids overlapped and spotted on nitrocellulose. Bound antibody was detected using anti-mouse IgG HRP conjugate (Dianova) followed by staining using SuperSignal West Femto (Pierce). Mab 5G4 showed reactivity to peptides H22-H24 that defined the epitope as sequence (E)GVVHGV(A), amino acids 46-53 of α-synuclein.

Immunochemistry
5G4 can't bind to A53T mutant of α-synuclein.

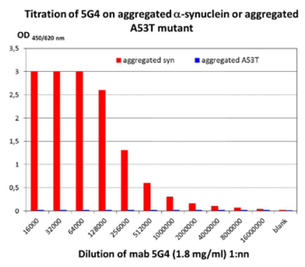

Figure 2 | Reactivity of mab 5G4 to aggregated preparation of human α-synuclein compared to mutant A53T of human α-synuclein, both coated on ELISA plate. Mab 5G4 was diluted 0.1ng/ml up to 0.1 pg/ml and bound antibody was detected using polyclonal anti-mouse-IgG-HRP.

Immunochemistry
5G4 is able to bind aggregated α-synuclein only.

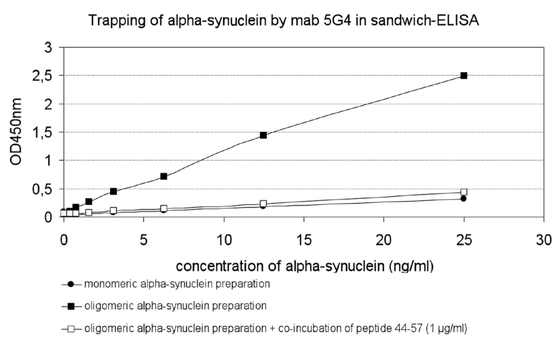

Figure 3 | Trapping of α-synuclein by mab 5G4 from monomeric and aggregated preparation, respectively. Detection of bound antigen was performed using HRP conjugated mab 10D2. Inhibition experiment with peptide containing amino acids 44-57 of α-synuclein is shown using a concentration of 1 µg peptide per ml in co-incubation with the aggregated α-synuclein preparation.

Immunostaining
5G4 reveals only pathological structures without background or synaptophysin-like staining pattern.

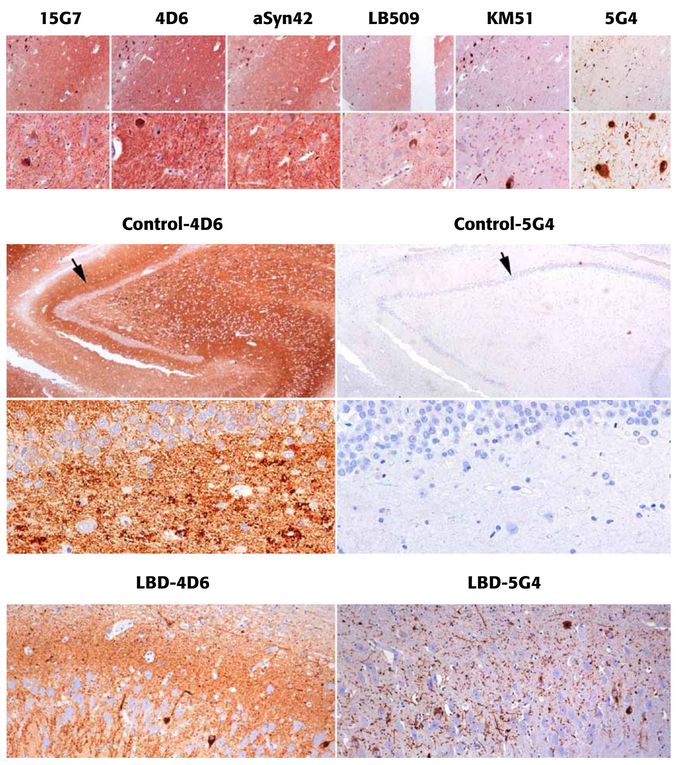

Figure 4 | Except for 5G4 all antibodies showed background or normal synaptic staining ("synaptophysin-like immunoreactivity") in at least one TMA (upper panel). In areas with large synapses, the presence of synaptic staining can be misinterpreted as small aggregates (middle panel, region indicated with arrow enlarged). Normal synaptic staining can disturb interpretation of pathological structures as well (lower panel).

Immunostaining
5G4 is useful in cases with very long formalin fixation.

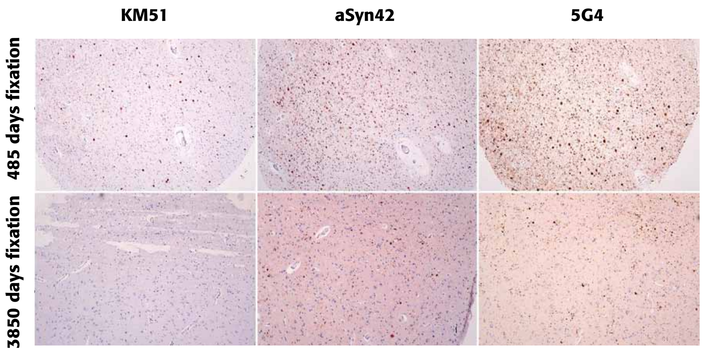

Figure 5 | Immunostaining in TMA-cores with different fixation time.

Immunoblotting
Difference between controls and Lewy body disease

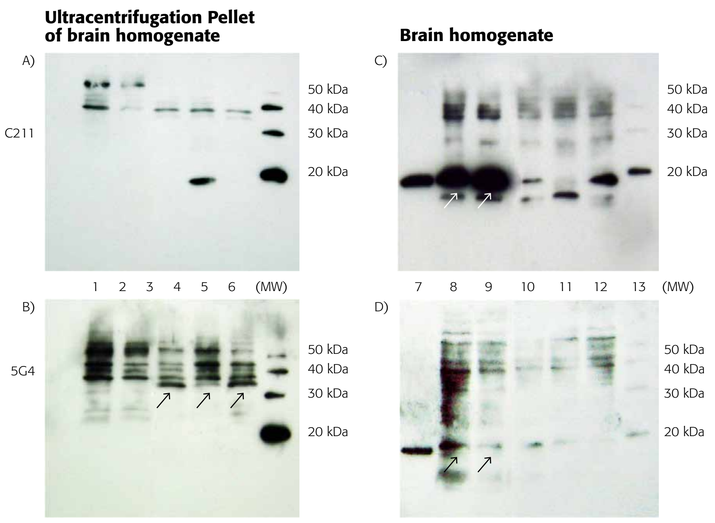

Figure 6 | Western blotting of α-synuclein in brain using two different antibodies: Syn211and 5G4.
Ultracentrifugation pellet of brain homogenates: A) Immunostaining with syn211 did not reveal any significant difference between control cases (lanes 1 and 2) and Lewy-body disease cases (lanes 3,4 and 5). **B)** Immunostaining with 5G4 antibody highlighted the presence of an additional band (black arrows) Around 35 kDa in Lewy-body disease cases. **Total brain homogenates: C)** Monomeric a-synuclein (black arrows) was highly detected in the two control cases (lanes 8 and 9) with Syn211 antibody, whereas it was very weak in the three DLB cases (lane 10,11,12). **D)** Monomeric a-synuclein (black arrows) was faintly detected in the same cases as c) with the 5G4 antibody.

Immunohistochemistry
mAb 5G4 reveals more pathological structures

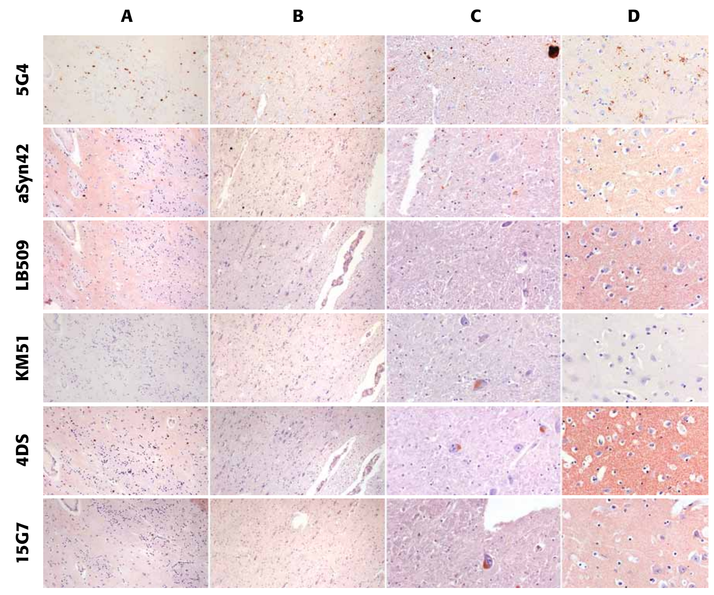

Figure 7 | Immunostaining in TMA-cores with different antibodies (A: MSA; B, C, D: Parkinson`s disease).

CONCLUSION

5G4 antibody seems to be suitable to offer new perspectives for the development of diagnostic assays for detecting disease-associated α-synuclein in biological samples.

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