Optimizing Western Blots for the Detection of Endogenous α-Synuclein in the Enteric Nervous System

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Résumé en anglais	Background:Alpha-synuclein containing inclusions in neurons, the characteristic pathological lesions of Parkinson’s disease (PD), are not limited to the central nervous system, but also affect the enteric nervous system (ENS). This suggests that the ENS offer some potential as a surrogate of central nervous system pathology and that it may represent an original source of biomarkers for PD. However, the usefulness of α-synuclein detection in gastrointestinal biopsies as a biomarker for PD is still unclear, as the different immunohistochemical methods employed to date have led to conflicting results. Objective:Our aim is to propose an optimized immunoblotting method for the detection of endogenous α-synuclein in the healthy ENS that may be used to supplement the immunohistochemical analysis. Methods:Primary culture of rat ENS and homogenates of human small intestine were analyzed by Western Blot using seven different α-synuclein and phospho-α-synuclein antibodies along with two methods that increase α-synuclein retention on blot membranes, namely incubation of the membranes with paraformaldehyde (PFA) or treatment of samples with the crosslinker dithiobis[succinimidylpropionate] (DSP). Results:A moderate improvement in the detection of endogenous enteric α-synuclein was observed following membrane fixation with PFA for only two of the seven antibodies we tested. Immunodetection of total and phosphorylated α-synuclein in the ENS was markedly improved when samples were treated with DSP, regardless of the antibody used. Conclusions:Our results demonstrate that the detection of α-synuclein in the gut by Western Blot can be optimized by using methods for enhanced membrane retention of the protein along with the appropriate antibody. Such an optimized protocol opens the way to the development of novel biomarkers for PD that will enable a quantification of α-synuclein in gastrointestinal biopsies.
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