Proteasome-Directed Camelid Nanobodies Promote the Degradation of a-Synuclein as a Potential Parkinson's Disease Therapeutic

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The purpose of this study was to screen several VHH nanobodies for effective intracellular binding to a-Synuclein (Syn), whose aggregation is associated with Parkinson’s disease. Previously, it was shown that intrabodies fused to a proteasomal targeting motif (PEST), can degrade their intracellular target in both Huntington’s and Parkinson’s diseases. Seven VHH nanobodies were subcloned into constructs that consisted of the nanobody and either no PEST motif (H), a PEST motif (P), or a Scrambled PEST motif (S). The seven PEST motif constructs were screened for their ability to degrade Syn compared to VH14-P, a single domain intrabody that degrades Syn efficiently in neuronal cells. The initial screening revealed that only Syn87-P degraded Syn to a greater extent than VH14-P. Interestingly, all Syn87 constructs degraded wild type Syn and Syn-eGFP in cells transfected with either protein, indicating that Syn87 intrinsically stimulated Syn degradation. Syn87's ability to promote the degradation of Syn was verified by inhibiting the proteasome and autophagy pathways. As expected, Syn87-P was shown to degrade Syn through the proteasome. Syn87-H and Syn87-S were shown to degrade Syn through autophagy. Viability was accessed through flow cytometric analysis. Cells transfected with either GFP or Syn-GFP and either Control, Syn87-P, or VH14P were stained with propidium iodide to label dead cells. Syn87P and VH14P reduced Syn attributed cell death from 22.59% to below GFP control levels 15.78%. PEST fusion to nanobodies offers a rapid method of screening nanobodies for effective binding to antigen in vitro. These in vitro data suggest that Syn87 may be a potential therapeutic for PD, although future in vivo studies are required.

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