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Title: Selective targeting of intracellular misfolded, pathogenic TDP-43 with rationally designed intrabodies

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Background: Cytoplasmically mislocalized aggregates of TDP-43 have been implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD) and limbic-predominant age-related TDP-43 encephalopathy (LATE) through direct toxicity, loss of function of normal TDP-43, induction of misfolding of other neuronal proteins, and prion-like, cell-to-cell propagation of disease. We generated monoclonal antibodies (mAbs) selective for the misfolded form of TDP-43 and derived single chain intrabody constructs to allow for intracellular targeting of pathogenic TDP-43 without interfering with physiological forms of TDP-43 important for normal cell function.

Methods: Monoclonal antibodies (mAbs) were generated by immunization with an unfolded N-terminal domain (NTD) linear epitope predicted to become exposed in cytoplasmically mislocalized aggregates of TDP-43 but otherwise buried in natively folded TDP-43. The selectivity of mAbs for pathogenic, aggregated TDP-43 was assessed by immunocytochemistry (ICC) of HEK293FT cells transfected with mutant TDP-43 (ΔNLS-TDP-43) and by immunohistochemistry (IHC) on patient samples. Single chain (scFv) intrabody constructs were derived from selective antibodies and HEK293FT cells were co-transfected with plasmids encoding intrabodies and misfolding ΔNLS-TDP-43. The selective interaction of expressed intrabodies with cytoplasmic ΔNLS-TDP-43 aggregates was assessed by ICC, and clearance of the aggregates was evaluated by western blot analysis of cell lysates.

Results:
ICC showed mAb reactivity with cytoplasmic aggregates of transfected ΔNLS-TDP-43 but not endogenous wild-type nuclear TDP-43. IHC of ALS and FTLD CNS sections confirmed immunoreactivity specifically with pathogenic TDP-43 in patient samples. Similarly, co-transfection of ΔNLS-TDP-43 and scFv intrabody constructs showed intracellular interaction of intrabodies with misfolded cytoplasmic aggregates of TDP-43 but not with normal, nuclear TDP-43. In agreement with a lack of interference with normal TDP-43, the robust expression of intrabodies did not affect cell viability. Intracellular clearance of the aggregates was observed in western blots of the cell lysates and was enhanced

by the inclusion of a lysosomal targeting signal in the intrabody constructs.

Conclusions: Immunization with an NTD epitope of misfolded TDP-43 gave rise to mAbs selective for pathogenic vs physiologically important forms of TDP-43. Intrabody constructs derived from these mAbs were able to promote clearance of intracellular pathogenic TDP-43 without toxicity to the cells.