Antibody-based therapy to overcome TDP-43 proteinopathy

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Introduction

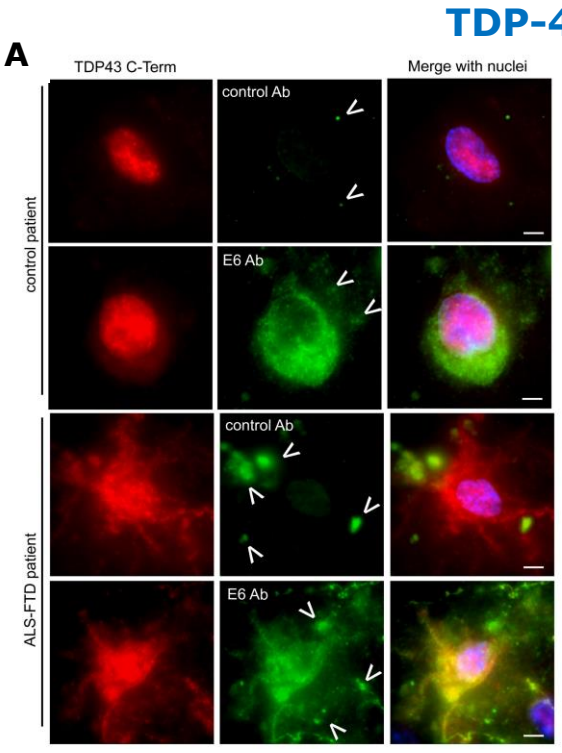
TDP-43 proteinopathy is an event characterized by a consistent cytoplasmic mislocalization and aggregation of the protein TDP-43, and a pathological hallmark of Amyotrophic Lateral Sclerosis (ALS) and FrontoTemporal Lobar Degeneration (FTLD). Different studies highlighted the sensitivity of the RRM1 domain in inducing TDP-43 proteinopathy (Chang et al. 2013; Shodai et al. 2013), and its role in activating the NF-kB pathway (Swarup et al. 2011).

Objectives and methods

To overcome this toxic effect, we developed two antibody-based therapeutic approaches, specifically directed against the RRM1-domain of TDP-43. We generated a monoclonal full-length antibody (E6) and tested its therapeutic efficacy in the TDP-43^{A315T} mouse model (Pozzi et al. 2020). From E6 full-length antibody we also derived a single chain antibody (VH7Vk9) that we virally delivered into two mutant TDP-43 mouse models (Pozzi et al. 2019).

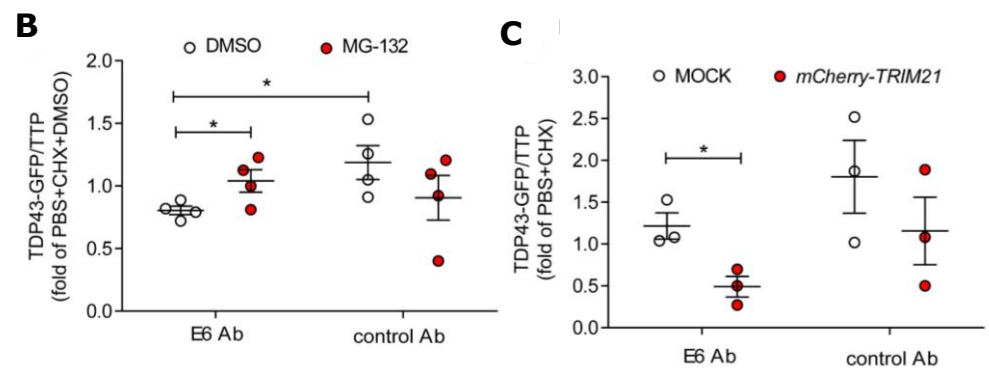
Results

1. E6 specifically recognizes cytoplasmic TDP-43



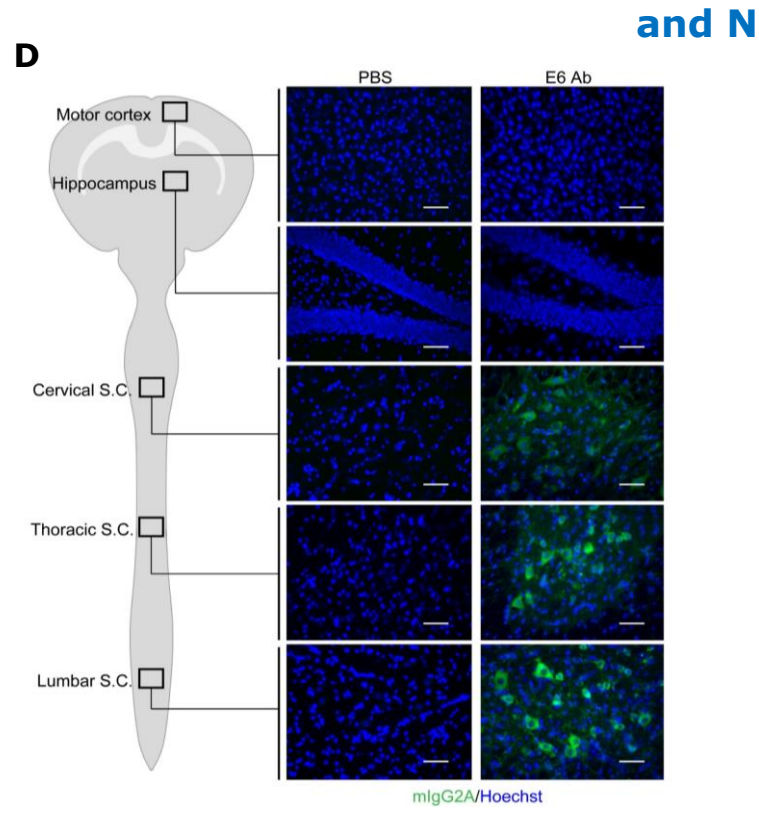
(**A**) E6 Ab labeled cytoplasmic and aggregated TDP-43 in FTLD patient neurons. Prefrontal cortex of a control non-degenerative patient and a FTLD patient. E6 or the isotype control Ab were 488 labelled. Scale bar: 5 µm. Lipofuscin non-specific spots were visible in all channels and are marked as arrowheads.

2. E6 reduces cytoplasmic TDP-43 by TRIM21/proteasome pathway

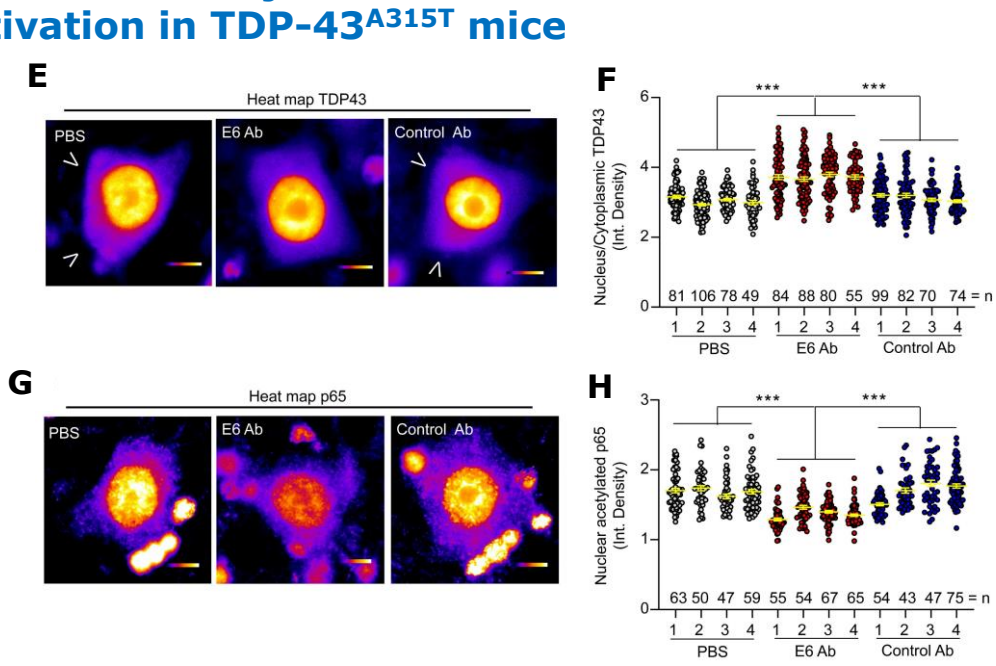


(**B,C**) E6 reduced TDP-43 levels. The pathway is regulated by the proteasome and TRIM21. Western blot quantification of TDP-43 in the cytoplasmic fraction of treated cells normalized on total transferred proteins (TTP). Data are represented as mean ± SEM; *n* = 3-4 independent experiments (dots). **P* < 0.05 by unpaired *t* test analysis.

3. E6 diffuses in spinal cord after repeated intrathecal injections and reduces TDP-43 mislocalisation and NF-kB activation in TDP-43^{A315T} mice

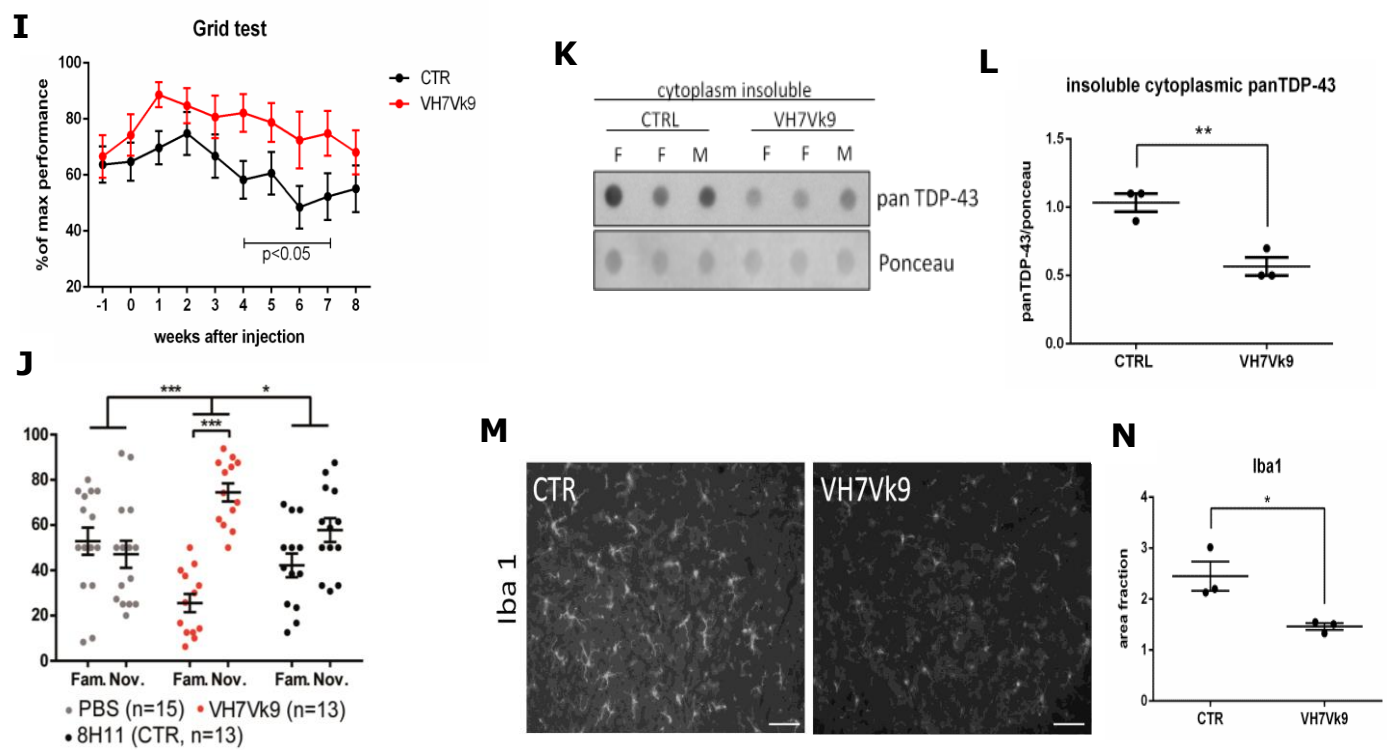


(**D**) E6 antibody (green) merged with nuclei (blue) in different regions of the CNS after 5 weeks of repeated IT injections. Scale bar: 50 µm.



(**E,G**) Representative high-magnification colorimetric heatmap images of TDP-43 (**E**) and p65 (**G**) immunofluorescence. Scale bar: 10 µm. Graph represent quantification of nuclear to cytoplasmic integrated density of TDP-43 signal (**F**) or nuclear integrated density of p65 signal (**H**) in single large neurons (area>250mm^2) of lumbar spinal cord ventral horns. Data are represented as mean ± SEM; number of counted neurons (dots) from 4 independent mice (numbered 1–4) is shown in the graph; 1-way ANOVA, ****P* < 0.0001 by Tukey's multiple comparison test.

4. E6-derived single chain antibody (named VH7Vk9) improves motor and cognitive performances, reduces TDP-43 aggregation and inflammation in TDP-43 mutant mice



TDP-43^{A315T} and ^{G348C} mice (9 months of age) were injected intrathecally with scAAV2/9 expressing VH7Vk9 or CTR ScFv (8H11, anti-GFP). TDP-43^{A315T} mice (n=18), receiving VH7Vk9, showed improvements in motor performances (**I,** grid test), Two-way Anova, **P*<0.05 by Fisher's LSD. TDP-43^{G348C} treated with VH7Vk9 showed improved memory tasks (**J**, novel object recognition), 2-way ANOVA followed by Sidak's test. (**K,L**) VH7Vk9 TDP-43^{A315T} treated mice (n=3) showed decreased levels of insoluble TDP-43 in the lumbar spinal cord, ***P* < 0.01 by unpaired *t* test analysis. Reduced microgliosis (**M**) was observed by quantification (**N**) of Iba1 staining in lumbar spinal cord of VH7Vk9 TDP-43^{A315T} treated mice (n=3), **P* < 0.05 by unpaired *t* test analysis.

Conclusions

We demonstrated for the first time the feasibility and efficacy of two antibody-based approaches against the RRM1-domain of TDP-43 in reducing TDP-43 proteinopathy and rescuing motor and cognitive deficits in ALS/FTLD mouse models.

References

1) Chang C, et al. *FEBS Lett.* 2013;587(6):575–582. **2)** Shodai A et al. *J. Biol. Chem.* 2013;288(21):14886–14905. **3)** Swarup V et al. *J. Exp. Med.* 2011;208(12):2429–2447. **4)** Pozzi S, et al. *J. Clin. Investig. Insight* 2020; **5)** Pozzi S et al. *J. Clin. Invest.* 2019;129(4):1581–1595.