TDP25 aggregation in motor neuron and muscle cells is rescued by chaperone overexpression.

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease involving both upper and lower motor neurons (MNs). As target of MNs, muscle cells role in ALS has always been investigated. ALS can appear as familial or sporadic form, and in the vast majority of patients motor neurons have been observed proteinaceous inclusions containing TDP43. TDP43 inside cytoplasmic aggregates has been found cleaved into C-terminal fragments of 35 and 25 kDa highly aggregation prone. In this work we firstly investigate the aggregation propensity of TDP43 and its ALS-associated fragments TDP35 and TDP25 in both motor neuron like (NSC34) and muscle like cells (C2C12). We found that TDP43 forms physiological oligomers retained in filter retardation assay (FRA) due to the interaction of the N-terminal domain in the nucleus. To correctly visualized TDP25 aggregates we used the NP40 extraction, and we observed the greatest fraction of TDP25 isolated in the NP40 insoluble fraction. Then we studied the degradation of TDPs species, noting that they were mainly degraded *via* proteasome, while autophagy contribution is less important. Targeting the degradation of aggregation-prone species is a promising strategy to counteract ALS and we increase protein degradation by mean of particular chaperones such as Bag1, Bag3 and HspB8. These proteins, in complex with Hsp70, direct cargos alternatively to proteasome (Bag1) or to autophagy (HspB8 and Bag3, in complex). Overexpressing Bag1 we found that both in NSC34 and C2C12 TDP25 aggregation was rescued, due to an increased degradation *via* the proteasome. Similar results were obtained in both models targeting autophagy by overexpressing HspB8 and Bag3.

Concluding, we showed that muscle cells are a site of misfolded protein aggregation as well as MNs. Importantly, we demonstrated that indirectly targeting degradative pathways by overexpressing chaperones could be beneficial against the formation of TDP25 aggregates both in MNs and muscle cells.