Amyotrophic lateral sclerosis related proteins are actively removed by HspB8.

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Amyotrophic lateral sclerosis (ALS) is a motor neuron disease caused by the presence of proteins that loose their proper conformation. TDP43 is one of the most important ALS-related protein. TDP43 has been found mislocalized and aggregated in the cytoplasm of neurons of ALS patients. In ALS the aggregates contain also TDP43 fragments of 35 and 25 kDa.

In these study we analyzed the biochemical behavior of TDP43 and its disease-associated fragments in NSC34 cells. By immunofluorescence we observed that TDP43 fragments had a higher clearance than the full length form, but when unsufficiently removed they form large inclusions in the cytoplasm. We performed an NP-40 extraction and we found that the total amount of NP40 insoluble TDP25 was greater than the soluble fraction; TDP43 or TDP35 protein levels in the two fractions were comparable. We inhibited the degradative systems (proteasome and autophagy) and found that the three TDPs forms were all degraded by the proteasome; interestingly, we found that only TDP25 accumulated in presence of autophagy inhibition.

The heat shock protein B8 (HspB8) is a molecular chaperone that we recently linked to the autophagic routing of misfolded and aggregated proteins. We overexpressed HspB8 in presence of the three TDPs and found that it reduced the NP40-insoluble TDP25 species in FRA.

Surprisingly, when HspB8 was silenced we found a comparable reduction in the NP-40 insoluble species of all TDPs. We obtain similar reduction in TDPs accumulation by silencing Bag3 a co-chaperone required for HspB8 activity.

In conclusion, we found that HspB8 may exert a protective role in presence of misfolded aggregated-prone proteins. We also found that the suppression of an autophagic re-routing pathway, probably activates other degradative pathway that could be important to understand the molecular mechanisms triggered by misfolded proteins.

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