Effects of HSP proteins on FUS aggregates modulation

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Abstract:

FUS is an RNA-binding protein, member of FET family proteins and is involved in several cellular processes such as splicing, translation and mRNA transport. FUS is also recruited very early at DNA damage sites and it is a substrate for ATM and DNA-PK, two apical kinases important in DNA damage repair.

Mass spectrometry analysis revealed that upon etoposide treatment, a genotoxic agent that causes DNA double strand breaks (DSBs), some proteins increased their affinity for FUS. Among these proteins, two proteins members of HSP protein family were identified. HSP proteins are part of the chaperone machinery. This system is important to maintain a correct proteostasis in the cell. In particular, the role of chaperone machinery in this process is to correctly fold proteins in order to reach their native conformation, by which they can be functional. This process starts immediately during protein translation and involves diverse chaperones. Proteins that have not been properly folded, could also re-enter this machinery to possibly reach the correct conformation.

The aim of this work is to investigate the possible roles of the increased interaction between FUS and HSP proteins. Aggregation of mislocalized FUS seems to require his prion-like domain and is a hallmark of amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases. The first point of this project is to understand if FUS aggregates after inducing DSBs and if HSP proteins could modulate this protein aggregation. To evaluate this, we are performing *filter retardation assay*, that consists on using vacuum pump to let the sample pass through a 0.2µm nitrocellulose membrane and then detecting FUS aggregates retained by the membrane. Moreover, we want to investigate the subcellular localization of FUS aggregation and the possible change of localization of HSP proteins upon treatment with the genotoxic agent. We are assessing these phenomena through cell *fractionation protocols* and *fluorescence microscopy*.

As a future perspective, it would be interesting to investigate the relationship of HSP proteins with other FET family proteins and with TDP-43 and SOD1, two genes also commonly mutated in ALS together with FUS.