Effects of HSP proteins in DNA damage response

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Abstract:

The genome is constantly subjected to exogenous and endogenous sources that can alter genomic integrity. Cells have developed two main pathways to repair Double Strand Breaks (DSB), the Homologous recombination (HR) which is used when cells are in the S / G2 cycle phase and the sister chromatid is required as a template to repair the DNA damage, and the Non-Homologous end joining (NHEJ) which is used when cells are in the G1 phase of the cell cycle and during the ligation process of the broken ends, a certain number of nucleotides are lost and consequently genetic information may be altered. Both mechanisms require the intervention of different activators and effectors of DNA Damage Response (DDR).

FUS is an RNA binding protein (RBPs), member of FET family proteins that 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 also a substrate of ATM and DNA-PK, two apical kinases important in DNA damage repair. It is possible that the progression of the ALS disease caused by the loss of motor neurons is due to the activation of the apoptosis pathway, triggered by the accumulation of DNA damage and inefficient repair mechanisms.

Mass spectrometry analysis revealed that upon induction of DSB (by treatment with Etoposide, ETO), some proteins increased their affinity for FUS, including two proteins members of HSP. HSPs are molecular chaperones that allow proteins to achieve proper folding. The aim of this work is to investigate the possible reason of the increased interaction between FUS and HSP proteins during DNA damage.

The first aspect that we want to assess is whether these HSP proteins could affect cell viability after genotoxic treatment. We decided to evaluate this effect by performing *trypan blue assay* upon treatment with several concentration of the ETO for 18h.

Finally, we want to assess whether these HSP proteins could have a role in DNA damage repair, as could be suggested by the increased interaction with FUS. To verify this hypothesis, we also decided to use *immunofluorescence* to detect the phosphorylation of key DNA damage-related proteins, in order to observe the possible differences in cells transfected with HSPs compared to the control cells upon DSB induction. As a future perspective, it would be interesting to investigate the relationship of HSP proteins with other FET family proteins (EWS and TAF15).