Investigating the role of TAF15 in the DNA Damage Response

Lombardi S.[1], Colella M. [1], Rocha Levone B.[1], Barabino S. [1]
E-mail: silvia.lombardi@unimib.it
[1] Università degli Studi di Milano-Bicocca, Italy

Keywords: RNA-binding proteins, DNA damage response, TAF15

Abstract:

To maintain genome integrity cells have evolved a complex network of mechanisms, comprehensively known as the DNA damage response (DDR). RNA and RNA-binding proteins (RBPs) are strongly involved in the DDR and, among the RBPs, the FET (FUS, EWS, TAF15) family of proteins are emerging as important players. Recent studies, including a paper published by our group (Levone et al. 2021), have described FUS function in the DDR, whereas the role of EWS and TAF15 is still not clear.

The aim of this project is to investigate the role of the FET protein TAF15 in the DDR, through the characterization of TAF15-knockout (KO) HeLa cells and with a focus on double-strand break (DSB) response.

Preliminary microirradiation experiments in wild-type (WT) Hela cells revealed that TAF15 is promptly recruited to DNA damage sites, reaching a peak within two minutes from irradiation. Viability and proliferation assays showed that the absence of TAF15 does not affect cells sensitivity to genotoxic agents, including camptothecin (topoisomerase I inhibitor), etoposide (topoisomerase II inhibitor), bleomycin (radiomimetic) and hydroxyurea (replication inhibitor). Analysis of DDR pathway activation through western blotting showed no major alterations in KO cells, except for the phosphorylation of the histone H2AX that was reduced compared to WT cells. Accordingly, the study of DNA damage foci formation showed that KO cells form less γH2AX foci after treatment with etoposide. Finally, microirradiation of WT and KO cells did not revealed significant differences in the recruitment of DDR apical factor KU80 or of the other FET proteins (FUS and EWS).

Overall, our results indicate that TAF15 is directly involved in the DDR, but it is not required for resistance to different genotoxic agents, suggesting a dispensable or redundant role or complementation by other proteins. Notably, KO cells showed defects in H2AX phosphorylation and foci formation, but at present no other major alterations of the DDR have been found. Future studies will investigate further the possible causes and effects of H2AX lower phosphorylation. Moreover, the dependency of TAF15 from DDR kinases and the downstream steps of DSB repair in TAF15-KO cells will be studied.