Role of RNA helicases in genome stability:

Genome stability is critical for the faithful transmission of genetic information. The maintenance of genome integrity is a complex and highly regulated process involving numerous molecular machineries. Most of them are directly involved in DNA repair and metabolism, but in the last two decades several factors involved in transcription and RNA metabolism have also been involved. Among these, RNA helicases emerge as versatile enzymes that participate in genome maintenance by controlling the homeostasis of R-loops, three nucleic acid structures consisting of a DNA-RNA hybrid and the displaced single strand of DNA. Specific R-loops have a known physiological function, but most of them are unscheduled and can interfere with DNA replication, transcription and repair thus compromising genome integrity.

RNA helicases of the DEAD-box family belong to the helicase superfamily 2, which contain a series of core domains highly conserved among the family members. There are over 35 DDX helicases in humans which are highly conserved in structure. They act in different processes of RNA metabolism, which in many cases are not yet well defined, and they do not have a redundant function. Interestingly, depletion of many of these helicases, such as, DDX5, DDX21, DDX39B and DDX47, have being reported to prevent or resolve R-loop accumulation and their associated genome stability phenotypes. However, not much is known about the function of each of them that could explain such a similarity of phenotypes. DDX5 is a key player in resolving unscheduled R-loops specially at DNA damage sites. The removal of these R-loops has been shown to be favoured by BRAC2 that stimulate DDX5 activity. In contrast, DDX47 is a nucleolar RNA helicase that helps to prevent R-loop accumulation mainly at ribosomal DNA. To understand deeply the mechanisms by which these helicases prevent or resolve R-loops in vivo, we are developing different strategies to identify the functional differences of specific RNA helicases in genome instability. Using CRISPR Cas9 technology we generated HCT-116 and RPE1 cell lines KO for the DDX5 helicase gene. We were unable to generate DDX47 KO cells, consistent with an essential role of some of them for cell viability, as previously described for DDX21. Here we present the construction and validation of the DDX5 KO cell lines and a preliminary characterization that light up differences between the role of DDX5 in cancerous and not cancerous cell lines.

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