



The involvement of DIS3L2 in nonsense-mediated mRNA decay and its functional networks in colorectal cancer

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Objectives: This project aims to analyze the mechanism by which DIS3L2 is involved in nonsense-mediated decay, as well as the DIS3L2 functional network involved in colorectal cancer tumorigenesis.

Methodology: The final step of cytoplasmic mRNA degradation proceeds in either a 5'-3' direction catalyzed by XRN1 or in a 3'-5' direction catalyzed by the exosome. In yeast, Dis3/Rrp44 protein is the catalytic subunit of the exosome. In humans, there are three paralogues of this enzyme: DIS3, DIS3L1, and DIS3L2. However, DIS3L2 exoribonuclease activity is independent of the exosome. Important findings have shown that they are involved in growth, mitotic control, and in important human diseases, including cancer. For example, DIS3L2 inactivation was associated with mitotic abnormalities and altered expression of mitotic checkpoint proteins (Astuti et al., *Nat Genet.* 2012, 44:277-84). In another study, DIS3L2 downregulation was found to enhance colorectal (CRC) cancer stem cell properties in vitro (Liu et al., *Onco Targets Ther.* 2017, 10:2367-2376).

Despite that DIS3L1 and DIS3L2 exoribonucleases localize in the same compartment where NMD occurs, nothing is known about their role in this process. In order to unveil the role of DIS3L2 in NMD, we have been performed its knockdown and measured the mRNA levels of various natural NMD targets. Our results show that some NMD targets are highly stabilized in DIS3L2-depleted cells. In addition, mRNA half-life analyses indicated that these NMD targets are in fact direct DIS3L2 substrates. We also observed that DIS3L2-mediated decay depends on the terminal uridylyl transferases (TUTases) Zcchc6/11 (TUT7/4) activity. Together, our findings establish the role of DIS3L2 and uridylation in NMD. In this project, we aim to go further into the understanding of the mechanism by which DIS3L2 is involved in NMD. Furthermore, we aim to analyze how DIS3L2 regulates the human transcriptome, and how it is involved in colorectal oncogenesis. We are currently performing an extensive characterization of the DIS3L2 mRNA targets, using DIS3L2 knockdown, RNA deep sequencing, and target RNA immunoprecipitation assays on normal colorectal cells, or in CRC cell lines, subjected to several stress stimuli, including nutrient deprivation and hypoxia. Bioinformatics and gene ontology analyses of transcripts from these cells will elucidate new cellular pathways regulated by DIS3L2 and/or by its targets. In this project, we aim to progress in this analysis, as well as to investigate how DIS3L2, and/or its targets and corresponding functional networks, are involved in proliferation, cell cycle progression, survival and motile and invasive proprieties of CRC cells.

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