Dissecting the DIS3L2 target-specificity of transcripts committed to nonsense-mediated decay in human cells

Juan F. García Moreno[1,2], **Miguel Carvalho[1,2]**, Rafaela Lacerda[1,2] and Luísa Romão[2]
[1]Department of Human Genetics, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal
[2]Universidade de Lisboa, Faculty of Sciences, BioISI – Biosystems and integrative Sciences Institute, Lisboa, Portugal

Introduction

- **Nonsense-mediated mRNA decay (NMD)** is a pathway that recognizes transcripts harboring premature translation-termination codons (PTCs), leading them to degradation, while also regulating the expression of certain physiological mRNAs.
- One of the branches of the NMD pathway is characterized by the involvement of the **3'-to-5' exoribonuclease DIS3L2**. This protein degrades different RNAs independently of the exosome, following **uridylation** at the 3' end by the terminal uridylyl transferases TUT4 and TUT7[1][2].

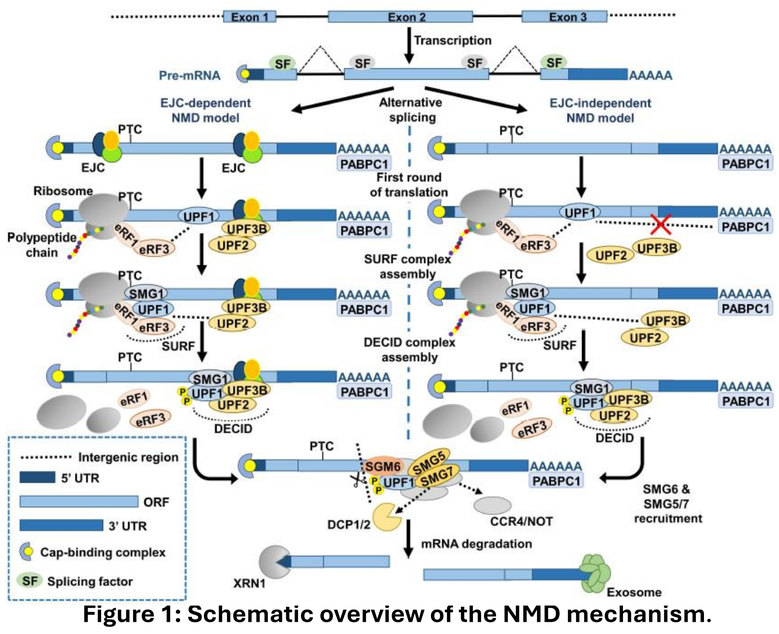


Figure 1: Schematic overview of the NMD mechanism.

Aims

We aim to characterize the mechanisms involved in DIS3L2/NMD-target specificity, uncovering the features shared by DIS3L2/NMD-degraded transcripts.

Results

Our **RNA-seq data,** obtained and validated[3], containing the transcripts upregulated upon DIS3L2 knockdown, were compared with a validated NMD-target set[4]. About **7%** of DIS3L2-sensitive transcripts overlap with known NMD-targets.

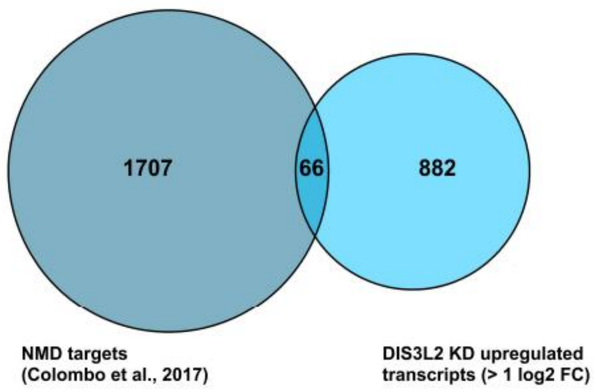
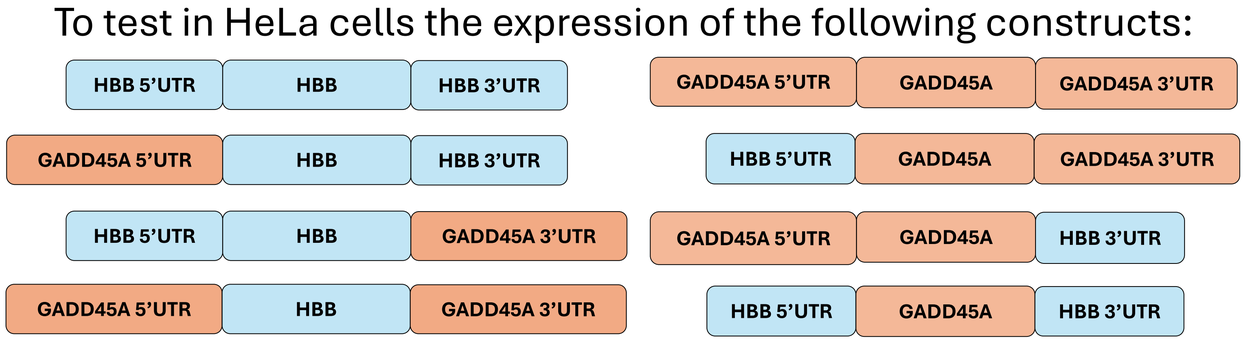


Figure 2: DIS3L2 regulates a set of transcripts that are NMD-targets. Representative Venn diagram containing the set of NMD-targets detected by Colombo and colleagues[4] (left circle) and the set of upregulated transcripts showing more than 1 log2 fold change (FC) after depletion of DIS3L2 in SW480 cells (right circle). There is an overlap containing 66 transcripts that are both NMD- and DIS3L2-targets.

Next Approaches

To test in HeLa cells the expression of the following constructs:



Methods

1. RNA was extracted from the SW480 cell line (colorectal cancer type IV);
2. RNA samples were sent to Stab Vida (Lisbon, Portugal) for sample preparation and sequencing;
3. RNA libraries were prepared from three independent biological replicates for each condition (DIS3L2 or luciferase knockdown);
4. Libraries were run on an Illumina Hiseq 2500 sequencing platform;
5. Differentially expressed genes (DEGs) were identified by analyzing read counts per gene. Gene expression data handled with DESeq2 package was statistically analyzed using a Wald test, which creates lists of DEGs with adjusted p values using the Benjamini-Hochberg procedure [5];
6. Specific features [5' and 3' untranslated region (UTR) lengths, presence of introns in the 3'UTR, 5' and 3'UTR GC- and AU-contents and presence of upstream open reading frames (uORFs)] were analyzed in the DIS3L2/NMD-targets, *versus* the NMD-targets, DIS3L2-sensitive targets, or the remaining transcriptome by a two-tailed unpaired t-test used to compare the means of single control groups to single test groups;

Results

DIS3L2/NMD-targets have a higher GC-content in the 3'UTR compared to the transcriptome and a shorter 5'UTR length compared to the remaining DIS3L2-targets

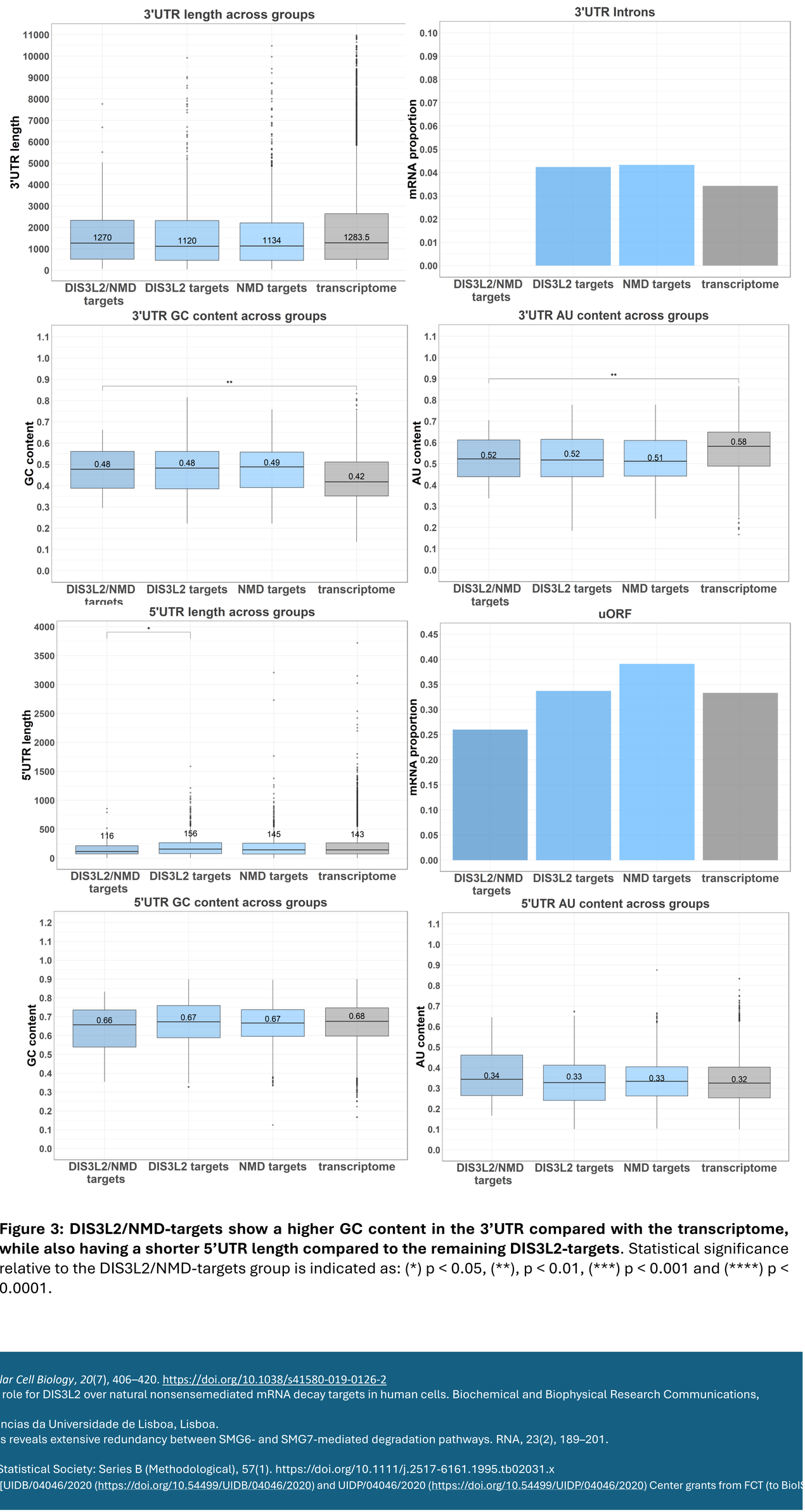


Figure 3: DIS3L2/NMD-targets show a higher GC content in the 3'UTR compared with the transcriptome, while also having a shorter 5'UTR length compared to the remaining DIS3L2-targets. Statistical significance relative to the DIS3L2/NMD-targets group is indicated as: (*) $p < 0.05$, (**), $p < 0.01$, (***) $p < 0.001$ and (****) $p < 0.0001$.

References:
[1] Kurosaki, T., Popp, M. W., & Maquat, L. E. (2019). Quality and quantity control of gene expression by nonsense-mediated mRNA decay. *Nature Reviews Molecular Cell Biology*, *20*(7), 406–420. https://doi.org/10.1038/s41580-019-0126-2
[2] da Costa, P. J., Menezes, J., Saramago, M., García-Moreno, J. F., Santos, H. A., Gama-Carvalho, M., Arraiano, C. M., Viegas, S. C., & Romão, L. (2019). A role for DIS3L2 over natural nonsensemediated mRNA decay targets in human cells. Biochemical and Biophysical Research Communications, 518(4), 664–671. https://doi.org/10.1016/j.bbrc.2019.08.105
[3] Juan F. García-Moreno (2023). *The involvement of* DIS3L2 *in nonsense-mediated mRNA decay and its functional networks in cancer*. Faculdade de Ciências da Universidade de Lisboa, Lisboa.
[4] Colombo, M., Karousis, E. D., Bourquin, J., Bruggmann, R., & Mühlemann, O. (2017). Transcriptome wide identification of NMD-targeted human mRNAs reveals extensive redundancy between SMG6- and SMG7-mediated degradation pathways. RNA, 23(2), 189–201. https://doi.org/10.1261/rna.059055.116
[5] Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society: Series B (Methodological), 57(1). https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Acknowledgments: This work was partially supported by Instituto Nacional de Saúde Doutor Ricardo Jorge and Fundação para a Ciência e a Tecnologia [UIDB/04046/2020 (https://doi.org/10.54499/UIDB/04046/2020) and UIDP/04046/2020 (https://doi.org/10.54499/UIDP/04046/2020) Center grants from FCT (to BioIS recipient of a fellowship from the BioSys PhD programme (SFRH/BD/114392/2016) from FCT.