CASC3 DEPLETION IN HEK293 CELLS CAUSES UPREGULATION OF NMD-SENSITIVE mRNA TRANSCRIPTS

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The exon junction complex (EJC) is a protein complex composed of four core components-EIF4A3, RBM8A, MAGOH, and CASC3- and several peripheral components. During splicing, it is deposited ~20–24 nucleotides (nt) upstream of exon-exon junctions [1]. Besides its ability to influence splicing decisions, the EJC also plays a role in the nonsense-mediated mRNA decay (NMD) pathway, namely-it serves as a mark of a premature termination codon (PTC): during translation, EJCs are displaced from the mRNA transcript by ribosomes; in the case of premature termination, EJCs downstream of PTCs are not removed and can trigger nonsense-mediated mRNA decay (if they are located at least ~50-55 nt. downstream of the PTC), leading to the degradation of the transcript [2]. Some sources suggest that CASC3 is essential for the EJC-dependent activation of NMD [3, 4] by showing that NMD efficiency for PTC-containing reporter mRNAs decreased in the absence of CASC3; others imply that CASC3 plays a minor role in NMD and only for certain targets [5]. However, the exact function of CASC3 in relation to NMD activation is not known.

To investigate the function of CASC3 in NMD, we first generated HEK293 cell lines containing 1-480 residue truncated CASC3 constructs derived from either the wild type (WT) CASC3 or from a mutant (two mutant were used: an F188D/W218D mutant that has binding with EIF4A3 impaired and cannot join the EJC; and an NES mutant, which has a mutated nuclear export signal). For this, we stably integrated cumate-inducible CASC3 WT (wild type), 188/218, and NES (as well as a FLAG-EmGFP construct as control) into CASC3 knock-out HEK293 cells. The 1-480 residue stretch contains all domains of CASC3 that are essential for its function [4]. After obtaining sufficiently confluent cultures, we induced the expression of the

constructs and performed mRNA extraction and cDNA synthesis. Meanwhile, from the differential expression analysis results (DESeq2) we found genes that showed a significantly higher expression level in the KO than the control. Besides being upregulated in the KO, some of these genes displayed an additional interesting feature: an exon inclusion. Finally, we selected two genes-HNRNPL and WHAMM- and designed PCR primers for them (situated in the exons adjacent to the included ones: exons 6 and 7, exons 5 and 6, respectively) in order to test these exon inclusion events in our cDNA samples and to verify whether there is a correlation between the lack-of-function/absence of CASC3 and the presence of these alternatively spliced transcripts. Primers were designed in such a manner that the additional exon would be amplified together with a small sequence of the adjacent introns, resulting in DNA fragments of different lengths if the exon were included or not. The cDNA obtained previously was amplified in a PCR reaction using the designed primers and subsequently loaded on an agarose gel (Figure). The inclusion of an exon would result in a larger DNA fragment on the gel.

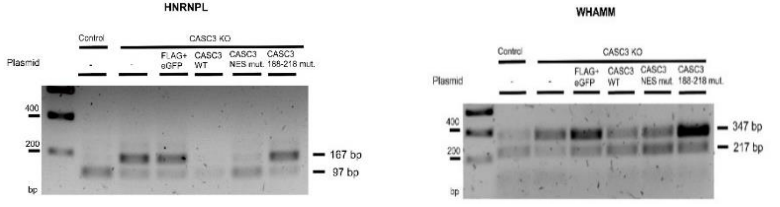

Figure. KO-rescue experiment: agarose gels with DNA fragments obtained by PCR with primers designed for the specific events. (A) HNRNPL. (B) WHAMM

For HNRNPL, we observe that the positive control (normal HEK293 cells) shows a band for the shorter fragment (i.e. no exon inclusion), whereas the two negative controls (CASC3 KO with no additional plasmid or a FLAG-EmGFP plasmid) have a significant band for the larger fragment (i.e. exon inclusion). The phenotype was rescued by the addition of WT CASC3, as we can see a profile similar to the positive control (although weaker, probably due to a lower amount of DNA). The CASC3 NES mutant was also able to restore the activity,

whereas the 188/218 mutant was not. In the case of WHAMM, we witnessed an analogous profile, although the endogenous levels of the alternatively spliced transcript seemed to be higher, as the band was noticeable even in the positive control. These results indicate that binding of CASC3 to EIF4A3 is essential for its function. Starting from the findings reported by Gehring et al. (2009) and Gerbracht et al. (2019) that CASC3 serves as a bridge between the EJC and the NMD machinery, we surmised that these transcripts might be targeted by NMD, hence they are present in low quantities in normal cells, whereas in the absence of CASC3 function they are stabilised and can be found in larger numbers (as seen in Figure). Furthermore, this hypothesis can be (partially) proved by the presence of PTCs which we found in the included exons, which are necessary for the activation of NMD. Based on these results, we hypothesise that the upregulation of alternatively spliced transcripts featuring an exon inclusion that we observed is due to impaired NMD, caused by CASC3 absence/loss-of-function. Furthermore, CASC3 must be bound to the EJC (and the mRNA) in order to perform its function in NMD activation.

In summary, we found that the depletion of CASC3 leads to the accumulation of some NMD-sensitive alternatively spliced transcripts, suggesting that CASC3 is needed for the activation of NMD (at least for a subset of mRNAs).

References:

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