

Abstract 5

UNDERSTANDING THE MOLECULAR PATHOLOGY OF UPF3B MUTATIONS

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Non-sense mediated mRNA decay (NMD) is a universal RNA surveillance pathway that among other functions degrades mRNAs bearing premature termination codons (PTC). We have recently showed that mutations in UPF3B, an important member of this pathway, caused syndromic and nonsyndromic mental retardation (MR). To assess the impact of UPF3B null mutations and identify relevant genes regulated by NMD, we performed expression profiling using RNA isolated from control and patient lymphoblastoid cells using Affymetrix Human U133 Plus 2 and Exon 1.0 ST arrays. Compared to controls, 633 genes were significantly down-regulated in patients (30% up, 70% down, false discovery rate = 10%), including down-regulation of UPF3B. Hence, NMD is only partially compromised in the absence of UPF3B as its own PTC containing mRNA is degraded. Comparison with previous microarray studies from UPF1, UPF2 or UPF3B knock down cell lines in human, fly and yeast generated minimal overlap. Such low correspondence might be explained either by tissue type and array platform differences or the technology (siRNA) used. We have identified bona-fide targets of the UPF3B dependent NMD pathway and suggest that up-regulation of some of these genes, e.g. ARHGAP24 contributes to some aspects of the phenotype seen in patients with UPF3B mutations. ARHGAP24 is a negative regulator of Rho-GTPase and has role in regulating cell polarity. We show that PC12 cells over-expressing a particular isoform of ARHGAP24, which we identified to be most likely regulated by NMD, failed to differentiate into neuronal-like cells upon treatment with the nerve growth factor. We have also identified a novel mechanism of posttranscriptional regulatory switch between the two UPF3 paralogs, UPF3B and UPF3A. In the absence of UPF3B, the UPF3A protein is stabilised and elicits NMD. Interestingly, the amount of UPF3A protein varies between patients and appears to negatively correlate with the severity of the disorder.

Acknowledgements: This work has been supported by Australian NH&MRC grant to JG.