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Preview

# A protein regulated by UBE3A PEGs a potential biomarker

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New research from Pandya and colleagues[1] identifies PEG10 as a UBE3A-regulated protein that may underlie pathophysiology in Angelman syndrome neurons. PEG10 is a secreted protein, and this work suggests that it may be a potential biomarker for Angelman syndrome therapeutics under development.

Loss of function from the maternally inherited allele of *UBE3A* causes Angelman syndrome (AS), a severe neurodevelopmental disorder affecting approximately 1/20,000 live births.[2,3] UBE3A is a HECT domain E3 ubiquitin ligase that ubiquitinates proteins, targeting them for degradation by the proteosome. Despite two decades of studying the role of UBE3A in the pathophysiology of AS using murine and cell culture models, the ubiquitination targets of UBE3A in neurons largely remain elusive. In this issue of *Cell Reports Medicine*,[1] an interdisciplinary group led by Ravi Jagasia and Nikhil Pandya leveraged human AS induced pluripotent stem cell (iPSC)-derived neurons to identify proteins whose abundance changed in response to altered UBE3A levels. This work ultimately identified PEG10 as a potential biomarker to reflect UBE3A protein levels and may have uncovered a role for PEG10 in AS pathophysiology.

Pandya et al.[1] first quantified expression of 7,000 proteins in AS and control iPSC-derived neurons using tandem mass tag (TMT)-MS3-based deep proteomic profiling, to identify proteins differentially expressed between AS and control neurons. While this can potentially identify direct UBE3A targets, it can also identify other proteins differentially expressed due to developmental differences caused by AS. To winnow out the UBE3A-regulated proteins, *UBE3A* was modulated using antisense oligonucleotides (ASOs) targeting either UBE3A itself or UBE3A-ATS, a non-coding RNA that negatively regulates UBE3A. UBE3A is encoded by an imprinted gene.[4] The paternally inherited allele of *UBE3A* is silenced by transcription of a non-coding RNA, *UBE3A-ATS*.[5] ASOs targeting *UBE3A-ATS* can derepress the paternal *UBE3A* allele, and thus restore *UBE3A* mRNA and protein in AS neurons.[6] This a promising therapeutic approach for AS. Conversely, ASOs targeting *UBE3A* can reduce the *UBE3A* mRNA and protein in control cells. ASO-mediated modulation of *UBE3A* levels was used to narrow down the list of putative UBE3A targets—proteins that were increased when UBE3A was depleted in control neurons and decreased when UBE3A was restored in AS neurons. Among the list of UBE3A targets, they identified PEG10 and its binding partners, TCAF1 and Retroposon-like 8 (RTL8)-A, -B, and -C as the most robustly changed proteins following modulation of *UBE3A* levels. PEG10 was also upregulated in AS post-mortem brain samples compared to normal controls.

UBE3A was shown to regulate PEG10 in a post-translational manner. Yeast two-hybrid and immunoprecipitation assays confirmed a direct interaction between UBE3A and PEG10. Although UBE3A did not ubiquitinate PEG10 in a bacterial ubiquitination assay, UBE3A-mediated regulation of PEG10 was dependent upon the proteasome. Importantly, the transcriptomes of AS neurons following PEG10 downregulation was remarkably similar to AS neurons following UBE3A reinstatement, suggesting that PEG10 contributes to the pathophysiology in human AS neurons. PEG10 was shown to be recruited to stress granules, where it was found to interact with many RNAs, and was secreted in extracellular vesicles (EVs), and potentially influence cargo loaded into these small compartments.[1] Overexpression of PEG10 can influence the numbers of EVs and proteins/RNAs loaded into EVs. *In utero* electroporation studies in mice suggested a neuronal migration defect upon PEG10 overexpression, further evidence for potential involvement of PEG10 in the pathophysiology of AS.

Interestingly, regulation of imprinted expression of *UBE3A* is conserved between mouse and humans, and AS mice have many phenotypes that parallel those seen in children with AS. Surprisingly, Pandya and colleagues found that murine PEG10 is not expressed highly in mouse neurons and is not upregulated in the brains of AS mice. They speculate that PEG10 may not explain phenotypes in the AS mouse model. However, it is also possible that the regulation of PEG10 by UBE3A serves a purpose other than regulation of neuronal function. The host-defense theory of genomic imprinting first suggested by Denise Barlow[7] posited that parent-of-origin-specific expression of some genes arose to ensure silencing of repeated and retroviral-like sequences that invaded mammalian genomes. Although the literature primarily points to other examples,[8,9] PEG10 is a retrotransposon-like, GAG-containing protein that is also encoded by an imprinted gene. Could UBE3A be yet another linchpin in host-defense from invasive DNA elements? If so, perhaps murine UBE3A exerts its regulation on PEG10 at a different stage of development, other than neurogenesis. Nonetheless, this work by Pandya et al. strongly indicates a role for

UBE3A in modulation of PEG10 levels in human neurons.

The list of putative UBE3A targets is a rich starting point for identifying the ubiquitination targets for UBE3A. The list of proteins differentially expressed following *UBE3A* modulation ranges from approximately 70 to approximately 225 proteins, providing the first evidence of the magnitude of impact for loss of *UBE3A* on the proteome. These estimates are likely conservative, given the inherent difficulties in quantifying proteins expressed at low levels and the heterogeneous nature of iPSC-derived neuronal cultures. Since loss of *UBE3A* underlies AS, the identification of protein targets is essential to connect UBE3A with the physiological deficits in AS neurons and eventually to behaviors in humans and animal models. Future studies will hopefully corroborate or refute these targets and shed light on the associated cellular processes which may underlie various AS phenotypes. For now, one of the most robust targets, PEG10, may provide valuable utility as a biomarker for therapeutic approaches for AS based on *UBE3A* replacement.

## DECLARATION OF INTERESTS

The authors have a patent application (PCT/US19/52272) related to Angelman syndrome therapeutics and a financial conflict of interest with Ovid Therapeutics.

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