TDP-43 represses cryptic exon inclusion in the FTD–ALS gene *UNC13A*

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1. 簡述論文的概要與重大發現：

大腦和脊髓神經元核中 RNA 結合蛋白 TDP-43 的耗盡是神經退化性疾病肌萎縮側索硬化症 (ALS) 和額顳葉癡呆 (FTD)的標誌性病理特徵。TDP-43 其中的主要功能是作為 RNA剪接過程中隱含外顯子包含的阻遏物。*UNC13A* 中的單核苷酸多態性與 FTD 和ALS 發現有正相關性，但這些變異如何增加疾病風險尚不清楚。此篇作者展現了 TDP-43 抑制了*UNC13A* 中的一個 cryptic exon。而在人腦細胞、神經細胞和源自誘導萬能幹細胞的運動神經元中 knock down TDP-43 會導致*UNC13A* 出現一個 cryptic exon，並降低正常的 UNC13A 蛋白質的表現量。此篇的數據也提供了 FTD 和ALS 遺傳風險因素之一（即*UNC13A* 遺傳變異）與 TDP-43 的功能喪失出關係。

2. 對論文內容的提問：

在本篇研究發現 TDP-43 phosphorylation 會導致 TDP-43 位於細胞質而非細胞核，但詳細原因尚不清楚，健康的人與 ALS-FTD 患者其 TDP-43 的差異何在，為什麼 TDP-43 會被 phosphorylation 並且使其無法進入核內執行 alternative splicing。

3. 論文的缺點與評價：

本文不僅發現了 *UNC13A* 會受到 TDP-43 的調控，更讓人發現 intron 內的鹼基及序列是不可忽略的要素；若 TDP-43 dysfunction 狀態下會使 *UNC13A* 出現一個 cryptic exon 可能會導致 nonsense-mediated decay，最終使 UNC13A level 下降而形成神經退化性疾病。而作者運用 mini gene reporter constructs 去了解 SNP 與 TDP-43 所執行的 alternative splicing 之間的關係。

作者並未說明 *UNC13A* 的 cryptic exon 為什麼會降低 UNC13A 蛋白質的表達量，也未將其定序確定是否含有 stop codon 以至於無法產生 UNC13A protein。

Article

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A hallmark pathological feature of the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) is the depletion of RNA-binding protein TDP-43 from the nucleus of neurons in the brain and spinal cord[1]. A major function of TDP-43 is as a repressor of cryptic exon inclusion during RNA splicing[2–4]. Single nucleotide polymorphisms in *UNC13A* are among the strongest hits associated with FTD and ALS in human genome-wide association studies[5,6], but how those variants increase risk for disease is unknown. Here we show that TDP-43 represses a cryptic exon-splicing event in *UNC13A*. Loss of TDP-43 from the nucleus in human brain, neuronal cell lines and motor neurons derived from induced pluripotent stem cells resulted in the inclusion of a cryptic exon in *UNC13A* mRNA and reduced UNC13A protein expression. The top variants associated with FTD or ALS risk in humans are located in the intron harbouring the cryptic exon, and we show that they increase *UNC13A* cryptic exon splicing in the face of TDP-43 dysfunction. Together, our data provide a direct functional link between one of the strongest genetic risk factors for FTD and ALS (*UNC13A* genetic variants), and loss of TDP-43 function.

TDP-43, encoded by the *TARDBP* gene, is an abundant, ubiquitously expressed RNA-binding protein that normally localizes to the nucleus. It has a role in fundamental RNA-processing activities, including RNA transcription, alternative splicing and RNA transport[7]. A major splicing regulatory function of TDP-43 is to repress the inclusion of cryptic exons during splicing[2,8–10]. Unlike normal conserved exons, these cryptic exons occur in introns and are normally excluded from mature mRNAs. When TDP-43 is depleted from cells, these cryptic exons get spliced into messenger RNAs, often introducing frame shifts and premature termination, or even reduced RNA stability. However, the key cryptic splicing events that are integral to disease pathogenesis remain unknown.

STMN2—which encodes stathmin 2, a regulator of microtubule stability—is the gene whose expression is most significantly reduced when TDP-43 is depleted from neurons[3,4]. *STMN2* harbours a cryptic exon (exon 2a) that is normally excluded from the mature *STMN2* mRNA. The first intron of *STMN2* contains a TDP-43 binding site. When TDP-43 is lost or its function is impaired, exon2a gets incorporated into the mature mRNA. Exon 2a harbours a stop codon and a polyadenylation signal—this results in truncated *STMN2* mRNA and eightfold reduction[3] of stathmin 2. Aberrant splicing and reduced stathmin 2 levels seem to be a major feature of sporadic and familial cases of ALS (except those with *SOD1* mutations)[3,4] and in frontotemporal lobar degeneration (FTLD) due to TDP-43 proteinopathy[11] (FTLD-TDP). The discovery of *STMN2* cryptic exon splicing in ALS and FTLD-TDP highlights a key mRNA target—we aimed to identify other possible mRNA targets.

To discover cryptic splicing targets regulated by TDP-43 that may also have a role in disease pathogenesis, we used a recently generated RNA sequencing (RNA-seq) dataset[12]. To generate this dataset, fluorescence-activated cell sorting (FACS) was used to enrich neuronal nuclei with and without TDP-43 from postmortem brain tissue from patients with FTD and ALS (FTD–ALS); RNA-seq was performed

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