The dual functions of the extreme N-terminus of TDP-43 in regulating its biological activity and inclusion formation

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Abstract

Introduction: TAR DNA-binding protein-43 (TDP-43) is the principal component of ubiquitinated inclusions in amyotrophic lateral sclerosis (ALS) frontotemporal lobar degeneration with TDP-43-positive inclusions (FTLD-TDP) (1,2). TDP-43 contains four functional domains, which include a nuclear localization signal (NLS) and two RNA recognition motifs (RRMs) within the N-terminal half of the protein, as well as a nuclear export signal (NES) and a glycine-rich region in the C-terminal half. The NLS and NES regulate the shuttling of TDP-43 between the nucleus and the cytoplasm (3), while the RRM1 and RRM2 are responsible for binding to nucleic acids, such as UG repeats (4,5). The glycine-rich region mediates protein–protein interactions between TDP-43 and other hnRNP members (6). Since the C-terminal region of TDP-43 harbors almost all known ALS-associated TDP-43 mutations (7), and contains Q/N-rich domains that promote TDP-43 aggregation (8,9), research has mostly focused on the C-terminal region of TDP-43. As a result, the functions of TDP-43's N-terminalregion remain largely unknown.

Methods: To bridge this gap in our knowledge, we utilized in-cell cross-linking, *CFTR* mini-gene splicing, immunofluorescence, neurite outgrowth and computer-assisted models to evaluate the functions of TDP-43 N-terminus in regulating its folding, self-interaction, biological activity and aggregation.

Results: We determined that the extreme N-terminus of TDP-43, specifically the first 10 residues, regulates folding of TDP-43 monomers necessary for proper homodimerization and TDP-43-regulated splicing. Indeed, deletion of these 10 residues, and even mutations of key residues within this sequence, impairs TDP-43 homodimer formation and result in the loss of TDP-43-regulated splicing. Despite such beneficial functions, we discovered an interesting dichotomy: full-length TDP-43 aggregation, which is believed to be a pathogenic process, also requires the extreme N-terminus of TDP-43.

Discussion: Our findings indicate that the extreme N-terminal region of TDP-43 is crucial for maintaining the normal conformation and biological activity of TDP-43 under physiological conditions. In disease, our data would suggest that the extreme N-terminal region of TDP-43 mediates full-length TDP-43 oligomerization and aggregate formation. This would result in a loss of functional TDP-43 due to sequestration of wild-type TDP-43 into insoluble inclusions, and perhaps a toxic gain of function resulting from the generation of TDP-43 oligomers and aggregates.

Figure 1

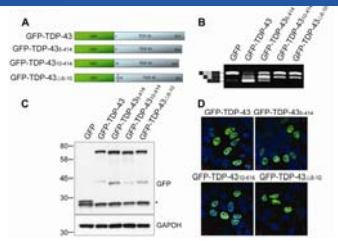

Figure 1. The first 10 N-terminal residues of TDP-43 are required for its biological activity.

Figure 2

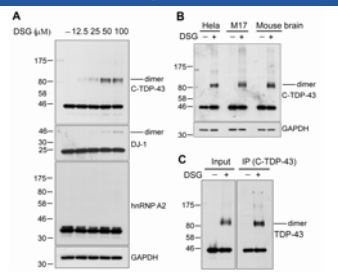

Figure 2. The first 10 N-terminal residues of TDP-43 are required for the formation of a functional TDP-43 homodimer.

Figure 2

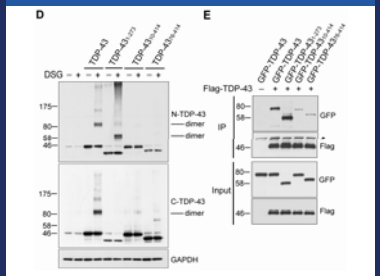

Figure 2. The first 10 N-terminal residues of TDP-43 are required for the formation of a functional TDP-43 homodimer.

Figure 3

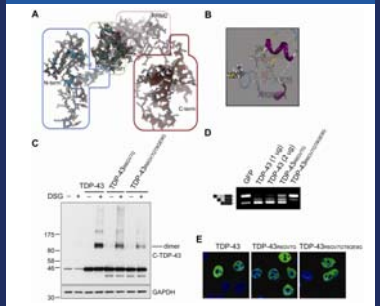

Figure 3. Arg6, Val7, Thr8 and Glu9 are required for TDP-43 homodimerization and splicing regulation.

Figure 4

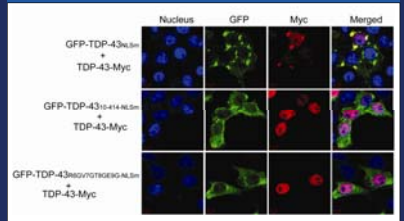

Figure 4. The extreme N-terminus of TDP-43 is required for aggregation of full-length TDP-43.

Figure 5

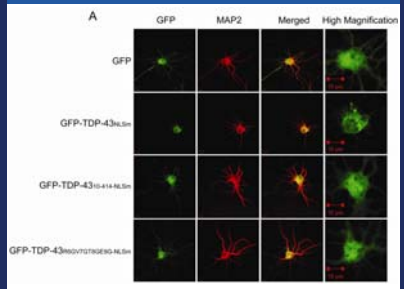

Figure 5. The extreme N-terminus of TDP-43 mediates full-length TDP-43 aggregation.

Figure 5

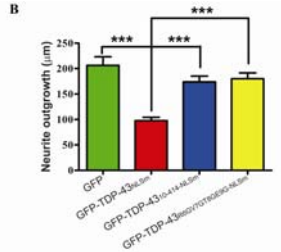

Figure 5. The extreme N-terminus of TDP-43 mediates full-length TDP-43 aggregation.

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