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COMPLEX COACERVATION AND MEDICAL SCIENCE

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ABSTRACT

Complex coacervation [formation of a dense macroion-rich phase (the coacervate) in equilibrium with a dilute macroion-poor phase (continuous phase or supernatant)] is a particular case of associative phase separation that occurs when oppositely charged macroions (or polyelectrolytes) are mixed. Since the pioneering work of Bungenberg de Jong and co-workers on gelatin–acacia gum complex coacervation in the 1920–40s, coacervates have received increasing research interest because a variety of mature and emerging technologies depend critically on the association of oppositely charged polymers or particles. To relate complex coacervation to medical science, disruption of normal intracellular complex coacervation, or gain of function mutations leading to abnormal complex coacervation, can cause disease. Benedek[1] used the term “molecular condensation disease” to broadly describe cataracts and other pathologies associated with abnormal protein phase separation. “Cold” cataracts are the reversible opacification of the lens by liquid–liquid phase separation of cytoplasmic crystallin proteins. As another example, the leading cause of fronto-temporal dementia may be a protein condensation disease caused by mistranslated arginine-rich dipeptide repeat proteins that insert or dissolve into liquid phase- defined intracellular compartments, disrupting their normal function.[2] Conceivably, better understanding of the physical chemistry of complex coacervation of biomacromolecules and the role of specific functional groups could lead to treatments for pathological intra- cellular phase separations.

Abnormal accumulation of TAR DNA-binding protein43 (TDP-43) is a pathological hallmark of amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease characterized by a selective loss of motor neurons, and a subtype of frontotemporal lobar degeneration (FTLD-TDP).[3] Recently, a number of studies revealed that many kinds of RNA-binding proteins (RBPs), including TDP-43, spontaneously develop granule-like structures via a liquid–liquid phase separation (LLPS) mechanism.[4,5] LLPS is a process in which proteins and nucleotides abruptly segregate into two distinct phases, enabling the formation of intracellular membrane-less organelles,[6] such as p-bodies[7] and stress granules.[8,9] Under physiological conditions, LLPS enables to achieve high local concentrations for molecular interactions and rapid chemical reactions, and allow fast changes of molecules upon signaling for facilitating various intracellular biological processes, e.g., transcriptional regulation and signal transduction.[10] However, once excess amounts of proteins are accumulated together with dysregulation of LLPS, the complexes quickly transform into pathological inclusions that are often found in neurodegenerative diseases.[11,12] ALS causative gene products, including FUS, TIA-1, and, of course, TDP-43, are proposed to form aggregates via LLPS. Consistent with this hypothesis, recent studies have discovered that optical multimerization of cytoplasmic TDP-43 induces the aggregation and sequestration of endogenous nuclear TDP-43 into the cytoplasmic aggregates that are dependent on LLPS.[13,14] As shown in Figure 1 A, B, 1,6-hexanediol (1, 6-Hd) drastically dissociated the cytoplasmic aggregates of RBPs, mutant FUS and TIA1. Consistent with the decreased number of RBPs-induced aggregates, sequestration of TDP-43 into the aggregates was also significantly prevented (Fig. 1C). These observations suggest that LLPS drives the cytoplasmic co-aggregation of RBPs and TDP43. In contrast, 1, 6-Hd did not influence either the number of aggregates of microtubule-related proteins (MRPs; mutant PFN1 and UBQLN2) or coaggregates with TDP-43 (Fig. 1 A–C), suggesting that a mechanism independent of LLPS drives the co-aggregation of MRPs and TDP-43.

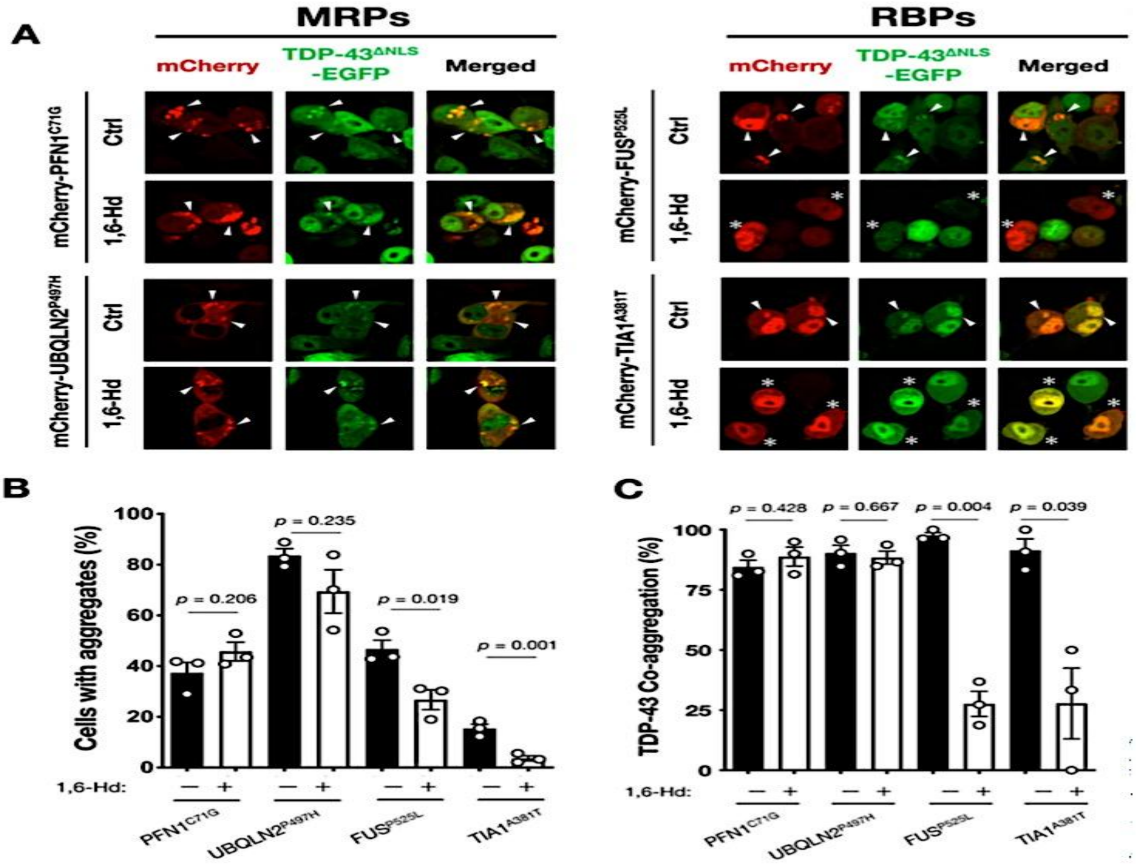

Figure 1: Liquid–liquid phase separation (LLPS) is involved in RBPs-induced TDP-43 co-aggregation. Administration of 1, 6-hexanediol (1, 6-Hd), an inhibitor of LLPS, dissociated TDP-43 co-aggregates with RBPs but not with MRPs in Neuro 2a (N2a) cells expressing TDP-43$^{\Delta NLS}$-EGFP. Representative images are shown in A. Both the ratio of the cells with TDP-43 aggregates (B) and TDP-43 co-aggregation (C) were reduced, specifically in the cells expressing RBPs with 1, 6-Hd. Arrowheads indicate co-aggregates of the RBPs and MAPs with TDP-43$^{\Delta NLS}$-EGFP. Whereas, asterisks indicate the cells without co- aggregates of the RBPs by the 1, 6-Hd treatment. More than 50 cells in each condition were analyzed for the quantification. Data are expressed as mean ± SEM (n = 3). This figure was reproduced from Watanabe, S.; Inami, H.; Oiwa, K.; Murata, Y.; Sakai, S.; Komine, O.; Sobue, A.; Iguchi, Y.; Masahisa Katsuno, M.; Yamanaka, K. Aggresome formation and liquid–liquid phase separation independently induce cytoplasmic aggregation of TAR DNA-binding protein 43. *Cell Death Dis.*, *11* (10), 909 (1-15), [15] Copyright 2020, Springer Nature.

The C9orf72 mutation is an expansion of a GGGGCC (G4C2) repeat in intron 1 of the gene. In unaffected individuals the G4C2 is repeated 2 to 23 times, whereas in those with the mutation, the sequence is expanded to contain hundreds to thousands of repeats.[16,17] Due to its location upstream of the coding region, the mutation can lead to a reduction in the levels of the protein that it encodes,[18,19] which is involved in the regulation of endo-lysosomal trafficking and autophagy.[20-22] However, a common finding from murine C9orf72 knockout models is the lack of neurodegeneration or TDP-43 pathology—a key pathological feature of C9orf72- ALS/FTD (frontotemporal dementia).[23-28] There is now substantial and clear evidence that a disruption in the phase separation behavior of proteins and RNA involved in the formation of liquid-like membraneless organelles explains much of the major pathological phenomena associated with C9orf72-ALS/FTD (Figure 2). Gain-of-function mechanisms associated with the G4C2 repeat expansion in C9orf72–G4C2 repeat RNA and the arginine rich DPRs poly-GR and poly-PR—undergo phase separation themselves and perturb the phase separation of LCD (low complexity sequence domains) containing proteins, resulting in abnormal membraneless organelle formation and dissolution, impairing their physiological functions and leading to neurodegeneration. Further pathological phase separation induced by the arginine rich DPRs is strongly associated with TDP-43 dysfunction and aggregation, the major pathological hallmark of C9orf72-ALS/FTD correlating with neuronal cell death. The targeting of abnormally phase separated condensates using small molecules or gene therapy provides a novel strategy for future therapeutics, although a greater understanding is needed of phase separation in order to design targets that are both beneficial and precise.[29]

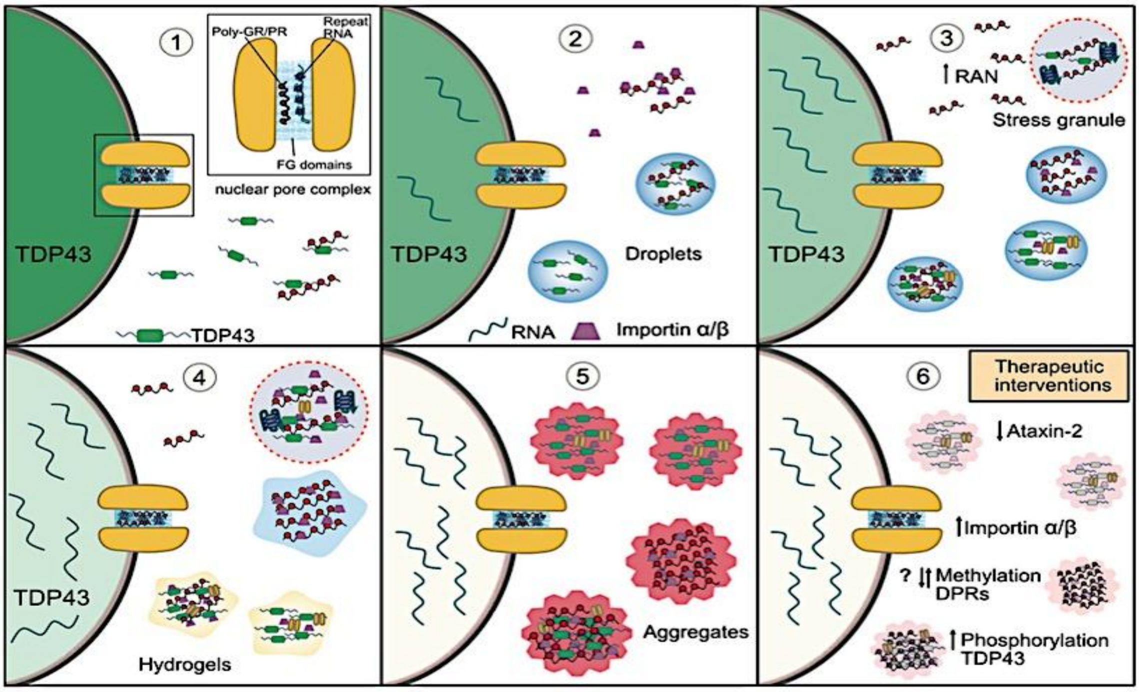

Figure 2: How disruptions in phase separation and membraneless organelles may lead to TDP-43 aggregation in *C9orf72*-ALS/FTD and possible therapeutic strategies. (1) *C9orf72* arginine rich DPRs and G_4C_2 repeat RNA bind nuclear pore proteins with phenylalanine rich repeats (FG domains) and result in nucleocytoplasmic transport dysfunction and mislocalization of TDP-43 to the cytoplasm. (2) Interaction between cytoplasmic TDP-43 and the arginine rich DPRs results in the LLPS of TDP-43 in the cytoplasm. Impaired nucleocytoplasmic transport also results in an accumulation of the importin-α/β complex (the import receptor for TDP-43) in the cytoplasm where it is also bound by the arginine rich DPRs and results in their reduced solubility. This begins a vicious feedback loop as impaired nuclear import of TDP-43 further increases levels of cytoplasmic TDP-43, whose LLPS is potentiated by a nuclear retention of mRNA from impaired nuclear export. (3) Cellular stress and the direct interaction of arginine rich DPRs and G_4C_2 RNA with stress granule proteins (including TDP-43) promotes phase separation and the formation of stress granules. The arginine rich DPRs also induce condensation of importin-α/β. TDP-43 droplets recruit importin-α/β complexes and nuclear pore proteins further impairing nucleocytoplasmic transport, resulting in more TDP-43 accumulation in the cytoplasm and depletion of nuclear TDP-43. Both stress granule accumulation and cytoplasmic TDP-43 also enhance RAN translation of the arginine rich DPRs. (4) The stress granules induced by the arginine rich DPRs and repeat RNA have reduced dynamics which entraps TDP-43, import receptors and nuclear pore proteins. Persistent TDP-43 and DPR-importin-α/β droplets are likely to mature into more solid-like states such as hydrogels, further immobilizing these proteins. (5) TDP-43 in solid-like states and within stress granules, and also DPRs, mature into pathological insoluble aggregates which further sequester proteins involved in nucleocytoplasmic transport. Thus, the disruption of phase separation and membraneless organelles leads to a cascade of vicious feedback loops which result in depletion of nuclear TDP-43 and its accumulation and aggregation in the cytoplasm in disease. (6) Therapeutic targeting of stress granules by reducing ataxin-2 levels, manipulating post-translation modifications such as methylation of the DPRs and phosphorylation of TDP-43, or increasing importin-α/β to reduce excessive LLPS may enhance the solubility of TDP-43, help to reduce its aggregation and ameliorate the pathological cascade in *C9orf72*-ALS/FTD and other TDP-43 proteinopathies. This figure was reproduced from Solomon, D. A.; Smikle, R.; Reid, M. J.; Mizielinska, S. Altered Phase Separation and Cellular Impact in C9orf72-Linked ALS/FTD. *Front. Cell. Neurosci.*, *15*, 664151 (1-24),[29] Copyright 2021, Solomon, Smikle, Reid and Mizielinska.

Notes

The authors declare no competing financial interest.

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