Degenerative neuromuscular diseases: Crucial gene and cell machinery discoveries

Degenerative neuromuscular diseases: from gene to cell machinery discoveries toward new therapeutic horizons

Amyotrophic lateral sclerosis (ALS) remains an elusive disorder both in term of pathogenesis and therapy. Its heterogeneity seems one of the major obstacles against the development of effective therapies, but also points to the need of personalized interventions based on the tailoring of medical treatment to the individual characteristics of each patient.

Currently the progress in gene discovery and cell biology has elucidated some putative key pathogenic mechanisms at least in genetic cases, opening a window upon a better hope for future disease-modifying therapies. The landscape of genetic causes of ALS is rapidly enriching, and in 2015 several new genes appeared on the stage, such as *CHCHD10*, *NEK1*, and *TBK1*. The latter gene seems to be particularly interesting, both considering how it was identified and its possible pathogenic mechanism. Cirulli and co-workers[1] utilized a whole-exome sequencing of Caucasian 2,869 ALS patients and 6,405 controls. They ran a standard collapsing analysis in which the gene was the unit of analysis, and coded individuals according to the presence or absence of qualifying variants in each sequenced gene according to several different genetic models. Results were then corrected for multiple testing. Several already known ALS genes were also identified, namely *SOD1, VCP* and *TARDBP*, confirming the quality of the analysis. *TBK1* encodes for TANK-binding kinase 1, a protein involved in autophagy and inflammation, two pathogenic mechanisms already proposed for ALS. Interestingly, TANK-binding kinase 1 binds and phosphorylates both optineurin and p62, two proteins respectively encoded by the ALS-related genes *OPTN* and *SQSTM1*, and seems to be an important component of the aggresome pathway required for the removal of pathological ribonucleoprotein inclusions. Patients with *TBK1* mutations are characterized by different phenotypes, including pure motor ALS, frontotemporal dementia (FTD), and ALS-FTD.

Another important step toward unravelling the molecular mechanisms underlying ALS is the identification by two independent groups[2,3] of defective nuclear transport in subjects carrying a GGGGCC (G_4C_2) hexanucleotide repeat expansion (HRE) in the first intron of the *C9ORF72* gene. This genetic defect is found in 50% of familial and 5% of sporadic ALS patients. Zhang and colleagues[2] and Freibaun and colleagues[3]

identified RanGAP, a protein interactor of G_4C_2 in a *Drosophila* model, as a key regulator of nucleocytoplasmic transport acting at the level of the external apparatus of the nuclear pore. The nuclear/cytoplasmatic (N/C) gradient of RanGAP was altered in the *Drosophila* model of C9ORF72 and it was successfully rescued by sense strand antisense oligonucleotide treatment. This novel pathogenic mechanism provides a promising new pathway for alternative therapeutic approaches in ALS.

Further advances in the understanding of pathogenic mechanisms of degenerative neuromuscular diseases and identification of putative treatment pathways are expected to come from the analysis of DNA transcription control. Altered RNA processing machineries causes several diseases, including neurodegenerative and mitochondrial disorders. ALS caused by mutation in *TARDBP* gene and spinal muscle atrophy type 1 and 2 (SMA) are examples of RNA splicing alteration related diseases. Alternative RNA splicing plays the key role to allow individual genes encoding for multiple proteins. This mechanism of proteome widening has enormous biological influence and huge level of complexity. However, to what extent single nucleotide variants (SNVs) can influence RNA alternative splicing is only partially known. Xiong and colleagues,[4] using an original machine-learning bioinformatic approach, discovered that more than 20,000 SNVs, including missense, nonsense and even synonymous SNVs, could regulate the number of exons in cell-specific mRNA. The authors successfully validated their novel computational model in SMA, the second most frequent autosomal-recessive disease of childhood and the most common cause of death in infancy. Their reliable prediction model expands the potentialities of genome-wide association studies through the identification of new disease-causing RNA splicing alteration that could represent putative therapeutic targets.

Altered balance between protein synthesis and degradation leading to intracellular accumulation of misfolded protein aggregates and failure of clearance mechanisms are emerging mechanisms in ALS and Charcot-Marie-Tooth neuropathy. Endoplasmic reticulum stress plays a key role in regulating the proteostasis through the activation of the unfolded protein response. A first-line adaptive response against misfolded protein accumulation is the phosphorylation of the eukaryotic translation initiation factor 2 (eIF2α) that leads to decreased protein synthesis. The enhancement of this self-limiting response seems crucial to rescue cells from misfolded protein accumulation. Das and colleagues[5] demonstrated that sephin

1 selectively binds and inhibits the regulatory subunit of eIF2α phosphatase and safely prevents molecular, morphological and motor impairment in superoxide dismutase 1-linked ALS and myelin protein zero-linked Charcot-Marie-Tooth (CMT1B) transgenic mouse models. These findings, while providing a specific background for disease-modifying treatment of ALS and CMT1B, open new scenarios on focused mechanism-related therapies for a broader range of diseases associated to misfolded protein accumulation.

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