How can an understanding of the C9orf72 gene translate into amyotrophic lateral sclerosis therapies?

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1. Summary

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease with few available treatments. The discovery of the mutation in the C9orf72 gene as the most common cause of familial ALS has provided new treatment targets. Here, we discuss how an improved understanding of pathogenic mechanisms of ALS caused by the C9orf72 mutation has led to the development of new therapeutic approaches.

2. Introduction

Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease of motor neurons with average survival of 2–5 years. Treatment options are limited to riluzole, a glutamate release inhibitor that extends lifespan an average of 3 months, and edaravone, a free radical scavenger that slows ALS progression in early disease [1]. The limited efficacy of these medications highlights the importance of identifying new treatment targets for ALS. Studying the pathogenesis of the 5–10% of ALS caused by mutations in known genes has provided insight into the underlying molecular mechanisms of ALS. Of these, autosomal dominant inheritance of a GGGGCC hexanucleotide repeat in the first intron of the C9orf72 gene is the most common familial cause of ALS [2], making nucleotide repeat expansion (NRE) in C9orf72 (C9-ALS) the leading known cause of ALS, and an appealing treatment target.

3. Pathogenic mechanisms of NRE in C9orf72

Multiple pathogenic mechanisms have been proposed for the C9orf72 NRE, which is typically less than 10 repeats in healthy individuals and several hundred repeats in C9-ALS. The NRE inhibits transcription of the C9orf72 gene; however, the lack of neurodegeneration observed in C9orf72 knockout mice suggests that loss-of-function is insufficient for motor neuron degeneration, at least in animal models [3]. Most evidence points to a gain-of-toxic-function induced by the NRE, though it remains unclear whether the major pathogenic mechanism is due to the NRE contained within the transcribed RNA itself, potentially sequestering RNA-binding proteins, or from repeat-associated non-AUG (RAN) translation of the NRE leading to production of dipeptide repeat (DPR) proteins [3]. It may be that these two mechanisms have synergistic effects, potentially combined with C9orf72 loss-of-function.

The recent discovery that a major cellular process affected by mutated C9orf72 is nucleocytoplasmic transport (NCT) [4–6] has opened promising avenues of research. Using a genetic screen in a Drosophila model expressing GGGGCC repeats, RanGAP, a major regulator of NCT, was identified as a suppressor of NRE toxicity [4]. RanGAP is a GTPase-activating protein (GAP) localized to the cytoplasmic face of the nuclear pore complex (NPC) that stimulates the hydrolysis of RanGTPase in the cytoplasm, favoring importin-mediated nuclear import of proteins containing a nuclear localization sequence (NLS). Importantly, RanGAP is inhibited by the GGGGCC repeat, leading to mislocalization of Ran to the cytoplasm in fly models and also in induced pluripotent stem cells differentiated into neurons (iPSN) from patients with C9orf72 mutation causing ALS [4]. This alteration of the Ran gradient has been shown to disrupt the nuclear import of NLS-containing proteins in fly and iPSN models of C9-ALS. Additionally, accumulation of nuclear transport proteins within stress granules (SGs) in response to cellular stress may be a common pathogenic mechanism in multiple neurodegenerative diseases including C9-ALS [7,8], and thus SGs are a promising therapeutic target upstream of NCT disruption.

Intriguingly, accumulating evidence indicates that NCT may also be disrupted in sporadic ALS. For example, mislocalization of TDP-43 from the nucleus to the cytoplasm in motor neurons is the pathogenic hallmark of >98% of all cases of ALS. Moreover, cytoplasmic TDP-43 oligomers themselves can disrupt NCT [9]. In fact, abnormal staining of nucleoporins (proteins that comprise the NPC required for NCT) has been seen in sporadic ALS brain tissue, indicating that NCT disruption may be a common pathway in ALS.

3.1. Therapeutics to target nuclear export

Given that disruption of NCT is likely a critical event in C9-ALS pathogenesis, attention has turned to devising strategies to restore NCT function. Many proteins contain both a NLS and a nuclear export signal (NES) and shuttle in and out of the nucleus. Since impairment of RanGAP favors cytoplasmic localization of these proteins, compounds that block nuclear export to
prevent cytoplasmic mislocalization of nuclear proteins may rescue deficits caused by disruption of nuclear import. In fact, selective inhibitors of nuclear export (SINE compounds) have been developed by Karyopharm Therapeutics and are currently in phase 3 clinical trials for malignancies. These drugs specifically inhibit Exportin-1 (XPO1), a protein that exports NES-containing proteins from the nucleus into the cytoplasm. Interestingly, SINE compounds suppress nucleocytoplasmic transport deficits and neurodegeneration in a Drosophila model of C9-ALS [4]. SINE compounds improve primary neuron survival and partially improve motor function in rats overexpressing TDP-43 [10]. In fact, a similar SINE compound, KPT-330, has been used in a phase 1 trial with dose escalation for multiple myeloma with promising results [11], indicating tolerability for SINE compounds in humans. Biogen has acquired the SINE compound KPT-350 which crosses the blood brain barrier for study in C9-ALS models, and a phase I trial in sporadic ALS is underway [12].

4. Antisense oligonucleotides

Antisense oligonucleotides (ASOs) are synthetic nucleic acids designed to target and alter mRNA that show great promise for targeted treatment of inherited neurologic diseases [13]. The FDA recently approved ASOs for treatment of both Spinal Muscle Atrophy (SMA) and Duchenne Muscular Dystrophy (DMD). ASOs can be used to inhibit the effects of genetic mutations that cause a toxic gain-of-function, or they can modulate the splicing or expression of genes whose loss-of-function causes disease (as in SMA and DMD). ASOs are a promising therapeutic modality; however, targeted delivery to specific tissues is limited, and they need to be delivered intrathecally to allow delivery to the brain and spinal cord.

ASOs have been used in animal models of genetic causes of ALS with promising results. Mutations in SOD1 (superoxide dismutase 1), the second most common known genetic cause of ALS, are thought to exert neurotoxicity through a toxic gain-of-function. In preclinical studies, intrathecal delivery of ASOs decreased SOD1 protein in the brain and spinal cord and significantly slowed disease progression in a transgenic mouse for SOD1 [14]. A subsequent phase I trial targeting SOD1 demonstrated safety [15], and a phase I-II trial on a second generation ASO targeting SOD1 (BIIB067; IONIS-SOD1RX) is currently ongoing. Interim analysis of this trial showed slowed clinical progression by BIIB067 treatment as measured by the ALS Functional Rating Scale-Revised in patients with SOD1-ALS. Furthermore, an ASO targeting Ataxin2, required for SG formation, rescues neurodegeneration in a TDP-43 mouse model of ALS [16], suggesting that ASOs inhibiting SG assembly may have therapeutic efficacy in sporadic ALS.

4.1. ASOs and C9orf72

Several animal studies have found beneficial effects using ASOs to target the C9orf72 hexanucleotide expansion. While evidence suggests that toxic gain-of-function by C9orf72 NRE is the primary mechanism of toxicity in C9-ALS/FTD, there is increasing evidence that loss of C9orf72 function may contribute to pathogenesis. Therefore, an important goal with targeting C9orf72 is to decrease mutant C9orf72 while maintaining expression of the normal allele. Supporting the feasibility of this approach, ASOs designed to target the expanded GGGGCC repeat without decreasing C9orf72 RNA levels significantly reduced the number of expanded repeat RNA foci per cell and percentage of cells containing RNA foci in fibroblasts and iPSCs derived from patients with the ALS C9orf72 mutation [17]. Of note, targeting C9orf72 with ASOs decreased the DPR poly-GP in iPSC cells [18]. Further, single dose ASO treatment of transgenic mice overexpressing the C9orf72 mutation rescued performance on behavioral tasks [18]. Given the above findings, Ionis Pharmaceuticals and Biogen are partnering on a phase 1 clinical trial using the ASO BIIB078 which began in January 2019. Since poly-GP levels in the cerebrospinal fluid (CSF) appear to be a stable biomarker for GGGGCC repeat expression in C9-ALS patients [19], measuring poly-GP may be used to confirm ASO target engagement in clinical trials. The clinical trial plans to enroll 80 patients with ALS, and patients will be treated with varying intrathecal dosing regimens and followed for a year to determine safety, tolerability, and pharmacokinetics (clinicaltrials.gov; NCT03626012).

5. Conclusions and future directions

Recent research exploring mechanisms of the pathogenesis of the C9orf72 mutation has led to the discovery of new treatment targets now in development for both C9-ALS and sporadic ALS. The discovery of nucleocytoplasmic disruption in C9orf72 ALS has led to a Biogen-sponsored phase I trial of a SINE compound first developed by Karyopharm Therapeutics. Other small molecules such as those disrupting SG assembly or the NRE tertiary structure are also in development for C9-ALS [20]. Furthermore, humanized monoclonal antibodies targeting the DPRs produced by the NRE are also being studied in animal models. Finally, the landmark success of ASO treatment for SMA suggests that the application of ASOs to ALS is a promising avenue of research, with clinical trials underway. These novel therapeutic approaches show great promise for future ALS therapeutics.

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Declaration of interest

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This paper shows that SINE compounds improve survival and motor function in iPSC and animal models of ALS.

This study shows that stress granule formation is required for NCT disruption in cell and fly models of C9-ALS and suggest that SG inhibitors are promising therapies.