● PERSPECTIVE

Amyotrophic lateral sclerosis disease modifying therapeutics: a cell biological perspective

Amyotrophic lateral sclerosis (ALS) is a progressively fatal neuromuscular disorder classically characterized by loss of upper and lower motor neurons from the cortex to the spinal cord. Diagnosed patients have a median survival of about 3 years and death usually results from eventual respiratory failure. The motor neuron disorder is clinically complex, and its diverse clinical presentations and multiple deciphered underlying pathologies indicate that it is a syndrome with overlapping disease phenotypes. 90–95% of ALS cases are sporadic and largely idiopathic. However, the 5–10% of cases that are hereditary in nature (fALS) has now a large number of implicated mutations and associated genetic variants (Renton et al., 2014). The first ALS causing mutation identified occurs in the Cu-Zn superoxide dismutase gene *SOD1*, but SOD1 mutations account for only about 20% of fALS. The subsequent identifications of other common mutations in the RNA/DNA binding proteins TAR DNA-binding protein 43 (TDP-43) and fused-in-sarcoma (FUS) resolved a pathological finding of ubiquitinated neuronal inclusions that are prominent in tissues of ALS subjects as well as those diagnosed with frontotemporal dementia (FTD), the latter primarily characterized by progressive neuronal loss in the frontal lobes. The more recently identified hexanucleotide (GGGGCC) repeat expansion in the intronic region of C9orf72 is the most common mutation in fALS and FTD (up to 40% of cases, also found in ~9% of sporadic ALS) (Bennion Callister and Pickering-Brown, 2014). These findings consolidated the ALS-FTD link, and precipitated a fresh notion that these disorders, as they were classically and neurologically defined, are basically different ends of a disease spectrum.

Given the devastating nature of the disease, many preclinical testings and clinical trials have gone into searching and testing potential therapeutic agents and disease modifiers, but thus far with no significant advances (DeLoach et al., 2015). The only FDA/EMA-approved disease-modifying therapy for some 20 years now is riluzole (6-(trifluoromethoxy)-2-aminobenzothiazole), proposed to act against glutamatergic excitotoxicity. Riluzole only prolongs median survival by 2–3 months, and does not reverse the course of motor neuron degeneration. Survivor prolonging intervention in ALS is limited to physical therapy and assisted ventilation at late stages. Like many other major neurodegenerative disorders, the varying degrees of therapeutic successes seen in animal models have not yet translate into significant benefits for human subjects. However, in some rare cases there are modest benefits, and newer, more disease mechanism targeting strategies are constantly being developed. There are a number of excellent recent reviews that have exhaustively documented the various preclinical and clinical trials of ALS therapeutics, and the readers are referred to these for a more detail update (DeLoach et al., 2015; Blasco et al., 2016). In this short perspective, I focus instead on the generic strategies underlying ALS therapeutics that have failed in clinical trials, and highlighted some possible neuronal cell biology based confounding factors against the expected beneficial effects. I then discuss the potential benefits of targeting ALS-specific pathology, and the possibility of enhanced benefits with combinatorial therapy.

Attempts at promoting motor neuron survival and possible caveats: Perhaps the most generic strategy in countering ALS is to attempt to keep the motor neurons alive, halting any neuromuscular denervation and hope for re-innervation. Pro-survival signaling mechanisms from neurotrophic factors are well known and these are effective in attenuating neuronal death in many *in vitro* and *in vivo* models of neurodegeneration. Likewise, vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), granulocyte-colony stimulating factor (G-CSF) and insulin-like growth factor 1 (IGF-1) have all been shown to attenuate motor neuron death, prolong survival and confer varying degree of preservation on motor function in ALS animal models, mainly mutant SOD1 transgenic mice or rats. However, all the larger phase II/III trials of BDNF, CNTF and G-CSF have not shown any benefits. For IGF-1, a double-blind, placebo-controlled, randomized study of 266 patients from multiple centers with treatment over 9 months revealed a very modest 26% slower functional impairment for the IGF-1 group compared to the placebo group (Lai et al., 1997). A phase II trial for VEGF is apparently underway after a demonstration of safety in a phase I trial. On the whole, it appears that these neurotrophic factors, when administered alone, will confer a very modest benefit at best to disease progression after symptom onset. Difference between rodent models and humans aside, perhaps the biggest confounding factor with this approach is that the point(s) of action is somewhat downstream of the cause of neuropathology and retrospective in terms of neuronal death induction. Neuroprotection *via* pro-survival signaling does not shut off the continuing pathological insult, and when given after certain survival thresholds were crossed would be too late to reverse the demise of compromised neurons.

Attempts at reducing general neuronal/neuromuscular pathology and possible caveats: Another, semi-generic type of strategy is to target common cellular pathologies that underlie multiple neurodegenerative diseases. These processes include chronic oxidative stress and mitochondrial dysfunction, endoplasmic reticulum (ER) stress, as well as neuroinflammation. Given the overlapping disease phenotype incurred by the different mutations in fALS, all of the above are likely relevant to the sporadic form of the disease to varying degrees. Of note, however, ALS trials with some of the most accessible drugs or compounds that have shown benefits in animal models have been disappointing. Pertaining to improving mitochondrial function, coenzyme Q10, olesoxime (TRO19622) and dexpramipexolehave all shown no benefits at phase II/III trials. The general antioxidant tocopherol (vitamin E) and N-acetylcystine were likewise ineffective. Trials with anti-inflammatory molecules and compounds such as NSAID celecoxib (Celebrex), thalidomide minocycline, the peroxisome proliferator-activated receptor gamma (PPARγ) agonist pioglitazone and erythropoietin showed that none of these confer any measurable benefits.

The fact that such a wide range of drugs and compounds with low toxicity and documented efficacy in antioxidant or anti-inflammatory action have all failed in phase II/III ALS clinical trials warrants a rethink of our perceived notions of how these reagents might be helpful. Again, the most likely reasons for failure may be that these interventions came too late for the subjects and acted far too downstream. However, there are two additional points that may be worth some consideration here. Firstly, neurons are highly active cell types in terms of mitochondrial respiration, which generate a good amount of reactive oxygen species (ROS). While it is generally true that externally imposed oxidative stress promotes neuronal dysfunction and demise, this is most pathologically relevant in situations of acute CNS injury, where high levels of reactive oxygen and nitrogen species (ROS/RNS) were generated in an acute and extended manner. On the other hand, mild elevation of ROS production has been shown to underlie preconditioning-induced neuroprotection (Ravati et al., 2001), and recent works in invertebrate models have indicated that mild ROS could be neuroprotective (Li et al., 2016) and indeed contributes towards lifespan

extension. In this regard, ROS induced signaling of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway is well known to be neuroprotective. Should an initial ALS-relevant insult on motor neurons generate mild ROS, any preconditioning effect that this may serve could in fact be wiped out by the broad action antioxidants used.

A second relevant consideration pertains to the actual benefits of suppressing neuroinflammation, with most of the compounds used targeting nuclear factor kappa B (NF-κB) signaling. Like oxidative stress, neuronal death resulting from glial cell-mediated CNS tissue inflammation is most prominent in the aftermath of injuries such as physical trauma and ischemia. There is also little doubt that microglia induction of motor neuron death occurs *via* NF-κB activation in these glial cells. However, what is often ignored is the fact that CNS neurons have a constitutive high level of NF-κB activation with RelA/p65 nuclear expression (Bhakar et al., 2002). This is likely a result of persistent neural activity and is critical for neuronal survival. In this regard, drugs that disrupt NF-κB signaling would also non-specifically (*i.e.*, regardless of CNS cell type) deprive CNS neurons of this constitutive mode of survival sustenance.

Targeting specific pathological mechanisms associated with ALS mutations and "personalized" combinatorial therapy: If our main courses of generic therapeutic strategies all failed at clinical trials because they carry some potentially confounding factors, how do we move ahead? First of all, in order to be able to halt disease progression, it would be critical to be able to target upstream, ALS-specific pathological mechanisms. In this regard, advances in the basic understanding of ALS pathology would be very helpful. Gain of toxicity in the most common fALS lesion that affects also sporadic ALS patients, C9orf72 hexanucleotide expansion, is likely due to sequestration of RNA binding proteins and/or translation into toxic dipeptide repeat proteins. Proof of principle that these could be alleviated by antisense oligonucleotides has been recently demonstrated (Jiang et al., 2016). A gene silencing approach could also be applicable to attenuating the toxicity of misfolded SOD-1, TDP-43 and FUS. On the other hand, agents that promote upregulation of chaperones (such as those of the heat-shock protein family) that may curb protein misfolding and their toxic accumulation, or those that could enhance their clearance *via* macroautophagy, would also be particularly useful against these pathologies. With regards to chaperone and autophagy induction, a potential route of therapeutics is the sirtuin activating compounds (STACs), such as resveratrol (Tang, 2016), which is yet to be tested in ALS patients.

Many factors contribute to drug efficacies in a trial, and pharmacological dosing and regimen refinements could certainly help. However, if there is a lesson to be learned from past failures, it would be that single-agent therapies, attractive as these may be in terms of convenience, may simply not work. We need therefore to up the level of sophistication, and use drugs in combination. In this regard, the drugs may target different pathological pathways, and their effects would simply be additive. An ALS pathological mechanism specific drug, in combination with an adjunct therapy that either attenuates toxic proteins or one that promotes pro-survival signaling, may predictively be one that would show a measurable benefit in clinical trials. It is also possible for the drugs to act synergistically, with one enhancing the effect of the other. Alternatively, instead of focusing solely on motor neurons, reagents that promote muscle function may also be of value. An example is tirasemtiv, a fast skeletal muscle activator that increases efficiency of muscle contraction, which is currently in phase III trial after some promising phase II results (Shefner et al., 2013).

What does the future hold? Moving forward, our cumulative and constantly improving knowledge of mutations and genetic variance that predispose one to ALS, coupled with the affordable price of whole genome sequencing, may allow clinical breakthroughs in two conceivable ways. The first is better ALS biomarkers for early diagnosis and more precise prognosis, not just for fALS but also possibly for sporadic ALS. The latter possibility stems from the fact that the more common fALS mutation, C9orf27 hexanucleotide expansion, is also identified in some sporadic cases, thus attesting to genetic tractability of the sporadic disease. More importantly, these may eventually allow specific tailoring of prophylactic therapies to susceptible individuals.

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