Molecular Therapy
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Commentary

Editing out the polymers: Toward a gene therapy for FENIB

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Rare genetic diseases pose a tremendous challenge in terms of developing a specific cure for each condition. Genetic editing holds promise in this direction, although the delivery of genetic correctors and the occurrence of undesired mutations remain major challenges. The manuscript by Konishi and colleagues[1] reports a strategy to correct a pathogenic variant of neuroserpin (NS) responsible for a rare but severe type of neurodegeneration called familial encephalopathy with neuroserpin inclusion bodies (FENIB). This disease is characterized by the formation of pathological polymers of mutant NS that aggregate within the endoplasmic reticulum (ER) of neurons to form characteristic inclusion bodies (IBs).[2] FENIB manifests with seizures, cognitive and motor impairment, and ultimately death, with no possibility of treatment or cure. This work shows, for the first time, a reduction in polymer deposition and clearance of NS polymers in cell culture models, as well as phenotypic improvement, upon correction of the mutation with a genetic tool (ABEs [adenine base editors]).

NS is a serine protease inhibitor (serpin) mainly produced by neurons of the central and peripheral nervous systems, where it participates in brain development and, in the adult brain, network remodeling by regulating synaptic plasticity.[3] Six point mutations have been identified so far in NS that cause FENIB, all of which affect protein stability and lead to polymer formation. The mechanism underlying NS polymerization has not been fully elucidated yet, but it may be similar to the one described for alpha1-antitrypsin (A1AT), which causes A1AT deficiency. In both diseases, a conformational change of the mutant protein causes pathological intermolecular interactions, which end up in the formation of stable chains of mutant serpin proteins, the pathological polymers. Interestingly, the instability caused by the mutation is proportional to the degree of polymerization and the associated clinical symptoms and inversely correlated with the age of disease onset. Studies in cellular and animal models of FENIB have demonstrated that part of the mutant NS is degraded by ER-associated degradation (ERAD) and part is secreted from the cells, but most of it escapes degradation and secretion and forms eosinophilic IBs typical of polymer deposition, most abundant in cortical and subcortical neurons. The presence of NS polymers is toxic, as it stimulates oxidative stress, mitochondrial alterations, and limited neuroinflammation.[4]

In this study, the authors established several cell culture models of FENIB using HEK293T cells as well as neurons derived from induced pluripotent stem cells (iPSCs) and driving the expression of wild-type or polymerogenic G392E NS by knockin or overexpression of a GFP-fused version of the proteins, under a constitutive or inducible promoter, with variable results. Indeed, some of the results obtained depend on the specific cell model used, limiting the impact of some of their findings. Using iPSC-derived neurons with inducible expression of G392E NS, they found that early termination of mutant NS overexpression led to polymer clearance, while halting the expression at a later stage prevented further deposition without achieving a decrease in polymer load. This observation is one of the most interesting results and, having been performed in neurons, one that is relevant to human disease. It suggests that stopping mutant NS expression in the neurons of patients at the onset of clinical symptoms, after years of polymer accumulation, would not be resolutive. To be useful, the correction should be made before intraneuronal polymer levels become toxic, so it may be more appropriate for children known to carry a disease-causing mutation before the onset of symptoms. This becomes particularly relevant as the authors demonstrate that genetic correction of the single point mutation can be achieved using ABE technology, converting the pathogenic adenine to guanine. Following correction, the authors observed reduced intracellular polymer accumulation in HEK293T cells and an improvement in the dendritic phenotype observed in iPSC-derived neurons carrying the pathogenic variant. The authors also addressed the issue of ABE delivery to neurons, showing that an engineered virus-like particle with enhanced neuronal specificity achieved good correction efficiency in iPSC-derived neurons.

In the past, attempts have been made to block the polymerization of mutant NS. Embelin, a small natural compound, was shown to prevent NS polymer formation and dissolve NS polymers *in vitro*, but its poor solubility and non-specific effects made it ineffective when tested in a cellular model of FENIB.[5] Although blocking the polymerization process would certainly slow disease progression, it would not rescue the inhibitory function of NS, which is affected by the mutation. The same applies to approaches aimed at reducing mutant NS levels, such as RNA interference or antisense oligonucleotides. NS inhibits tissue plasminogen activator (tPA), a serine protease regulating neuronal excitability and facilitating the propagation of seizures. Reduced inhibition by mutant NS has been postulated to favor the epileptic phenotype observed in patients with FENIB. Therefore, in addition to the clearance of the polymers deposited in neurons, an effective therapy must restore the protein levels of active NS necessary to fully rescue the manifestation of the disease. The strategy presented by Konishi et al., by correcting the disease-causing mutation using

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the ABE-mediated approach, promises to achieve this, although this type of editing still needs to be improved to achieve high levels of correction while avoiding bystander mutations.

In conclusion, the findings of Konishi and collaborators provide evidence that genetic editing prevents polymer accumulation and rescues dendritic arborization in cell culture models of FENIB and that early reduction of mutant protein expression allows the cell to remove existing polymers. In addition, the authors show promising advances to improve the efficiency of base editing in neuronal cells, as the ABE-mediated approach circumvents the need for an active cell cycle, as in the case for CRISPR-Cas9, and virus-like particles, which are thought to be less immunogenic and more specific compared to viral delivery systems, are able to deliver the editing system to iPSC-derived neurons. The next challenge will be to test engineered virus-like particles for delivery of ABEs in an animal model of FENIB and then enable safe and efficient translation of the therapy to patients with FENIB.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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