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CLINICAL IMPLICATIONS OF BASIC RESEARCH

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Finding a Treatment for ALS — Will Gene Editing Cut It?

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A key challenge in human medical genetics is developing the ability to suppress the expression of mutant genes that cause diseases that are transmitted as dominant traits. This is particularly true for the neurodegenerative disorders; many such disorders have dominantly inherited genetic forms and are not reversible with current therapies. One example is amyotrophic lateral sclerosis (ALS): approximately 15% of cases can currently be attributed to dominant, high-penetrance gene variants,[1] and many more ALS-causing genes probably remain to be discovered.

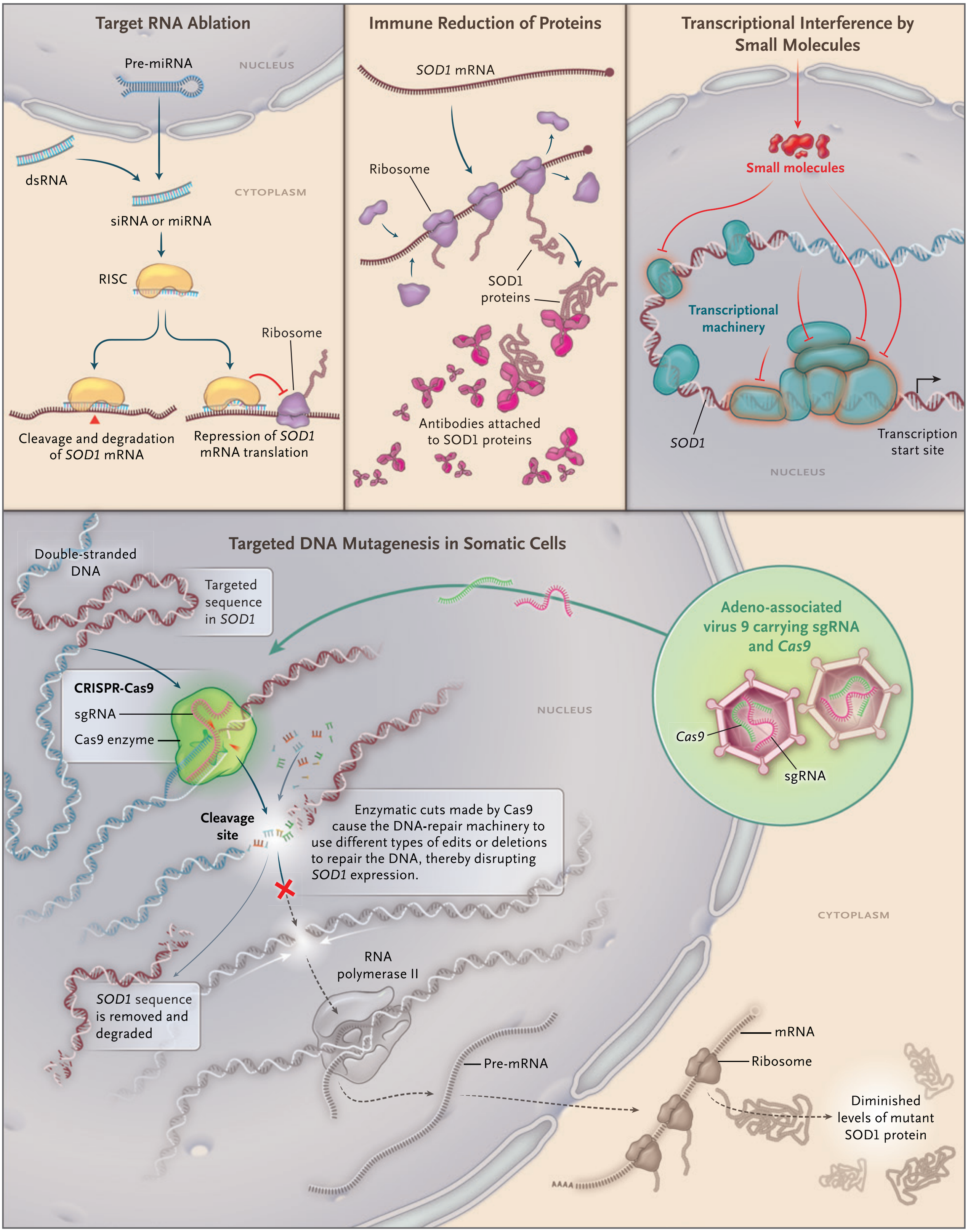
There are four approaches to suppressing the toxic effects of etiologic genes: the use of microRNA or antisense oligonucleotides (ASOs; complementary DNA or RNA sequences designed to pair with the target sequence and activate RNA degradation) for ablation of the RNA transcribed from the gene, reduction of the burden of the mutant protein (e.g., immune-mediated reduction), interference with the transcriptional process with the use of small molecules, and somatic-cell mutagenesis — that is, back-mutating the gene in the appropriate nongermline cells to its wild-type form (Fig. 1). Several reports have documented that the first three of these methods are feasible. The great advantage of the last approach is that correction of the mutant DNA eliminates downstream abnormalities and is at least in theory a one-time intervention.

However, somatic-cell mutagenesis has been elusive. Methods involving the use of specialized enzymes (e.g., zinc-finger nucleases) that are targeted to specific regions of DNA have proved to be inefficient. Particularly exciting, therefore, is the debut of methods that are based on enzymatic clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 gene editing.[3,4] CRISPR-Cas9, together with a single guide RNA (sgRNA), makes up a complex that targets a specific gene sequence — complementary to part of the sgRNA — for precise gene editing and correction. The challenge is delivering this editing system in vivo.

A study recently reported by Gaj and colleagues involved use of adeno-associated virus 9 (AAV9) to deliver CRISPR-Cas9 to transgenic mice that carried multiple copies of a human mutant *SOD1* (in which glycine is replaced by alanine at position 93).[2] ALS develops in these

Figure 1 (facing page). Four Ways to Suppress Gene Expression.

There are four approaches to suppressing the toxic effects of a mutated gene: ablation of RNA, immune reduction of protein, transcriptional interference with small molecules, and DNA mutagenesis. In target RNA ablation, sequence-specific pre-microRNA (pre-miRNA), which is encoded in the nucleus and sent to the cytoplasm, or externally introduced pre-miRNA or double-stranded RNA (dsRNA) is cleaved by the Dicer protein into 21-bp sequences; these sequences can be incorporated into the RNA-induced silencing complex (RISC). RISC then binds to the target sequence and ablates or represses it. RNA degradation can also be achieved by delivering single-stranded, modified DNA that targets the messenger RNA (mRNA); RNAse H then recognizes and degrades the RNA target. Immune reduction depends on antibodies that are directed toward the pathogenic protein. In transcriptional interference with small molecules, drugs that block enhancers or activators of transcription are used to reduce or prevent protein production. CRISPR-Cas9 mutates DNA by cutting through double-stranded DNA at a specific gene sequence, to which it is directed by a single guide RNA (sgRNA). After the cut is made, the DNA-repair machinery of the cell automatically tries to fix it, and it is at this juncture that different types of edits (e.g., deletions) can be introduced at the cleavage site. Gaj et al.[2] designed a CRISPR-Cas9 system to disrupt expression of human *SOD1* in a transgenic mouse model of amyotrophic lateral sclerosis; they packaged the CRISPR-Cas9 system into a virus vector and injected it into the facial veins of affected mice, after which levels of mutant protein declined in the lumbar and thoracic spines and the onset of disease was delayed. The abbreviation siRNA denotes small interfering RNA.


Target RNA Ablation
Pre-miRNA
NUCLEUS
dsRNA
CYTOPLASM
siRNA or miRNA
RISC
Ribosome
Cleavage and degradation of *SOD1* mRNA
Repression of *SOD1* mRNA translation
Immune Reduction of Proteins
SOD1 mRNA
Ribosome
SOD1 proteins
Antibodies attached to SOD1 proteins
Transcriptional Interference by Small Molecules
Small molecules
Transcriptional machinery
SOD1
Transcription start site
NUCLEUS
Targeted DNA Mutagenesis in Somatic Cells
Double-stranded DNA
Targeted sequence in *SOD1*
CRISPR-Cas9
sgRNA
Cas9 enzyme
NUCLEUS
Adeno-associated virus 9 carrying sgRNA and *Cas9*
Cas9
sgRNA
Cleavage site
Enzymatic cuts made by Cas9 cause the DNA-repair machinery to use different types of edits or deletions to repair the DNA, thereby disrupting *SOD1* expression.
CYTOPLASM
RNA polymerase II
SOD1 sequence is removed and degraded
Pre-mRNA
mRNA
Ribosome
Diminished levels of mutant SOD1 protein
AAAA

mice at approximately 90 days after birth, and the mice die at approximately 125 days. Within a day after the mice were born, Gaj et al. injected them through the facial vein with an AAV9 containing CRISPR-Cas9 and an sgRNA designed to disrupt *SOD1*, and systemic delivery was achieved. They detected a reduction in levels of mutant SOD1 protein in the lumbar spine and in the thoracic spine to 30% and 39%, respectively, of levels in untreated mice, with a 37% delay in disease onset (from a mean of 92 days after birth to a mean of 126 days). Although the rate of disease progression did not slow, the delay in onset prolonged overall survival by 25%. A disadvantage of this approach is that the activity of CRISPR-Cas9 is ongoing, which increases the risk of off-target effects, although these were minimal in the Gaj et al. study.

Several hurdles lie in the path between this proof-of-principle study in mice and the treatment of humans who have ALS. A key consideration is the timing of treatment. Gaj and colleagues delivered the gene-editing system immediately after birth, when the blood–brain barrier is most permeable, and before the disease was active. Although screening in utero for mutations in families with ALS might be possible, this would preclude treatment for people who have a genetic cause of ALS but do not have a family history; such cases are detectable only through population-level screening. The alternative is treatment after disease onset, but it is not clear whether that would be effective. Another consideration is that the gene-editing system used by Gaj et al. reduces the expression of both wild-type and mutant *SOD1* but is beneficial because insufficiency of wild-type SOD1 protein is not harmful. A benefit of this strategy is that one therapy can be used for any mutant *SOD1*. However, for other genes, an insufficiency of the wild-type protein could turn out to be pathogenic, in which case a strategy that targets only the mutated gene would be required. In that situation, since most variants causing ALS are rare, the paucity of persons harboring these variants would represent a challenge in clinical trial enrollment. As with other innovative therapies, another potential hurdle is the securing of regulatory approval.

There is evidence that the CRISPR-Cas9 approach is effective against structural changes to the genome, such as variations in the copy number of a gene; indeed, the mouse model in the Gaj et al. study harbors multiple copies of the transgene. In this regard, it is relevant that CRISPR-Cas9 inactivates multiple copies of porcine endogenous retrovirus in a kidney epithelial cell line. However, it is difficult to know whether treatment would be effective for common gene variations that have a small effect on risk or for the 1% of people with two rare variants underlying their ALS.[5] A final point is that it would also be instructive to inactivate variants that shorten survival (as opposed to increasing susceptibility): such a strategy could be effective in treating people after the onset of disease.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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