Correction of genetic disease by making sense from nonsense

Commentary

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Biological processes are mechanistically and architecturally conserved across all species. One of the best examples is the similarity between bacteria and humans in the protein synthetic machinery that catalyzes aminoacyl transfer to the COOH-terminal end of a growing polypeptide. As a consequence, aminoglycoside antibiotics disrupt translational fidelity in both species. Genetic and biochemical studies demonstrate that aminoglycoside antibiotics bind a specific site in ribosomal RNA and disturb codon-anticodon recognition at the aminoacyl-tRNA acceptor site. As a result, the ribosome introduces missense mutations and translates through termination codons in both bacterial and eukaryotic cells.

Growth of mammalian cells in the presence of an aminoglycoside antibiotic can suppress nonsense mutations (1). Suppression of a nonsense mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene by growth in gentamicin increased CFTR expression to 10–20% of the levels observed in control cells, and restored cAMP-activated chloride transport (2, 3). In this issue of the *JCI*, Barton-Davis et al. utilized the ability of gentamicin to disrupt translational fidelity to phenotypically correct a nonsense mutation in the Duchenne muscular dystrophy (DMD) gene in mice (4). This is the first in vivo application of this approach targeting a nonsense mutation in the *mdx* model.

Currently there is no treatment for DMD that results from defects in the dystrophin gene. Patients exhibit progressive muscle degeneration and die of respiratory or cardiac failure by their early-to-mid twenties. The *mdx* mouse has a nonsense mutation in the dystrophin gene, and has proved to be a useful model for testing different therapeutic modalities (5). Barton-Davis et al. demonstrate that gentamicin restores dystrophin levels to 10–20% of normal in all striated muscles examined. The accumulated dystrophin also led to assembly in the membrane of the dystrophin-associated glycoprotein complex. Although gentamicin did not completely restore dystrophin levels, the level produced was sufficient to protect against contraction-induced injury. This presents a significant first step toward the use of these antibiotics in treating a broad class of genetic diseases resulting from nonsense mutations.

Although gentamicin promotes translation read-through of the nonsense codon in dystrophin, it may also interfere with nonsense-mediated mRNA decay (6). All eukaryotic cells eliminate mRNAs that encode prematurely terminated products. This may protect cells from the production of nonfunctional or gain-of-function proteins. mRNAs that contain nonsense mutations more than 50 nucleotides upstream of the 3′-most exon-exon junction are selectively degraded by a process that requires protein synthesis. Stabilization of these mRNAs can occur if the ribosome translates through the termination codon (7, 8). The nonsense mutation in the *mdx* mouse reduces dystrophin mRNA levels to 10–15% of normal (9). Therefore, the beneficial effects of gentamicin may result from dystrophin mRNA stabilization. It will be important to study dystrophin mRNA levels in the muscle tissues to determine if these drugs exert their action by stabilizing the nonsense-containing mRNA. This appears likely, because gentamicin restored CFTR mRNA level in a cystic fibrosis (CF) bronchial epithelial cell line carrying a premature stop codon (3).

Unfortunately, long-term use of aminoglycoside antibiotics is associated with nephrotoxicity and ototoxicity. Despite extensive knowledge of the mechanism of action of these antibiotics in the lab, very little is known about what causes their toxicity in the clinic (10). It seems reasonable that general translation misreading would generate proteins with altered function that, if not folded properly, would be prone to form toxic aggregates. Alternatively, it is possible that mistranslated proteins may act in protein complexes in a dominant way to disrupt cellular functions or may display gains-of-function that are detrimental to the cell. In addition, aminoglycosides interfere with other RNA functions including self-splicing of group I introns (11), HIV replication by interaction with the Rev responsive element (RRE) (12), and hammerhead ribozyme cleavage (13). They are also known to disrupt plasma membranes and interfere with signal transduction involving phosphatidylinositol, possibly by inhibiting protein kinase C.

In the Barton-Davis study, not all animals responded to gentamicin, possibly owing to different rates of gentamicin metabolism. In addition, the animals that responded did so over a very narrow concentration range of gentamicin. Method of administration also influenced the outcome. As a consequence, extensive dosing studies may be required to maximize nonsense suppression and minimize toxicity.

Aminoglycosides that contain 2-deoxystreptamine (ring II) bind the decoding region in the 3′ end of the small ribosomal subunit rRNA (14, 15). However, the eukaryotic ribosome is significantly more resistant to these antibiotics, owing to a lower binding affinity. The aminoglycoside binding site in prokaryotic 16S rRNA has an A at position 1408 (*Escherichia coli* numbering), whereas the nucleotide in this position of the binding site in eukaryotic 18S rRNA is a C. The A1408 base pairs with A1493 and is required for high-affinity binding to ring I of the aminoglycoside (16). Substitution of A1408 to G in *E. coli* 16S rRNA confers resistance to aminoglycosides that contain a 6′ amino group on ring I, such as gentamicin, but has little effect on binding of aminoglycosides that have a 6′ hydroxyl group, such as paromomycin (17). The 6′ hydrogen bond donor on the aminoglycoside contacts the phosphodiester backbone between A1492 and A1493. Indeed, aminoglycosides with a 6′ hydroxyl group are most effective in stimulating

misreading in in vitro translation assays (18). Therefore, compounds such as paromomycin may be effective at lower doses and may display less toxicity than compounds such as gentamicin. An increased understanding of the role of glycoside rings in RNA binding will help the design of more specific compounds to target selective RNAs.

It may be possible to target drugs to the dystrophin mRNA to increase specificity and limit toxicity. For example, it might be possible to target the drug to muscle — perhaps by conjugation to compounds that bind to muscle fibers, such as acetylcholine derivatives. For diseases of the liver, it should be possible to target these compounds to the hepatocyte-specific galactose receptor by conjugation to galactose derivatives. It might also be possible to directly target ribosomes associated with the defective mRNA. For example, conjugation to antisense dystrophin oligonucleotides might provide greater specificity toward the defective dystrophin mRNA.

At present there is considerable enthusiasm and excitement for the potential of gene therapy to treat genetic disease. In weighing the potential advantages of gene therapy for DMD, there are several important considerations. First, gene therapy is significantly limited by its inability to efficiently transduce all muscle cells and to express significant levels of the protein (19). At present it is unrealistic to infect all muscles of the body by viral-mediated gene therapy. Transgenic expression studies in the *mdx* mouse demonstrate that a portion of the dystrophin gene is sufficient to provide a substantial increase in muscle function (20). In addition, significant improvement occurs with only 10–20% of normal dystrophin levels. Importantly, muscle function is much greater when all fibers express the transgene as opposed to only a fraction of the fibers (21). As a consequence, effective gene therapy would require efficient transduction of the majority of muscle fibers in a given muscle. Presently this is not possible. In contrast, gentamicin injection should provide systemic delivery to all muscle fibers, which might be why it was so effective in correcting the phenotype of the *mdx* mouse.

It is likely that clinical studies for gentamicin treatment of DMD resulting from nonsense mutations in the dystrophin gene will be initiated based on these exciting preclinical results. Many of the point mutations that result in DMD are due to nonsense mutations; missense mutations are rare, probably because no single missense mutation destroys dystrophin expression and/or function. It is estimated that as many as 10% of DMD patients have nonsense mutations. However, the gene defects in the majority of affected individuals have not been determined. Detection of nonsense mutations requires more extensive analyses than large gene defects, which are easily detected by routine PCR technologies. If nonsense suppression were to become a feasible treatment for genetic diseases resulting from nonsense mutations, then it would be important to identify the underlying gene defect in all patients.

DMD is an attractive disease to test the feasibility of nonsense suppression, because of the extensive clinical experience and defined clinical endpoints. The natural course of the disease is very well characterized, so that even a trial as short as 6 months may identify significant loss of progression or actual reversal of muscle function (22). In addition, periodic synthesis of dystrophin early in life may be expected to have a long-term beneficial effect, in part because of its long half-life.

The characterization of aminoglycosides on progression of DMD due to nonsense mutations is a first step toward the treatment of many diseases resulting from nonsense mutations. There are well over 600 Mendelian genetic disorders that could result from premature translation termination (23). Approximately 5% of patients with CF have nonsense mutations in the CFTR gene. In addition, the CF nonsense mutation Trp1282Stop is present in 60% of CF patients in the Ashkenazi Jewish population (24). Aminoglycosides might also prove to be beneficial in cancers that result from nonsense mutations in tumor suppressor genes, such as colon cancer. The promising results presented here should encourage studies in a variety of different genetic diseases.

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