

Gentamicin as gene therapy

Aminoglycosides are antibiotics that contain amino sugars linked to an aminocyclitol ring by glycosidic bonds. The drugs are polycations, poorly absorbed orally, only slightly protein bound, and are rapidly excreted by the kidneys. Aminoglycosides remain drugs of choice for life-threatening Gram-negative bacterial infections. However, serious toxicity to the kidney and the eighth nerve limits their use. Thirty years ago I became interested in the toxicity of gentamicin, which was still the most commonly employed aminoglycoside. Gentamicin's structure is shown in Fig. 1. My associates and I found that gentamicin is rapidly filtered and has a short plasma half-life, in rats only 30 min. To our surprise, we found that the half-life in kidney tissue was much longer, up to 100 h [1]. The drug was not concentrated in the medulla as we initially thought but instead in the cortex in proximal tubular cells. The proximal tubular cells formed bizarre lysosomal structures, termed cytosegresomes, before their departure through necrosis [2]. Silverblatt and Kuehn [3] speculated that a luminal receptor may be involved in aminoglycoside accumulation. Over 20 years later Willnow's group [4] showed conclusively that this

receptor is megalin, a large low-density lipoprotein-like receptor present in the kidney and the central nervous system.

Patients with cystic fibrosis commonly require treatment with aminoglycoside antibiotics. These patients are plagued with *Pseudomonas aeruginosa* upper respiratory infections and receive gentamicin and other aminoglycosides not only parenterally but also by means of inhalation. Clinicians were surprised how well the drugs were tolerated by children with cystic fibrosis and also observed that nephrotoxicity arose less frequently than clinically expected [5, 6].

The idea that my pediatrician friends may actually be engaging in "gene therapy" for cystic fibrosis while they were chasing away the *Pseudomonas* infections never occurred to me. Cystic fibrosis is caused by mutations in the gene coding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. CFTR is a member of the ATP-binding cassette family of transmembrane transporter proteins. Many mutations have been found that are responsible for cystic fibrosis. The mutations fall into five categories: stop codon mutations, missense mutations, frameshifts, in-frame deletions, and splicing mutations. The mutations all obviate CFTR function and thereby cause abnormal transport of both chloride and sodium across many types of epithelial tissues as well as disrupt other membrane-associated functions, including pH and ion regulation.

Hamilton [7] has published a brief commentary on how the mutations are likely to work. Nonsense, frameshift, splice-junction mutations, and premature stop mutations result in little or no CFTR protein translated from CFTR

Clinical Implications

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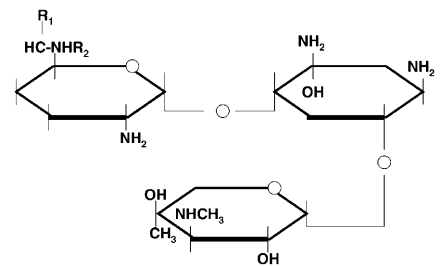


Fig. 1 The structure of the aminoglycoside antibiotic gentamicin C is depicted

mRNA. Class 2 mutations, such as the very common $\Delta 508$, result in a CFTR that is improperly processed or transported by the cellular transport machinery. Class 3 mutations result in CFTR proteins that are unable to function as chloride channels because of a regulatory problem. Class 4 mutations also result in a CFTR protein that cannot conduct chloride because of faulty transmembrane segments. Class 5 mutations cause early failure of protein biogenesis or impaired transcription, in part through faulty splicing of mRNA. A schema adapted from Hamilton is shown in Fig. 2. Thus far, replacing the function of the CFTR gene by inserting a new one has not been possible. However, since even a minimal increase in CFTR expression has a major ameliorative effect on the disease, overcoming trafficking defects pharmacologically has accrued great interest. Such compounds are currently under development.

About 10% of all patients with cystic fibrosis carry a premature stop mutation that is responsible for the dis-

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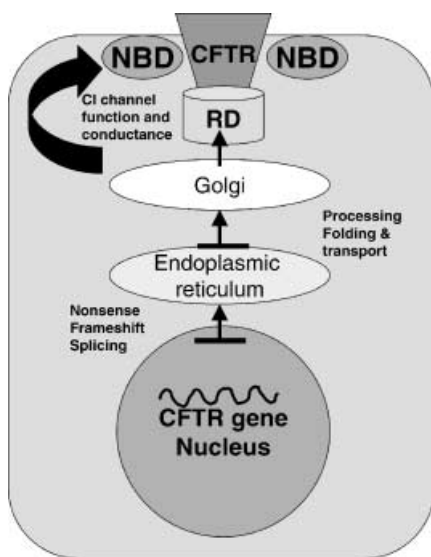


Fig. 2 The sites are shown at which mutations result in failure of the cystic fibrosis transmembrane conductance regulator (CFTR) regulator protein (adapted from [7]). *NBD* Nucleotide binding domain; *RD* is the regulatory domain. Gentamicin can induce “readthrough” of stop codon mutations

ease. Bedwell and colleagues [8, 9] reported that gentamicin can overcome the effects of stop mutations in the CFTR in cultured respiratory epithelial cells. Other investigators showed subsequently that aminoglycosides also increase the expression of full-length mRNA from a dystrophin gene that contained stop codon mutations in a mouse model [10]. A human trial of gentamicin therapy for cystic fibrosis has been published. The data are preliminary, but promising [11].

In the *Journal of Molecular Medicine*, Du et al. [12] construct a transgenic mouse from the CFTR^{-/-} gene disrupted background. The mouse was made transgenic for the human G542X cDNA under control of the intestinal fatty acid binding protein promoter. The idea was to develop a model in which human (h)CFTR expression could be relatively easily tested. The mice were treated with gentamicin or tobramycin in generous doses. Both aminoglycosides caused detectable

hCFTR protein expression at the apical surface of intestinal glands, although gentamicin was more effective. Short-circuit current studies showed that the system also worked functionally. In addition to demonstrating a proof-of-principle, the investigators provide a model with which to test compounds permitting a “readthrough” of stop codons.

Gentamicin and some other aminoglycosides thus have the ability to induce translational readthrough of stop codons. Howard et al. [13] performed an analysis of aminoglycoside-induced readthrough of stop codons in human tissue culture cells using a dual luciferase reporter system. Significant differences in the efficiency of aminoglycoside-induced readthrough were observed, with UGA showing greater translational readthrough than UAG or UAA. They also found that the nucleotide in the position immediately downstream from the stop codon had a significant impact on the efficiency of aminoglycoside-induced readthrough in the order C>U>A> or = G. Their studies showed that the efficiency of stop codon readthrough in the presence of aminoglycosides is inversely proportional to the efficiency of translational termination in the absence of these compounds.

The relevance of these ingenious studies is obvious. No one believes that gentamicin will have found a new job in the treatment of cystic fibrosis. However, as a model system to develop other compounds that perform this function more effectively with less toxicity, the old war-horse, gentamicin, has again risen to the occasion.

Respectfully,
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