Journal of Medical Sciences (2009); 2(1): 1-4

Editorial

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Suppression of Premature Stop Codons for the Treatment of a Subset of Patients with Genetic Disorders

H. Lee Sweeney, Ph.D.

Department of Physiology / B400 Richards Bldg., University of Pennsylvania School of Medicine, 3700 Hamilton Walk, Philadelphia, PA 19104-6085, USA

The concept of personalized medicine has been extended to recognize the fact that variations in the genetic makeup of individuals can greatly alter their responsiveness to drug treatments for a variety of disorders. An important corollary to this concept is that patients may have one of a number of different genetic diseases due to the same type of mutation in the disease-causing gene. This creates the possibility of a very different paradigm within personalized medicine, in which a drug can be designed to treat a specific genetic mutation, rather than a specific disease. The first example of this paradigm is drug-based suppression of a premature stop codon. There are a large number of individuals collectively afflicted by genetic disorders resulting form premature stop codons. Development of a drug-based therapy that can suppress this class of mutations could allow treatment of subsets of patients across a large number of genetic disorders, and thus address a substantial unmet medical need.

Correspondence
H. Lee Sweeney
Department of Physiology / B400 Richards Bldg., University of Pennsylvania School of Medicine, 3700 Hamilton Walk, Philadelphia, PA 19104-6085, USA
E-mail: Lsweeney@mail.med.upenn.edu

Within the patient populations of genetic disorders that arise as a consequence of mutations that result in the loss of a specific protein, premature stop codons (so-called nonsense mutations) are often the underlying defect.[1] These mutations, creating a UAA, UAG, or UGA codon in the coding region of the mRNA, result in premature translational termination. This causes production of a truncated protein that is non-functional and/or rapidly degraded. Furthermore, premature stop codons lead to mRNA destabilization through the process of nonsense-mediated decay (NMD) of mRNA.[1] Premature stop codons typically account for 5 to 20% of the individual cases of genetic disorders, including several cancers,[2,3] and their incidence is considerably higher (up to 70%) in populations with founder effects.[4]

Promoting read-through of premature stop codons with pharmacological agents was first investigated using aminoglycoside antibiotics. These drugs normally function by inhibiting bacterial protein synthesis at concentrations that do not alter protein synthesis in eukaryotic cells. However, it was first noted in yeast in the late 1970's, that higher concentrations of aminoglycosides could lead to suppression of premature stop codons in yeast.[5,6] These observations were extended to mammalian cells in culture.[7] This work first raised the possibility of the premature stop codon suppression as a possible treatment for human diseases, such as forms of thalassemia.[8,9] These observations were later extended to human cells.[10,11]

The first proof of principle experiments involving premature stop codon suppression in an animal model of a human disease utilized the mdx mouse.[12] This mouse is a model of the human disease, Duchenne muscular dystrophy, and importantly not only displays premature translational termination resulting in the

total loss of the protein known as dystrophin, but also shows significant nonsense mediated decay of the affected mRNA.[13] Thus this animal model faithfully reproduces both aspects of the disease-causing mutation that will be encountered in patients with disorders arising from premature stop codons.

The ability to suppress the effects of a disease-causing mutation and to generate therapeutic levels of missing protein with administration of the aminoglycoside, gentamicin generated considerable interest in moving toward clinical trials.[14] The gentamicin study in the mdx mouse[12] also provided insight into the types of diseases that might benefit from suppression of premature stop codons, stemming from the observation that production of the missing protein was at lower than normal rates and amounts. This was likely due to a combination of the suppression being inefficient and the mRNA levels being reduced (by NMD). This implied that there are at least two distinct scenarios in which premature stop codon therapies may be maximally effective: (1) Diseases in which a much lower than normal levels of the missing protein will be therapeutic, and (2) diseases in which the therapeutic protein has a very long half life and thus can accumulate to significant levels via premature stop codon suppression. Cystic fibrosis[15] and hemophilia[16] are examples of the first disease scenario, while Duchenne muscular dystrophy is an example of the second.[12]

Subsequent to the initial mdx mouse studies,[12] there were numerous studies performed in cultured cells and in mice aimed at demonstrating the feasibility of suppressing various human disease-causing premature stop mutations with aminoglycosides.[16-28] Importantly, a number of clinical trials also followed, using gentamicin in patients with diseases resulting from premature stop codons.[16,29-33] For example, topical application of gentamicin to the nasal mucosa of nonsense mutation-mediated cystic fibrosis (CF) patients for 14 days resulted in local CFTR protein production of the missing protein (CFTR) and functional improvements (chloride channel activity) resulting from the correct localization of the protein.[29-31] Similarly, intravenous gentamicin treatment in patients with stop codons in Duchenne muscular dystrophy[32] and hemophilia[16] promoted production of the missing protein.

The gentamicin proof-of-concept clinical experiments demonstrated that small molecules can promote read-through of premature stop codons and thus have the potential to suppress the disease-causing mutation in patients whose disease is caused by a premature stop mutation. However, the lack of oral bioavailability and the potential for serious renal and otic toxicities limit the clinical utility aminoglycoside therapy in a chronic disease. Indeed, positive results were not uniformly obtained in trials with gentamicin,[33] perhaps due to either under-dosing of the drug because of concerns with toxicity, or due to the variable heterogeneity of the gentamicin formulations.[34] Thus there was a clear need for a new class of drug, preferably not an antibiotic, that would be orally bioavailable and low in toxicity, and yet suppress premature stop codons with high enough efficiency to produce therapeutic levels of disease-associated proteins.

This need may have been met with the development of PTC 124.[35] PTC124 is a small molecule (<300 Da) that can be given orally and has shown low toxicity in animals and humans.[35,36] The drug was developed using high throughput screens with premature stop codons inserted into reporter genes in cultured cells, and then refined with medicinal chemistry efforts that utilized the mdx mouse as a human disease model. In experiments that paralleled the gentamicin studies in the mdx mouse, PTC124 was shown to produce dystrophin protein at levels sufficient to correct the major features of the disease phenotype.[35] Furthermore, experiments revealed that PTC selectivity suppresses premature stop codons, and not authentic stop codons, which likely is critical for the lack of toxicity. Based on this work, PTC124 has entered human trials in both Duchenne muscular dystrophy and Cystic Fi-

brosis. Interim Phase 2 results have been made available (http://www.ptcbio.com/3.1.1_genetic_disorders.aspx), and document production of therapeutic protein, as well as indications of efficacy, in a significant number of patients.

In summary, PTC124 may provide the first effective drug treatment for large number of patients afflicted with one of thousands of genetic diseases that can be caused by premature stop codons. As such it may allow us to forge a new paradigm in personalized medicine, in which patients are treated for a specific type of genetic mutation, rather than a specific disease.

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