Stony Brook University
The Graduate School

Doctoral Defense Announcement

Abstract

Targeting Nonsense-Mediated mRNA Decay for Cystic Fibrosis Therapy

By

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In spite of great advances in cystic fibrosis (CF) therapeutics, current therapies are not adequate for CF patients with the *W1282X* nonsense mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene that causes a severe form of CF. Overcoming very low expression of *CFTR-W1282X* mRNA due to nonsense-mediated mRNA decay (NMD) is a major hurdle in developing a therapy for this form of CF. CFTR-W1282X protein retains partial function, so increasing CFTR-W1282X protein levels by inhibiting NMD of its mRNA may contribute to CF therapy. Since the NMD machinery also regulates global mRNA expression, general inhibition of NMD may disrupt mRNA homeostasis and cause a broad range of detrimental effects in multiple tissues. Thus, a gene-specific NMD inhibition strategy may lead to an effective allele-specific therapy for CF. NMD requires the binding of protein complexes called exon junction complexes (EJCs) on spliced mRNA. An EJC bound downstream of a premature-termination codon (PTC) strongly enhances NMD of the target mRNA. Based on other studies and our own data, the *CFTR-W1282X* mRNA harbors multiple NMD-inducing EJCs. We previously showed that synthetic antisense oligonucleotides (ASOs) designed to prevent binding of multiple EJCs downstream of PTCs attenuate NMD in a gene-specific manner. These results suggested that a cocktail of ASOs could be used for stabilizing mRNA harboring certain disease-causing nonsense mutations. Using *CFTR* minigene NMD reporters, we identified lead ASOs that efficiently target individual EJCs downstream of the W1282X mutation. Combining the three lead ASOs specifically increases the expression of endogenous *CFTR* W1282X mRNA and CFTR protein in transfected human bronchial epithelial cells. All three EJCs >50 nucleotides downstream of the nonsense mutation have to be targeted for effective NMD inhibition by ASOs. Furthermore, the ASO cocktail increased the CFTR-mediated chloride current in human bronchial epithelial cells. These results set the stage for the development of an allele-specific therapy for CF caused by the W1282X mutation.

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Place: Hawkins Room, Wendt Building, Cold Spring Harbor Laboratory