

Cystic Fibrosis Research News

Title:

DECREASED MRNA AND PROTEIN STABILITY OF W1282X LIMITS RESPONSE TO MODULATOR THERAPY

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What was your research question?

The DNA in our cells contains the information passed from parents that makes each of us unique. Molecules called messenger RNAs (or mRNAs) are copies of this information and are used as templates for making full-length functional proteins. A nonsense mutation in DNA leads to a premature stop codon, which results in a shortened protein. Our cells have evolved a mechanism, called mRNA surveillance, for finding and destroying faulty mRNAs that contain a premature stop codon to prevent the synthesis of a potentially harmful, truncated protein. Our research questions were (i) Is *CFTR* mRNA with the nonsense mutation W1282X susceptible to mRNA surveillance? (ii) What measures could be taken to inhibit its destruction? (iii) Is the truncated CFTR protein with a W1282X mutation stable? (iv) Can CFTR modulators increase stability of W1282X protein?

Why is this important?

FDA-approved modulator therapies have transformed the treatment of CF; however, these treatments are not available for individuals with CF who harbor nonsense mutations such as W1282X. W1282X is one of the ten most common CF-causing mutations. Understanding *CFTR* mRNA surveillance in response to nonsense mutations, and identifying means to overcome *CFTR* mRNA decay are essential to develop treatments to improve the disease outcomes in individuals with CF caused by nonsense mutations. In this study, we measured the *CFTR* mRNA surveillance in an individual with CF who harbors two copies of the W1282X mutation, in comparison to her parents who carry one copy of W1282X each.

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What did you do?

First, we determined the *CFTR* mRNA abundance in the nasal cells of the individual with CF, and in her parents. Second, we sequenced all RNA molecules in the nasal cells of the individual with CF to determine which genes, other than *CFTR*, are differentially expressed compared to healthy controls and parents. Third, we treated nasal cells with NMDI-14, which inhibits degradation of mRNA. Lastly, we created an expression minigene (EMG) cell line model, which recapitulates mRNA surveillance, to assess the use of NMDI-14 and *CFTR* modulators as a treatment for individuals with nonsense mutations.

What did you find?

We found a marked reduction in the W1282X-*CFTR* mRNA abundance and protein stability in comparison to *CFTR* mRNA and protein with no CF-causing mutations. First, RNA isolated from the primary nasal cells showed significant reduction in the W1282X mRNA. Second, RNA-sequencing showed upregulation of a subset of mRNA surveillance genes. Third, inhibition of mRNA surveillance by NMDI-14 in the primary nasals cells resulted in the stability of W1282X-*CFTR* mRNA. Fourth, the cell line model demonstrated decreased stability of the W1282X-*CFTR* protein. Lastly, *CFTR* modulators in combination with inhibition of mRNA surveillance increased stability of W1282X-*CFTR* protein.

What does this mean and reasons for caution?

Taken together, we propose that evasion of mRNA surveillance should be considered in the development of therapies for individuals with CF carrying a W1282X mutation. However, it is important to be cautious doing this, because mRNA surveillance has a critical role in repressing the production of all truncated proteins, not just *CFTR*. Hence, inhibition of mRNA surveillance would allow other truncated proteins to exist, which could be harmful to the cells. Another point of caution is that this study included only one individual with CF, and repetition in more individuals to verify these results would be beneficial.

What's next?

A better understanding of the factors that control human mRNA surveillance will be essential to develop treatments that affect mRNA surveillance to improve disease outcomes. We suggest that future studies enroll extended family members to enable assessment of the role of genetic (and non-genetic) factors in mRNA surveillance.



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