The effect of the UPR and NMD mechanisms on the response to readthrough treatments

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Readthrough of nonsense mutations generates full length proteins. Variable response to readthrough treatment was found to correlate with CFTR transcript levels. Nonsense Mediated mRNA Decay (NMD) mechanism degrades nonsense carrying transcripts and affects the readthrough response. The CFTR protein is folded in the ER. Accumulation of unfolded proteins in the ER activates the Unfolded Protein Response (UPR), which alters the cellular transcription and translation. Since inefficient NMD leads to translation of truncated proteins that are not correctly folded, we hypothesize that:

1. Inefficient NMD leads to accumulation of truncated proteins in the ER that activates the UPR.
2. UPR further inhibits the NMD (which is translational dependent).

Results: Downregulation of the NMD by siRNA directed against hUPF1 induced the UPR, indicated by increased levels of UPR markers. Importantly, UPR activation by TM or DTT inhibited the NMD as revealed by increased levels of physiological NMD substrates. Subsequently, we studied 2 sisters homozygous for the W1282X mutations of whom one responded to readthrough treatments and had higher CFTR transcript level. Using SILAC we compared the entire proteome between cells derived from these sisters. Out of 6000 analyzed proteins, 440 showed a significant different level between the cell lines. At least 44 were ER proteins functioning in the UPR. Strikingly, a ~2 fold higher level of each of these proteins was found in the expressing and non-expressing experimental conditions we found an inverse correlation between expression and DNA methylation of each of the 3 analyzed zones of the 5 ENaC genes. They may be of interest for the comprehension of the mechanisms of NMD substrates. Subsequently, we investigated its consequences on mRNA level. We show that c.204 C reinforces an aberrant partial inclusion of STX1A intron 3, leading to nonsense mediated mRNA decay (NMD). Accordingly, c.204 C should be lower expressed, which is currently investigated by means of allele-specific quantification. Our results further indicate that STX1A mRNA expression is very low in epithelial tissues. Instead, we find STX1C, a splice-isoform of STX1A, to be up to 100 fold higher expressed than STX1A, indicating that not STX1A, but rather STX1C is an important player in CF pathophysiology. This finding additionally suggests that the exact role of STX1A in CFTR regulation should possibly be reconsidered.

Clinical and molecular characterization of the CF disease modifier syntaxin 1A (STX1A)

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Disease severity in cystic fibrosis (CF) varies greatly among patients, even when they carry the same CFTR mutation. This might partially be due to environmental factors, yet sibling studies strongly indicate that genes other than CFTR modify CF disease outcome. Syntaxin 1A (STX1A) has been reported as negative regulator of CFTR and other ion channels. We hypothesized that STX1A acts as CF modifier by influencing the remaining function of mutated CFTR. Applying linear mixed models to data obtained with the Bernese CF cohort revealed STX1A c.204 T>C to influence lung function parameters. Subsequent genotyping of c.204 T>C in the European CF sib and twin study revealed significant differences in allele frequency between mildly and severely affected sib pairs, thus confirming that the polymorphism modifies CF pulmonary disease.

Considering that c.204 T>C does not change the amino acid sequence of STX1A, we investigated its consequences on mRNA level. We show that c.204C reinforces an aberrant partial inclusion of STX1A intron 3, leading to nonsense mediated mRNA decay (NMD). Accordingly, c.204 C should be lower expressed, which is currently investigated by means of allele-specific quantification. Our results further indicate that STX1A mRNA expression is very low in epithelial tissues. Instead, we find STX1C, a splice-isoform of STX1A, to be up to 100 fold higher expressed than STX1A, indicating that not STX1A, but rather STX1C is an important player in CF pathophysiology. This finding additionally suggests that the exact role of STX1A in CFTR regulation should possibly be reconsidered.