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Editorial to Zaman et al. - S-Nitrosylation of CHIP Enhances F508Del CFTR Maturation

Putting CHIP(S) on the table – introducing nitrosothiols (SNOs) into the arena of CFTR modulation

C Addy1,2, Schock BC15.

1Centre for Experimental Medicine, Queen’s University of Belfast, Belfast, UK
2Northern Ireland Regional Adult CF Centre, Belfast Health and Social Care Trust, Belfast, UK

Corresponding author contact details:
Address: Queen’s University Belfast, Medical Biology Building, 97 Lisburn Road, Belfast BT9 6BL, UK
Phone: +44 2890 972258
Email: b.schock@qub.ac.uk

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Cystic Fibrosis (CF) is an autosomal recessive genetically based life-limiting disease, characterised by viscid secretions in multiple organ systems (1,2). The Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene, on chromosome 7, codes for a cAMP regulated chloride channel, on multiple epithelial surfaces, including the airways, pancreas, and intestine (1,2). Mutations affect the CFTR protein at any point through formation, maturation, intracellular trafficking, surface function and denaturation (1,2). Over 2000 mutations are described, with a phenylalanine deletion resulting in misfolded protein and accelerated degradation (F508del), the most common disease casing mutation (1,2). Defective CFTR function impairs chloride conductance, with associated defective sodium transport, across epithelial surfaces, generating the viscid secretions which are the hallmark of CF (1).

**Drug development and life expectancy**

Multi-disciplinary centre-based care, combined with therapeutic advances have dramatically increased life expectancy for people with CF (PWCF); with infants born today predicted to survive for almost 50 years (2). Precision therapies capable of modulating the underlying cellular defect (CFTR modulators) represent the most significant therapeutic advance in CF (2). With early diagnosis through new-born screening, an increased lifespan and CFTR modulation, clinical care is moving away from managing downstream complications, towards targeted therapies to prevent disease progression (1,2,4).

**CFTR modulators including combinations**

The first CFTR modulator, Ivacaftor, a CFTR potentiator, improves channel gating, at epithelial surfaces, potentiating chloride conductance and CFTR function, with resultant clinical improvement (3). Only 4% PWCF (2), carry “gating” mutations responsive to Ivacaftor, where sufficient defective CFTR traffics to the cell membrane (2,3), to provide substrate for Ivacaftor (1-3). Subsequently, combination therapy, combining a CFTR corrector, Lumacaftor or Tezacaftor, which promote CFTR production, with Ivacaftor to potentiate, has widened the range of mutations amenable to CFTR modulation, including F508del (4). The clinical benefits of current combination therapy require optimisation (2,5). Whilst early results of triple combination therapy (two correctors and one potentiator) appear promising, the search for CFTR modulators suitable for all PWCF continues (2,4,5). Gene agnostic CFTR amplifiers, which increase the protein substrate for CFTR modulators, may widen the scope for future combination therapies (5). Even with a portfolio of promising pre-clinical and clinical studies, a closer insight into molecular pathways associated with CFTR trafficking may provide necessary additional therapeutic targets (2,5).
Molecular pathway associated with F508del CFTR trafficking and maturation

Into this arena, in this issue, the study by Zaman et al. (6) highlights a novel alternative to current approaches to CFTR modulation. While correctors, potentiators and amplifiers all target the promotion of CFTR production (2,5), Zaman et al. focus on reducing ubiquitination and proteasomal degradation, increasing CFTR trafficked to the cell membrane (6). The F508del CFTR mutation, impairs protein folding and assembly, detected by the cell’s quality control (QC) system. Polyubiquitination of defective CFTR leads to its extraction from the endoplasmic reticulum (ER) (7).

Detection of defective CFTR in the cytoplasmic region is performed by the chaperone heat shock protein (cHSP)-70 (8). cHSP-70 interacts with the E3 ligase carboxy terminus of heat shock protein 70-interacting protein (CHIP), which in association with the E2 ligase UbcH5a ensures ubiquitination and transport of misfolded CFTR for degradation by the cells’ quality control system (ubiquitin-proteasome system, UPS) (7,8). Targeted therapies could inhibit ubiquitin-dependent degradation of CFTR, shielding it from the UPS machinery, with CHIP inhibition identified as a potential pharmacological target (9) (Figure 1).

Nitrosylation of CFTR

Nitric-oxide synthases (NOS) are abundant in the respiratory epithelium and are essential for the formation of stable bioactive s-nitrosothiols (SNOs), important in cellular signalling, -NO transfer and regulation of protein function (10). Dysregulation of SNO formation is implicated in multiple respiratory diseases (10), including cystic fibrosis. Nitrosylation inhibits E3 ligases (11), increases CFTR maturation (12) and chloride transport (13) in CF airway epithelial cells. Zaman et al. (6) hypothesized that SNOs could inhibit CFTR proteasomal degradation, through s-nitrosylation-dependent inhibition of CHIP. They show CHIP expression in primary cells and bronchial cell lines, with dose-dependent s-nitrosylation of CHIP after treatment with GSNO (S-nitrosoglutathione), reducing CHIP expression and activity. This leads to a reduction in CHIP-CFTR, decreasing CFTR ubiquitination, allowing F508del CFTR to escape the ER QC system and traffic to the cell membrane. This, for the first time, shows the mechanisms behind the effect of s-nitrosylation on CFTR expression (6) (Figure 1). Of all SNOs tested, s-nitrosoglutathione diethyl ester (GNODE) showed the highest effect on CFTR maturation, but in the current study was tested only in cell monolayers (6). To progress towards CFTR modulating therapies, cell permeable GSNO analogues, including GNODE, capable of replicating these findings in differentiated pseudostratified epithelium are required.

Bioavailability and bio-activation of SNOs, may be lower in pseudostratified epithelium, potentially reducing the overall effect of GSNO on CFTR expression (14).
Are the CHIP(S) ready for the table?

The modulation of the QC system may increase CFTR trafficked to the cell surface (6, 7), but the impact on CFTR dependent chloride transport and later denaturation, requires further investigation, both in-vitro and in-vivo (2,5). Previous in-vivo studies using SNOs within CF, focused on their anti-inflammatory, smooth muscle relaxant and bronchodilatory potential (15). A randomised trial of inhalational GSNO demonstrated tolerability, but only modest effects on oxygen saturations (15). Whilst Zaman et al. (6) focus on the potential of GSNO as an inhalational agent, all current CFTR modulators are oral, for systemic CFTR modulation (3,4). CF is a multi-system disease, and the systemic effects of CFTR modulators contribute significantly to their clinical benefits (1-5). Future studies are needed to investigate pharmacological modifications of QC systems and effects on proteasomal degradation of CFTR, which may differ reliant on cell and tissue dependent expression (7). With nitrosylation of CHIP being dose-dependent (6), optimising therapeutic ranges across cell types and individuals may be challenging. Furthermore, any adverse effects of nitrosative stress (excessive NO / reactive oxygen species) which can contribute to NO-induced neurotoxicity (11), must be excluded.

Conclusion

This study highlights a novel therapeutic target, nitrosylation of CHIP, with potential for innovative CFTR modulation. The selective modification of the QC machinery remains an attractive approach, which, with further exploration of therapeutic targets in the pathway shown by Zaman et al. (6), may generate systemic therapies for the benefit of PWCF. The potential for combination therapy, with other CFTR modulators, could optimise future clinical benefits (Figure 1). Zaman et al. (6) may have brought a future new player to the table, and further study will determine what impact this has on those already there.
**Figure 1:** CFTR modulation: Through the action of CHIP (carboxy terminus of heat shock protein 70-interacting protein), misfolded CFTR (deltaF508) is targeted towards proteasomal degradation (left). CFTR correctors enhance CFTR transcriptional and translational output allowing for some CFTR to be trafficked to the apical surface. Here, CFTR potentiators enhance the channel gating resulting in chloride conductance (right). In future CFTR amplifiers could increase the CFTR substrate available for CFTR correctors. Alternatively, nitrosylation of CHIP (nitrosothiols) leads to reduced CHIP expression and activity allowing misfolded CFTR to escape the quality control system, enhancing CFTR maturation. The addition of CFTR potentiators may provide further benefits.
References:


