Reaching the surface in CF

By Lev Osherovich, Senior Writer

Researchers at **The Scripps Research Institute** have found that inhibiting histone deacetylase 7 may improve ion transport in the lung cells of patients with cystic fibrosis by helping to properly fold mutant CFTR protein.¹ Although the precise mechanism is not yet known, **Proteostasis Therapeutics Inc.** is collaborating with the researchers to develop screens, and the company hopes to develop small molecule stimulators of protein folding to treat CF.

CF is caused by hereditary defects in cystic fibrosis transmembrane conductance regulator (CFTR), an ion pump that helps keep the lung epithelium hydrated and free of bacterial infections. Patients who inherit two copies of the $\Delta F508$ allele, which is responsible for two-thirds of CF cases, make a misfolded version of the protein that does not get transported to the cell surface and instead is flagged by the endoplasmic reticulum for degradation by the proteasome.

Biophysical studies have shown that the $\Delta F508$ protein is functional if artificially placed on the cell surface.² Thus, whereas previous efforts have sought to completely correct the CFTR gene, more recent approaches have focused on rescuing the defective protein from degradation and redirecting it to the cell surface.

Vertex Pharmaceuticals Inc. is pursuing the latter strategy with VX-809, a small molecule co-developed with **Cystic Fibrosis Foundation Therapeutics Inc.** that is in Phase IIa testing to treat $\Delta F508$ -linked CF. The compound corrects $\Delta F508$'s structure and triggers transport of the ion channel to the cell surface.

Now, a team led by William Balch, professor of cell biology at Scripps, has found a new way to avoid $\Delta F508$ degradation and facilitate its mobilization to the cell membrane by blocking histone deacetylases (HDACs) that regulate the chaperones responsible for folding CFTR.

Previous *in vitro* screens targeting $\Delta F508$ have found molecules that “stimulate CFTR activity, or structure correctors that enable its folding,” said Balch, who consults for and has invested in Proteostasis.

Modulating chaperone activity is “a completely new tack to solve the folding problem,” he said. “If the folding of a protein is wrong, anything that boosts the chaperone system even indirectly would help CFTR function.”

Proteostasis interim CSO Walter Newman said Balch's study shows that subtly perturbing the folding environment of a particular protein, in this case the defective version of CFTR, could lead to a treatment for a disease such as CF.

“Balch's data suggest that the trafficking of CFTR needs to be pushed in a certain direction,” said Newman. The approach therefore overlaps with the company's strategy of treating diseases by fine-tuning protein folding and trafficking, he said.

According to Newman, Proteostasis and Scripps are discussing a joint effort to identify HDAC7 inhibitors and to test them in CF.

CF suppressant

Based on earlier studies by Balch's team suggesting that proper CFTR folding was determined by HDAC-regulated chaperones,³ he hypothesized that small molecule HDAC inhibitors could help stimulate acceptable folding of $\Delta F508$ CFTR. This, in turn, was expected to increase CFTR trafficking to the cell surface and CFTR-mediated ion transport.

To test the hypothesis, the team used Zolinza vorinostat, a broad-spectrum HDAC inhibitor marketed by **Merck & Co. Inc.** to treat cutaneous T cell lymphoma (CTCL).

Cultured CF lung cells treated with vorinostat had higher CFTR expression and ion transport than mock-treated control cells, indicating that the drug allowed the defective CFTR protein to move to the cell surface and function correctly. Vorinostat treatment raised the activity of $\Delta F508$ to 28% of that found with wild-type CFTR.

The results were published in *Nature Chemical Biology*.

Newman said those seemingly modest improvements in surface levels of CFTR could translate to meaningful clinical outcomes. “Even restoring 25% of protein expression could be useful,” he noted.

Because vorinostat inhibits as many as 11 different HDAC homologs, Balch's team next used small interfering RNA to sort out which of the enzymes actually mediated the drug's effect on CFTR trafficking. The team found that siRNA knockdown of HDAC7 corrected the defect in $\Delta F508$ nearly as well as vorinostat, suggesting that this enzyme is likely the most relevant target of the Merck compound.

Elizabeth Joseloff, senior project manager in the medical department of the **Cystic Fibrosis Foundation**, which partially funded Balch's work, said that identifying a specific target is a key step because she thinks nonselective HDAC inhibitors may have too many off-target effects to be useful for treating CF in children, the main target population. Because so many biological processes are affected by HDACs, “developmental effects are a concern,” said Joseloff.

She also noted that CF therapies would most likely require chronic administration and that the effects of persistent HDAC inhibition are unknown.

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—Walter Newman,
Proteostasis Therapeutics Inc.

Alternatively, Balch thinks improved CFTR activity could be achieved with intermittent HDAC inhibition, citing experiments in the *Nature Chemical Biology* paper in which a brief burst of vorinostat led to higher levels of $\Delta F508$ on the surface of cultured cells for several days.

Chaperone hypothesis

A remaining question is how exactly HDAC7 inhibition improves the folding of the mutant CFTR.

Balch believes HDACs regulate the activity of heat shock protein 90 (Hsp90), a key component of the protein chaperone network that recognizes mutant CFTR as a problem protein.

"Hsp90 is an HDAC substrate," he noted. "By manipulating Hsp90 with specific HDAC inhibitors you can modulate its activity" to aid proper CFTR folding.

Chris Penland, director of research at the Cystic Fibrosis Foundation, said it may be too early to attribute the effects of HDAC inhibition to Hsp90 versus other chaperones.

"Other chaperones are also involved in targeting mutant CFTR for degradation," he said. "Whether Hsp90 is the culprit here remains to be seen."

Even if Hsp90 turns out to be the key actor, Proteostasis' Newman thinks it may not be an ideal target because of Hsp90's central role in coordinating the folding of many proteins. Instead, he said an unbiased screening approach could identify the best point of intervention in the chaperone network.

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man. "We may find that upregulating other chaperones could also be effective."

Balch's next step is to screen for selective HDAC7 inhibitors and to work out the mechanistic details of how HDAC7 inhibition affects the activity of Hsp90 and other chaperones on mutant CFTR folding and activity.

He noted that there is no mouse model of CF but said a recently developed pig model of the disease⁴ will be useful for testing therapeutic candidates.

Balch has filed patents on targeting HDACs to treat CF and said he is working with Proteostasis to develop screening procedures based on these patents.

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COMPANIES AND INSTITUTIONS MENTIONED

Cystic Fibrosis Foundation, Bethesda, Md.

Cystic Fibrosis Foundation Therapeutics Inc., Bethesda, Md.

Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.

Proteostasis Therapeutics Inc., Cambridge, Mass.

The Scripps Research Institute, La Jolla, Calif.

Vertex Pharmaceuticals Inc. (NASDAQ:VRTX), Cambridge, Mass.