Sponging out cystic fibrosis

By Lev Osherovich, Senior Writer

A Canadian team has found that compounds from sea sponges could be useful for treating cystic fibrosis. The compounds work in part by inhibiting a new family of targets for the disease: poly(ADP-ribose) polymerases.¹ The researchers now plan to work backward to uncover why blocking these polymerases improves the function of cystic fibrosis transmembrane conductance regulator, the mutated protein that causes cystic fibrosis.

CF results from loss-of-function mutations in cystic fibrosis transmembrane conductance regulator (CFTR), an ion channel that helps lubricate the epithelial lining of lungs, pancreas and intestine. Patients who inherit two mutated copies of *CFTR* develop thick mucus and are prone to severe respiratory infections and digestive problems.

The most common CF-associated CFTR mutation, $\Delta F508$, is a genetic deletion of a single amino acid within the protein. Whereas wild-type CFTR functions on the cell surface, the $\Delta F508$ protein is flagged as defective by cellular quality control mechanisms and becomes trapped in the endoplasmic reticulum (ER).

Notably, the $\Delta F508$ version of CFTR can still function as an ion channel if it can make it to the cell surface. Thus, one therapeutic strategy has been to find compounds that change the ER's metabolism to allow the mutant CFTR to proceed to the cell surface.

An alternative strategy, pursued by **Vertex Pharmaceuticals Inc.**, is to hit mutant CFTR directly with molecules that fix its structure, allowing the defective protein to pass muster with the ER quality control system.

Since 2001, a team led by David Thomas, chair of the Department of Biochemistry at **McGill University**, also has run screens in collaboration with **GlaxoSmithKline plc** to find small molecules that improve cell surface expression of CFTR.

Now, the McGill team has reported on two new hits coming out of those screens—latonduine A and latonduine B (ethyl). The two closely related compounds originally were isolated from the sea sponge *Stylissa carteri*.

"Most of the molecules found so far have been either direct structural modulators of CFTR or metabolic regulators of ER quality control," said postdoctoral research associate Graeme Carlile, the team's coleader. "This molecule, latonduine, falls into the latter category."

GSK researchers were not authors on the paper, and the company declined to comment on the work.

Under the C

Carlile and Thomas screened a library of 720 compounds from marine organisms and found that latonduine A and latonduine B (ethyl) increased the surface expression of transgenic $\Delta F508$ CFTR in hamster cells compared with vehicle.

The increase in cell surface expression of $\Delta F508$ CFTR allowed the protein to do its job of transporting chloride ions out of the cell. Latonduine A-treated lung epithelium cells from patients with CF had about half the ion secretion of cells expressing wild-type CFTR, whereas untreated cells had nearly zero ion secretion. Likewise, mice with the $\Delta F508$ Cfr mutation had higher levels of salivary secretion after latonduine A treatment than untreated controls. Because latonduine A was the better compound, it was used in subsequent experiments.

As latonduines did not appear to directly bind CFTR, Carlile and Thomas made a radiolabelled derivative of latonduine and used it to pull down a family of proteins that bound to it *in vitro*.

Those proteins all turned out to be members of the poly(ADP-ribose) polymerase (PARP) family. PARPs add the post-translational modifier poly(ADP-ribose) to a variety of proteins that regulate many basic cell processes.

More than a dozen PARPs have been identified in humans, and several have been implicated in cancer. At least three PARP inhibitors are in Phase II testing for cancer: olaparib (AZD2281) from **AstraZeneca plc**, veliparib (ABT-888) from **Abbott Laboratories** and rucaparib (CO-338)

from partners **Clovis Oncology Inc.**, **Pfizer Inc.** and **Cancer Research UK**. The anticancer effects of those molecules are thought to result primarily from inhibition of PARP-1 and PARP-2.

In contrast, Carlile and Thomas found that latonduines *in vitro* inhibited another family member, PARP-3, more potently than other PARPs. Moreover, partial knockdown of PARP-3 but not other PARPs enhanced the effect of latonduine A on $\Delta F508$ CFTR surface expression in cell culture.

Although PARP-3 knockdown made CF cells more sensitive to latonduine A, knocking down the gene in the absence of the drug did not by

itself improve $\Delta F508$ CFTR. This suggests there are other targets besides PARP-3 that contribute to the compound's effect.

Carlile suspects latonduine may act by inhibiting other PARPs in addition to PARP-3. He said one likely suspect could be PARP-16. In October, **Massachusetts Institute of Technology** researchers reported that PARP-16 resides in the ER and participates in the unfolded protein response, putting it in the right place to potentially play a role in CFTR trafficking.²

Results were reported in *Chemistry & Biology*.

PARP for the course

The question now is why reducing PARP activity has a beneficial effect on $\Delta F508$ CFTR.

"There's not much literature on PARP-3, but what we do know is that it works in the nucleus," said Carlile. "Prior to this work, we had no evidence of PARP involvement on CFTR metabolism."

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**—Graeme Carlile,
McGill University**

Carlile said that the first-generation latonduines used in his study are most likely not suitable as therapeutics because of their promiscuous binding to multiple PARPs. Thus, he plans to conduct SAR studies to find latonduine derivatives with even higher specificity for PARP-3 over other PARPs.

"I think the significance of the work is not so much about finding a corrector of $\Delta F508$ CFTR dysfunction but rather the identification of a potentially novel target," said Frederick Van Goor, head of biology in the CF research program at Vertex. "Using this molecule to pull out this target was not necessarily expected."

Vertex's VX-809 acts on mutant CFTR and corrects its structure so that it can leave the ER.³ The compound has completed Phase II testing in CF in combination with Kalydeco ivacaftor, a potentiator that enhances CFTR's ion transport activity.

Vertex markets Kalydeco for patients who carry a different CFTR mutation, G551D, which leads to impaired ion transport by the protein.

"As multiple targets are involved in CFTR processing and trafficking, it remains to be determined if hitting one target in particular, such as PARP-3, would have a sufficient effect," said Van Goor.

It is possible that latonduine derivatives with higher selectivity for PARP-3 might turn out to be less effective than the original promiscuous compounds. On the other hand, broad-spectrum PARP inhibitors run the risk of safety problems due to knock-on effects on multiple targets.

He suggested that further mechanistic cell culture studies are needed to uncover what happens to $\Delta F508$ CFTR when PARP-3 or other PARPs are inhibited. He also recommended testing latonduine derivatives in other cell culture and animal models that more closely resemble CF than the transgenic cell lines used by the McGill team.

Van Goor also noted that inhibiting PARPs could alter the trafficking of other proteins besides CFTR. Because of the concerns about knock-on effects on other proteins, he said Vertex has focused its screening efforts on compounds that are selective for CFTR.

Carlile said preliminary data suggest inhibiting PARP-3 does not affect the bulk flow of proteins out of the ER, "so there is some hope that this is a specific interaction with CFTR."

According to Carlile, the composition of matter and therapeutic use of latonduines were previously patented by **The University of British Columbia**. Researchers at that university were coauthors on the current study.

At least two other companies—Pfizer and **Proteostasis Therapeutics Inc.**—are developing small molecule therapeutics specifically for $\Delta F508$ CFTR.

Under a deal announced in November, Pfizer will receive up to \$58 million from **Cystic Fibrosis Foundation Therapeutics Inc.** (CFFT), the drug development affiliate of the **Cystic Fibrosis Foundation**, to develop preclinical candidates for $\Delta F508$ CFTR-associated CF. The co-development deal follows up on a 2007 deal between CFFT and FoldRx Pharmaceuticals Inc., which Pfizer acquired in 2010.

In May, CFFT partnered with Proteostasis to identify small molecule modulators of $\Delta F508$ CFTR folding and function.

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COMPANIES AND INSTITUTIONS MENTIONED

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