Halofuginone target ID

By Joanne Kotz, Senior Editor

A U.S. and South Korean research team has identified the molecular target of halofuginone, a small molecule antifibrotic and anti-inflammatory compound.[1] The findings could help guide the design of next-generation halofuginone analogs with improved pharmacological profiles and may help determine the specific indications most amenable to treatment with the analogs.

Halofuginone is a halogenated derivative of the plant alkaloid febrifugine, which is found in hydrangea roots. In preclinical animal models, halofuginone prevents fibrosis by decreasing fibroblast-mediated production of extracellular matrix proteins such as collagen and lowers inflammation by blocking the differentiation of autoimmune-mediating T helper type 17 (Th17) cells.

However, clinical development of halofuginone has been stymied by the molecule's poor drug-like properties and GI toxicity.

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—Mark Lupher, Promedior Inc.

"Halofuginone is an interesting molecule in the context of fibrosis research, as it is one of only a few small molecule compounds identified early on in the field that could inhibit fibroblast production of collagen. Ironically, we did not know the specific molecular target for any of these early compounds when they were identified. Although relatively potent in cell-based assays, halofuginone's poor oral bioavailability, questionable tolerance profile and limited patent life prevented it from being developed successfully," said Mark Lupher Jr., CSO at fibrosis company **Promedior Inc.**

"A new understanding of its specific molecular target, however, could show promise in enabling the identification of new chemical classes with superior pharmacologic profiles to the canonical halofuginone," Lupher said. "If a potent, safe and bioavailable analog could be identified, it would have potential in a large number of fibrotic and inflammatory indications."

A team led by Malcolm Whitman, Tracy Keller, Ralph Mazitschek and Chang-Yeol Yeo set out to identify halofunginone's target.

Whitman is a professor of developmental biology and Keller is an instructor in developmental biology at the **Harvard School of Dental Medicine**. Mazitschek is an assistant professor in the radiology department at **Harvard Medical School** and co-director of the chemical biology platform in the Center for Systems Biology at **Massachusetts General Hospital**. Yeo is an assistant professor in the division of life and pharmaceutical sciences at **Ewha Womans University**.

The researchers had a good starting point. In 2009, they reported in *Science* in collaboration with Anjana Rao that halofuginone induced the amino acid response pathway in fibroblasts and Th17 cells,[2] the two cell types through which halofuginone exerts its effects.

Rao is a professor at the **La Jolla Institute for Allergy & Immunology**.

The amino acid response pathway is triggered by nutritional stress that results from low levels of amino acids and leads to decreased levels of protein synthesis as a way to conserve nutrients and improve cellular survival.

Now, the researchers have probed the protein synthesis pathway to look for components that are altered or impaired in the presence of halofuginone.

An *in vitro* protein translation assay showed that halofuginone inhibited translation of a reporter gene only if the resulting protein contained proline residues. That result suggested halofuginone inhibited the prolyl-tRNA synthetase domain of glutamyl-prolyl-tRNA synthetase (EPRS), a dual-function tRNA that binds glutamate and proline residues and incorporates them into proteins. Indeed, halofuginone inhibited the prolyl-tRNA synthetase domain of EPRS with a K_i of 18 nM *in vitro*.

In cell culture, both the antifibrotic and anti-inflammatory effects of halofuginone were reversed by the addition of proline.

Collectively, the results confirm that EPRS is halofuginone's target. Results were published in *Nature Chemical Biology*.

Building a better inhibitor

Shelia Violette, VP of research at **Stromedix Inc.**, said it should now be possible to determine whether halofuginone's GI toxicity is due to effects on the amino acid response pathway or off-target effects.

Stromedix's STX-100, a humanized mAb against integrin $\alpha_v\beta_6$, will be tested in a Phase II trial to treat idiopathic pulmonary fibrosis (IPF) to be initiated later this year. In February, **Biogen Idec Inc.** announced it was acquiring Stromedix.

"If the data remain strong and indicate that the beneficial effects of halofuginone are related to activation of the amino acid response pathway and that toxicity can be dissected away, then one could build a series of chemical compounds around this target that have attractive drug properties, such as oral bioavailability, stability, half-life and pharmacokinetics," said Violette.

Indeed, Whitman said the team now plans to do just that, using proline rescue to investigate whether the molecule's toxicity is on target or off target.

Lupher said the identification of EPRS as halofuginone's target will enable two previously unavailable approaches for identifying new chemical classes of EPRS inhibitors. One option is *in vitro* high

throughput screening assays using the purified prolyl-tRNA synthetase domain of EPRS. The other is obtaining a crystal structure of halofuginone bound to EPRS to facilitate structure-based drug design.

"We're now thinking of how to distribute the drug better *in vivo*," Whitman said.

Mazitschek told *SciBX* the team already has identified halofuginone derivatives that retain activity and have better solubility and stability in solution.

The researchers are using these derivatives as a starting point for building prodrugs that are activated systemically to overcome GI toxicity. Finally, the team is developing topically active compounds that limit systemic exposure.

Mazitschek is a cofounder of **Shape Pharmaceuticals Inc.**, which has a topical histone deacetylase (HDAC) inhibitor in Phase I testing for cutaneous T cell lymphoma (CTCL).

Probing the pathway

Knowing halofuginone's target also will help investigations of the amino acid response pathway and its role in disease.

"The identification of a molecular target of halofuginone opens the door to asking more specifically how activation of the amino acid response pathway relates to the occurrence and progression of various human diseases. This information could help fine-tune the disease indications that might be affected by impacting this pathway," said Violette.

"There wasn't a good tool compound for the amino acid response, so it's been difficult to interrogate this highly conserved pathway," said Mazitschek. The team is now looking at the effects of perturbing the amino acid response pathway in rheumatoid arthritis (RA) and other preclinical models of fibrotic and inflammatory diseases, Whitman added.

Whitman said the team is also collaborating with an undisclosed company looking for druggable targets further downstream in the pathway.

At least one company has a halofuginone analog about to enter the clinic. By year end, **Halo Therapeutics LLC** plans to start Phase II testing of its HT-100 to treat Duchenne muscular dystrophy (DMD), in which muscle fibrosis occurs during disease progression.[3] The company said its compound lacks the GI issues of the parent molecule. Halo declined to discuss whether the paper has implications for HT-100's development.

The researchers have filed patent applications covering therapeutic uses of tRNA synthetase inhibitors in inflammatory and fibrotic indications and covering composition of matter of the new halofuginone derivatives. The IP is available for licensing through the **Harvard University Office of Technology Development**.

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