PCSK9 peptide inhibitors

By Lauren Martz, Staff Writer

Although at least 10 companies are chasing inhibitors of PCSK9 for decreasing low-density lipoprotein cholesterol, the compounds dominating the field are antibodies or siRNAs that require injection. Roche's Genentech Inc. unit and a separate team from Pfizer Inc. and The University of Queensland are betting that peptides might provide an oral competitor and have identified short peptide fragments that inhibit binding of PCSK9 to its target.^{1,2} Optimizing the potency and stability for oral delivery is the next challenge.

Genentech is not disclosing plans for future development of the peptides. Pfizer did not reply to enquiries regarding future plans for its compounds.

Figure 1. PCSK9-mediated regulation of LDLR levels. Proprotein convertase subtilisin kexin type 9 (PCSK9) is highly expressed in the liver and is a validated target for hypercholesterolemia. In *Chemistry & Biology*, researchers showed that truncated peptide analogs of the epidermal growth factor-like A domain (EGF-A) of the low-density lipoprotein receptor (LDLR) block receptor interaction with PCSK9 and may reduce circulating LDL cholesterol.²

[a] PCSK9 is a protease that is synthesized as an enzyme precursor. Following synthesis, PCSK9 undergoes autocatalytic cleavage, which is required for secretion from the cell. Although cleaved, the two domains of PCSK9 remain together.

[b(1)] When LDL cholesterol binds to LDLR, the receptor ligand is internalized, thus removing LDL cholesterol from circulation. When LDL binds a PCSK9-bound LDLR, the entire complex is internalized. PCSK9 then directs the LDLR to the lysosome, where it is degraded and prevented from recycling to the cell surface. The short peptides block the interaction between PCSK9 and its EGF-A-binding site on LDLR.

[b(2)] If an internalized LDLR is not bound to PCSK9, the receptor is recycled to the cell surface, where it continues to remove LDL cholesterol from circulation.

A number of companies are developing compounds that decrease PCSK9 activity, blocking synthesis of the protein with RNA-targeting therapeutics, mAbs or small molecules that inhibit PCSK9 binding to LDLR.

The low-density lipoprotein receptor (LDLR) decreases circulating LDL cholesterol by binding the lipid at the hepatocyte cell surface, causing internalization of the LDL-LDLR complex. Inside the cells, LDLR dissociates from LDL and recycles to the cell surface, whereas the lipid is degraded by the lysosome.

PCSK9 (proprotein convertase subtilisin/kexin type 9) is a key regulator of LDL that acts by decreasing surface levels of LDLR, which reduces uptake of the lipid from the blood.

PCSK9 is synthesized as an inactive proenzyme and undergoes autocatalysis to form the active protein that binds LDLR. When the PCSK9-bound LDLR binds LDL, the whole complex is internalized. PCSK9 then directs the bound LDLR to the lysosome, where it is degraded and prevented from recycling to the cell surface (see **Figure 1, "PCSK9-mediated regulation of LDLR levels"**).

The protein has thus become the focus of intense activity as a target for lowering circulating LDL by blocking the interaction between PCSK9 and LDLR.

Several PCSK9-targeted antibodies and siRNA candidates are in clinical and preclinical development for various dyslipidemias (see **Figure 1, "PCSK9-mediated regulation of LDLR levels"**). However, these compounds all require parenteral administration, and orally available inhibitors still lag far behind.

Serometrix LLC and **Shifa Biomedical Corp.** have disclosed preclinical small molecule programs for oral inhibitors of PCSK9. Serometrix is developing allosteric ligands of PCSK9 that disrupt normal protein folding to inhibit LDLR binding. Shifa is developing small molecules that block the autocatalytic cleavage of PCSK9 to prevent secretion from the cell and small molecules that block the interaction between PCSK9 and LDLR.

In general, protein-protein interactions do not lend themselves to small molecule inhibition. 3D crystallography shows that the site of interaction between PCSK9 and LDLR is relatively flat and does not have pockets that enable small molecule binding.³

Now, two separate teams have taken different approaches to exploring whether peptides could block the binding interaction better than small molecules, offering a potential alternative for creating oral inhibitors of PCSK9.

Short peptide inhibitors

David Craik and colleagues used rational drug design to create peptides that would competitively inhibit the interaction between PCSK9 and its binding site on LDLR, which lies in the receptor's epidermal growth factor-like A domain (EGF-A).²

Craik is laboratory head of the Chemistry and Structural Biology Division at the University of Queensland. The study also included researchers from Pfizer.

The team tested various truncated analogs of EGF-A and found a 26-amino-acid peptide analog lacking the C-terminal region that bound PCSK9.

To optimize the peptide's binding affinity, they introduced a gain-of-function mutation associated with genetic predisposition to hypercholesterolemia that enhances the binding of LDLR to PCSK9.

In binding assays, the modified analog bound PCSK9 with a K_d of $\sim 0.6 \mu\text{M}$, which was about twofold more potent than binding for full-length EGF-A. The peptide also competitively inhibited binding of PCSK9 to LDLR in *in vitro* assays and promoted recycling of LDLR to the cell surface in PCSK9-treated hepatocarcinoma cells. In the latter two assays, the peptide's potency was about five- to sevenfold lower than that of full-length EGF-A.

Results were published in *Chemistry & Biology*.

In the Genentech study, the team screened phage-display libraries for peptides that bound PCSK9 and then optimized the highest-affinity peptides using SAR studies.¹

The most potent peptide was a 13-amino-acid mimetic of EGF-A that had structural features comparable to those of the full-length EGF-A and bound PCSK9 via similar intermolecular contacts.

In binding assays, the phage display-derived peptide bound PCSK9 with a K_d of $\sim 0.7 \mu\text{M}$. This peptide also restored LDL uptake in hepatocarcinoma cells pretreated with PCSK9.

Data were published in *The Journal of Biological Chemistry*.

There is no overlap between the peptides identified in the two papers.

Peptide hurdles

Craik told *SciBX* that the next steps for his team include improving the potency and biopharmaceutical properties of the peptides. His team has not yet tested the peptides in animal models.

Converting peptides to oral therapeutics has proved difficult in many therapeutic areas.

Roger Newton, executive chairman and CSO at **Esperion Therapeutics Inc.**, said, "The biggest challenge with peptides is achieving oral delivery, which requires high stability, small size and potency. Unless the peptides are modified for protection, they will be proteolytically degraded in the stomach and intestines before they even have a chance at a therapeutic benefit."

Esperion has ETC-1002, a small molecule ATP citrate lyase (ACLY) inhibitor and AMP-activated protein kinase (AMPK) activator, in Phase IIb testing to treat hypercholesterolemia.

Serometrix CEO Michael Muehleemann said that one way to avoid the problems with peptide drug delivery is to convert small peptides into small molecules. However, he does not expect this strategy will work for the EGF-A peptides.

He told *SciBX* that his team looked at competitive inhibitors of EGF-A binding early in the PCSK9 program. Although the researchers identified some peptides that blocked binding, the activity decreased as they reduced peptide size to enable oral delivery.

Muehleemann added that based on the size and relatively flat nature of the orthosteric EGF-A site, there would be a high risk in trying to develop peptides small enough for conversion to small molecules.

Newton added, "The work provides great proof of concept, but this work is still *in vitro*. Taking the peptide therapeutics to the whole-animal level and proving efficacy is the next step and a big challenge."

He noted, "The take-home message is that it is possible to disrupt the interaction with small peptides, which is a significant advancement. However, the amount of optimization that the teams went through in these papers speaks to how hard it would be to develop an effective oral peptide."

If the teams can overcome the hurdles associated with designing oral peptides, the peptides could have benefits over existing cholesterol-lowering drugs and PCSK9 mAbs.

"There is a big market for statin alternatives. Two to seven million patients in America are intolerant of statins due to side effects such as muscle pain," said Newton. "Everyone in the field is looking for a compound that is an improvement over statins."

Craik did not disclose the patent status of his work. The work was sponsored by and licensed to Pfizer.

The patent and licensing status of the peptides from the *JBC* paper is unavailable.

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Contact: Daniel Kirchhofer, Genentech Inc., South San Francisco, Calif.
e-mail: dak@gene.com

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**—Roger Newton,
Esperion Therapeutics Inc.**

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Contact: David J. Craik, The University of Queensland, Brisbane, Queensland, Australia
e-mail: d.craik@imb.uq.edu.au
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COMPANIES AND INSTITUTIONS MENTIONED

Esperion Therapeutics Inc. (NASDAQ:ESPR), Plymouth, Mich.
Genentech Inc., South San Francisco, Calif.
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
Serometrix LLC, Pittsford, N.Y.
Shifa Biomedical Corp., Malvern, Pa.
The University of Queensland, Brisbane, Queensland, Australia