A bid to revive MMP inhibitors

By Chris Cain, Staff Writer

Matrix metalloproteinases, or MMPs, were once viewed as obvious drug targets—extracellular proteases that are selectively upregulated and play critical functional roles in many diseases. But MMP inhibitors failed in cancer trials a decade ago primarily because of poor specificity that led to severe musculoskeletal side effects. Now, researchers at the **State University of New York at Stony Brook** have identified small molecule inhibitors specific for MMP9 that block its protein-protein interactions rather than its proteolytic activity.[1]

The next steps for the Stony Brook team will be to design more potent inhibitors and convince potential partners the molecules have a clean safety profile.

Academics contacted by *SciBX* think previous failures of MMP inhibitors—most notably marimastat from British Biotech plc[2] (now part of **Vernalis plc**)—reflected a fundamental lack of biological and biochemical understanding of the targets.

"They didn't know the concentrations of inhibitors to use, they didn't know how to measure activity *in vivo*, they didn't know how to discriminate one enzyme versus another enzyme. It was like trying to spray a bull's eye with bullets with your eyes closed," said Alex Strongin, a professor in the Cancer Research Center at the **Sanford-Burnham Medical Research Institute** who has spent his career studying MMP biochemistry.

Barbara Fingleton, assistant professor of cancer biology at **Vanderbilt University Medical Center**, agreed. "What happened with the initial inhibitors was scary; people didn't even know how many MMPs there were. Now we know there are 24. They also had no way of confirming they were really inhibiting the right targets *in vivo*," she said.

"Unless we can deliver a new mechanism to the companies, they will be inert. Only a fool makes the same mistake twice, and together companies burned over $2 billion in clinical trials of MMP inhibitors," said Strongin.

A team led by Jian Cao, associate professor of medicine and pathology at Stony Brook, has identified a mechanism that might fit the bill.

In the past few years, Cao's lab and others have published that MMP9 has noncatalytic functions required for cell migration.[3,4] He told *SciBX*, "Cancer invasion and metastasis require proteinase activity to break up the extracellular matrix and enable cell-cell disassociation, but they also require the activation of signaling pathways that trigger cell migration. If you block either step, you block invasion."

Most previous compounds, including marimastat, worked by binding to the highly conserved MMP catalytic site. The compounds blocked proteinase activity but were not selective for individual MMPs.

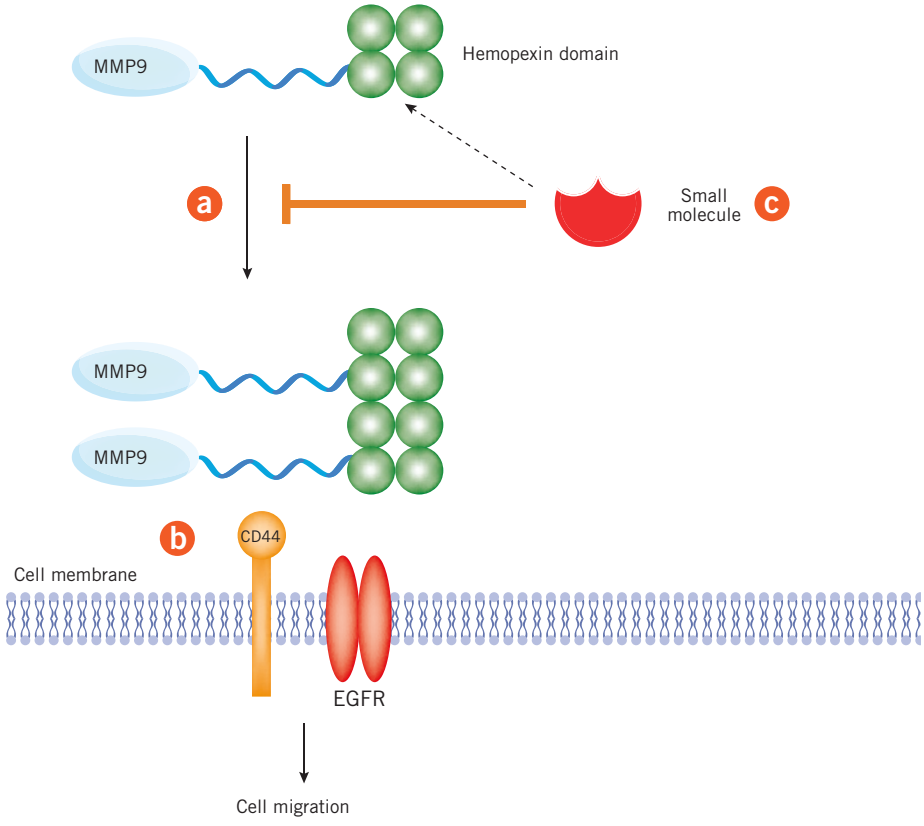

Figure 1. Blocking matrix metalloproteinase 9 dimerization inhibits cell migration. Matrix metalloproteinase 9 (MMP9) homodimerizes via its hemopexin domain [**a**], which promotes interaction with the cell surface glycoprotein CD44, activating the epidermal growth factor receptor (EGFR) [**b**]. A small molecule [**c**] was identified that bound specifically to the hemopexin domain of MMP9. This molecule did not inhibit MMP9 catalytic activity *in vitro* but blocked MMP9 dimerization, thus preventing its interaction with CD44 and lowering EGFR activation. This decreased both cell migration in cell culture and metastasis in a xenograft mouse model of breast cancer.

Cao's team used computational modeling to identify small molecules that could target MMPs with greater specificity. The group focused on the hemopexin domain, which is found in most MMPs and is required for MMP9-induced cell migration but is not as highly conserved as the catalytic site.

The hemopexin domain facilitates protein-protein interactions between MMPs and many of their substrates and regulatory proteins[5] (*see* **Figure 1, "Blocking matrix metalloproteinase 9 dimerization inhibits cell migration"**).

> **"This paper is a very fresh way of thinking about how to target metalloproteinases."**
> ***—Alex Strongin, Sanford-Burnham Medical Research Institute***

"The hemopexin domain of MMP9 shares about 25% identity with other MMP hemopexin domains, while its catalytic domain is roughly 65% identical," said Cao. "We hypothesized that we could selectively inhibit a single MMP by interfering with its hemopexin domain function."

A series of synthetic, pyrimidinone-derived lead compounds bound the hemopexin domain of MMP9, blocking its dimerization without inhibiting catalytic activity *in vitro*. Chimeric proteins containing the catalytic domain of MMP9 fused to the closely related hemopexin domain of MMP2 were not bound by the compounds, suggesting binding was specific for the MMP9 hemopexin domain.

In vitro the compounds blocked the migration of cultured breast cancer cells, and *in vivo* they inhibited metastasis in a xenograft mouse model of breast cancer.

Results were published in *Cancer Research*.

Beyond proof of principle

The study provides proof of principle that the hemopexin domain of MMPs can be hit by small molecules, but more potent compounds and additional preclinical testing will be required to make a convincing case for safety and efficacy.

"This paper is a very fresh way of thinking about how to target metalloproteinases," said Strongin. He appreciated the specificity obtained by targeting the hemopexin domain but added, "This paper is not bulletproof."

For example, said Strongin, the mice received repeated intratumoral injections of milligram concentrations of the compounds, providing proof of principle but little clinical relevance because the dosing was impractically high.

Jointly with his long-term collaborator Maurizio Pellecchia, Strongin and his lab are designing inhibitors of MMPs and are focused on blocking the dimerization of matrix metalloproteinase 14 (MMP14), another MMP strongly upregulated in cancer cells. Pellecchia is a professor of infectious diseases at Sanford-Burnham.

Cao told *SciBX* that larger-scale screens are underway to identify more potent inhibitors. His team is also collaborating with Francis Johnson, professor of chemistry and pharmacological sciences at Stony Brook, to synthesize more potent derivatives of these compounds.

For safety, the key will be showing that the new compounds are free of the musculoskeletal side effects that plagued the development of pan-MMP inhibitors.

"Before going any further, you would need to look at the effect of these compounds on joints, at least with histology, to see if there are any changes," said Vanderbilt's Fingleton. She said it is impressive that Cao's compound did not interact with the MMP2 hemopexin domain but noted there are many proteins that contain the domain. Thus, she said, further studies need to confirm the compound's specificity.

Fingleton's lab is identifying beneficial roles for MMPs in normal cellular processes. Also, she has previously published that MMP9 inhibition has varying effects in various mouse models of cancer metastasis.[6]

Finally, although noncatalytic roles have become more accepted for MMPs over the past five years, it remains to be demonstrated whether the compounds described in the new paper affect MMP9 catalytic activity *in vivo*.

"Many biological effects including intracellular signaling likely proceed when MMP9 is activated and cleaves protein substrates. To clarify this issue, studies are needed with catalytic mutants of MMP9 or knock-in mice expressing such enzymes," said Erkki Koivunen, associate professor of leukemia research at **The University of Texas M.D. Anderson Cancer Center**.

Fingleton noted that Mmp9 knockout mice do not have musculoskeletal defects, so it might not matter if these inhibitors also affect proteinase activity as long as they are specific for their target.

She also suggested that a knock-in mouse experiment with catalytically inactive Mmp9 would provide the best evidence for its noncatalytic function. She added that the *in vitro* test of catalytic activity used small synthetic peptide substrates, not the larger native proteins that would be encountered *in vivo*.

In vivo, these larger proteins are often targeted for cleavage by binding to the hemopexin domain, so it would not be surprising if these compounds had some effect in blocking MMP9 cleavage of its natural targets, she said.

Cao has filed patent applications covering methods of use and composition of the compounds, and he is seeking licensing and sponsored research partners for the project.

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