One-two punch against *Pseudomonas*

By Kai-Jye Lou, Staff Writer

University of Washington researchers have found that inactivating a gene that codes a two-component regulator in *Pseudomonas aeruginosa* reduced bacterial virulence and increased the efficacy of tobramycin treatment.¹ Now that the gene has been shown to be involved in aminoglycoside resistance, it offers a previously unknown target for new compounds to boost the efficacy of existing aminoglycoside antibiotics against *P. aeruginosa* and reduce the risk of dosage-related side effects from aminoglycoside treatment.

P. aeruginosa is a common cause of opportunistic nosocomial infections, and treatment usually involves a course of antibiotics. Although aminoglycoside antibiotics have remained highly effective, the incidence of resistant strains has been increasing.^{2,3} Moreover, the ability to use higher doses or prolong treatment with aminoglycosides like tobramycin is offset by the increased risk for renal toxicity and ototoxicity.

To combat the spread of resistant *P. aeruginosa* strains, a research group led by Colin Manoil, professor in the Department of Genome Sciences at the University of Washington, embarked on a search for new targets. Rather than attempting to identify targets for single agents—an effort that has produced only lackluster results for over a decade—the group searched for targets that could bolster the effect of existing antibiotics.

Manoil's group found that inactivating mutations in the *P. aeruginosa* gene coding for a two-component regulator—which they renamed *aminoglycoside resistance (amgRS)*—caused an 8- to 16-fold reduction in the minimal concentration of tobramycin required to inhibit visible bacterial growth in culture.

Two-component regulator systems allow bacteria to sense and respond to changes in environmental conditions. These systems consist of a histidine kinase sensor protein that receives environmental stimuli and a corresponding response regulator protein that mediates a cellular response.

amgRS was originally called *ompR-envZ* based on its homology to a two-component regulator system in *Escherichia coli*. In *E. coli*, the *ompR-envZ* regulator has been shown to modulate gene expression in response to changes in osmolarity.⁴ In contrast, Manoil's new data suggest that *amgRS* controls a membrane stress response that protects *P. aeruginosa* from envelope damage.

"The sequence similarity with *E. coli* *ompR-envZ* is beyond dispute, but its function appears to be very different," said Manoil.

To validate the findings from their genetic screen, the Manoil group showed that an inactivating mutation in *amgRS* increased the bactericidal activity of tobramycin by 100-fold in *P. aeruginosa* biofilm cultures compared with that in biofilms harboring bacteria that had wild-type *amgRS*. In 23 *P. aeruginosa* isolates from chronically infected cystic fibrosis (CF) patients, including 11 with infections that were highly resistant to tobramycin, inactivation of *amgRS* increased bacterial sensitivity to the aminoglycoside.

The results were published in the *Proceedings of the National Academy of Sciences*.

"We found a physical regulator in *P. aeruginosa* that is a promising combination drug target for increasing the efficacy of current aminoglycoside antibiotics," Manoil told *SciBX*.

"This target appears to be most important in *Pseudomonas aeruginosa*, and we definitely need new treatment approaches for *Pseudomonas* infections," said Michael Dudley, SVP of R&D and CSO at **Mpex Pharmaceuticals Inc.** "Aminoglycosides given systemically or even by the inhalation route are losing their effect due to the development of resistance. Being able to restore the efficacy of proven antibiotics with proven safety profiles is the main benefit that a combination therapy target could offer," he said.

"These findings could potentially give new life to these old favorites," said Prabhavathi Fernandes, founder, president and CEO of **Cempra Pharmaceuticals Inc.**

Mpex's MP-376, an aerosolized formulation of the fluoroquinolone antibiotic levofloxacin, is in a Phase II trial to treat chronic *P. aeruginosa* infections in CF patients and a Phase II trial to prevent pulmonary exacerbations in high-risk patients with chronic obstructive pulmonary disease (COPD). The company expects to have results from the CF trial this year and from the COPD trial in 2010.

Cempra's CEM-102, an oral antibiotic active against Gram-positive bacteria, is in Phase II/III testing to treat skin and skin structure infection (SSSI). The company also plans to take oral and i.v. formulations of its macrolide antibiotic candidate CEM-101 into a Phase II trial for community acquired pneumonia (CAP) next year.

New life for genomic approaches?

Manoil's work could revitalize efforts to apply genomic approaches to the discovery of new antibiotics.

For over a decade, companies and research groups have undertaken large-scale genomic screening to identify new antibiotic and antimicrobial targets. However, more than 125 screens run by 34 companies between 1996 and 2004 have not yielded any strong development candidates.⁵

Separately, **GlaxoSmithKline plc** has carried out 70 high throughput screening (HTS) campaigns in a massive 7-year effort between 1995 and 2001.⁶ Each HTS campaign cost the pharma about \$1 million and the entire effort only delivered five leads. In their report, company scientists wrote that "based on GSK screening metrics, the success rate from antibacterial HTS was four- to fivefold lower than for targets from other therapeutic areas at this time."

These lackluster results have caused many pharma and biotech to abandon or scale back their efforts against bacterial infections, and there is some debate as to whether all useful antibacterial targets have been discovered.^{7,8}

"There is a dramatic need for new antibiotics and new targets," said Manoil. "However, the search for brand-new standalone targets for bacterial infections has been a little disappointing. We have taken a more conservative route and looked for targets that can enhance the efficacy of existing antibiotics."

In their genetic screen covering 42,240 *P. aeruginosa* strains, the Manoil group identified insertion mutations in 71 genomic locations that could sensitize the bacteria to tobramycin. The researchers selected *amgRS* for further evaluation because inactivation of the gene caused the largest increases in tobramycin sensitivity.

"In a bigger sense, our work provides a new way for looking for antibiotic and antimicrobial compound targets," Manoil told *SciBX*. "In screening for this target, we used an approach that was not possible until a few years ago. What made the screen feasible was the availability of a sequence-defined mutant library with mutations in all of the nonessential genes of *P. aeruginosa*. It was thus possible to identify virtually all functions contributing to aminoglycoside resistance and determine that *amgRS* was the most important."

"The idea that you *can* use genomic approaches to discover new targets is a promising finding from the *PNAS* paper," said Cempres Fernandes.

Gerry Wright, director of the Michael G. DeGroote Institute for Infectious Disease Research and a professor in the Department of Biochemistry and Biomedical Sciences at **McMaster University**, said the approach "adds the ability to keep well-established treatment regimens and products, and perhaps decrease dose, which is important with this class of drug as there is some significant toxicity associated with it."

The interesting question, noted Wright, is whether the observed activity is restricted to *P. aeruginosa*, as this might make it less attractive to drug developers. "On the positive side, combinations that only take out one organism can be highly desirable as, in principle, this should lessen problems associated with broad-spectrum antibiotics such as GI side effects" he said.

Taking the target forward

Researchers contacted by *SciBX* think that continued development will hinge on the ability to identify a highly specific inhibitor of *amgRS* activity.

"The key next step is to find a molecule that can directly or indirectly inhibit *amgRS* and show that this synergizes with aminoglycosides," said Wright. "If such a compound can be found, then this study becomes very important in the drug discovery field."

"In a bigger sense, our work provides a new way for looking for antibiotic and antimicrobial compound targets."

—Colin Manoil,
University of Washington

However, identifying such a compound may not be so simple. "Despite the fact that many large drug companies and biotech have agreed that two-component systems make interesting targets for antibacterial agents, there are no drugs yet coming from screens," Wright noted. "This doesn't make this study any less valuable, it's just that the target they pulled out is not easy to deal with."

Although earlier screening efforts have yielded small molecule inhibitors of two-component regulators in other bacterial strains, the identified compounds have not been very specific. Manoil acknowledged that this potentially could be an issue but added that those studies were based on screens of decade-old chemical libraries. "There have been many advances since then," he told *SciBX*.

As another caveat, Fernandes noted that the developmental barriers for a compound intended for combination therapy will be bigger than those for a monotherapy. "You will have to do more extensive pharmacokinetic and toxicology studies, and if you plan to take the combination to clinical trials, you will need to have multi-arm studies evaluating each compound individually and in combination," she said.

Dudley and Fernandes both said it also would be important to evaluate the likelihood of *P. aeruginosa* acquiring resistance or adapting against the candidate combination therapy. "We will need to see studies looking at serial exposures to tobramycin in the *amgRS* mutant strain to evaluate the likelihood of acquiring resistance," Dudley told *SciBX*.

Manoil said his group is developing bacterial strains to screen for small molecule inhibitors against *amgRS*. His group also is working to identify other functions regulated by *amgRS* and noted that these could also be potential targets for antimicrobials.

The work in the *PNAS* paper is not patented.

Lou, K.-J. *SciBX* 2(33); doi:10.1038/scibx.2009.1264
Published online Aug. 27, 2009

REFERENCES

1. Lee, S. *et al. Proc. Natl. Acad. Sci. USA*; published online Aug. 10, 2009; doi:10.1073/pnas.0903619106
Contact: Colin Manoil, University of Washington, Seattle, Wash. e-mail: manoil@u.washington.edu
2. Poole, K. *Antimicrob. Agents Chemother.* **49**, 479–487 (2005)
3. Merlo, C.A. *et al. Chest* **132**, 562–568 (2007)
4. Heyde, M. & Portalier, R. *Mol. Gen. Genet.* **208**, 511–517 (1987)
5. Chan, P.F. *et al. Drug Discov. Today Ther. Strateg.* **1**, 519–527 (2004)
6. Payne, D.J. *et al. Nat. Rev. Drug Discov.* **6**, 29–40 (2007)
7. Silver, L.L. *Nat. Rev. Drug Discov.* **6**, 41–55 (2007)
8. Bumann, D. *Curr. Opin. Microbiol.* **11**, 387–392 (2008)

COMPANIES AND INSTITUTIONS MENTIONED

Cempra Pharmaceuticals Inc., Chapel Hill, N.C.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
McMaster University, Hamilton, Ontario, Canada
Mpex Pharmaceuticals Inc., San Diego, Calif.
University of Washington, Seattle, Wash.