Pheromonicin Biotechnology Ltd.

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Engineering next-generation antibiotics drawn from nature's toolbox

Pheromonicin Biotech's platform for developing a progressive league of antibody mimetics provides distinct advantages and a new therapeutic approach to combating infectious diseases and other indications.

Beijing-based Pheromonicin Biotech employs a proprietary platform that exploits knowledge of natural microorganism-targeting bacteriocins coupled to target-specific small antibody mimetics. "We link our antibody mimetics to colicin Ia, a bacteriocin produced by *Escherichia coli*, to create fusion proteins called pheromonicins," explained Xiao-Qing Qiu, the company's chief scientist. Bacteriocins are toxic proteins produced by bacteria to inhibit the growth of similar or closely related bacterial strains.

Pheromonicin Biotech's technology platform is based, in part, on natural bacterial toxins. About 20 years ago, Qiu helped to decipher the molecular structure and function of *E. coli*-specific colicin Ia, which prompts voltage-activated ion channels to form when brought in close proximity to a cell membrane. The ion channels create large pore openings that cause profuse cell leakage, constituting an effective mechanism for killing competing *E. coli* strains.

The platform also implements Qiu and colleagues' method for deducing just how much (or little) of an antibody's structure is necessary to target an antigen. The antibody mimetics engineered by Pheromonicin Biotech are substantially smaller than the multi-chain full-length immunoglobulin (150 kDa) and Fab fragment (55 kDa) that are more commonly applied to the development of therapeutics. Pheromonicin Biotech's mimetic is only 28 amino-acids long and weighs about 3 kDa, because it includes two complimentary determining regions (CDRs) and a portion of the framework region necessary to help the chains retain the antigen-recognition ability of their parent antibodies. The strategy maximizes the sequence search and design efficiency, while creating sufficient antigen specificity and binding affinity. The small size also minimizes the chance of the tag interfering with the core channel-forming activity of colicin Ia. "The channel-forming activity of colicin Ia is independent of the targeting receptor and the type of lipid bilayer; therefore, colicin Ia can be engineered to address different antigenic targets," Qiu explained.

Specificity and versatility by design

"Engineering target-specific pheromonicins is akin to fitting the right bit to a powerful and yet versatile drill," Qiu described. "Recombinant pheromonicins provide a new way to think about designing anti-microbial agents and other targeted therapies." Data from cell and animal assays demonstrated that Pheromonicin Biotech's mimetic peptides delivered the conjugated cytotoxin specifically to tumor cells, and that the peptide–toxin fusion protein mimetic penetrated further into the tumor tissue compared with the parental antibodies.

The potential advantages associated with pheromonicins extend beyond their specificity and penetrative abilities. As an anti-infective or anti-fungal, the high specificity and cell-killing ability demonstrated by the company's first attempt at harnessing colicin Ia for therapeutic application could potentially decrease the risk of a drug-resistant strain emerging[1]. The first pheromonicin was designed about 10 years ago using a naturally expressed pheromone sequence (AgrD) to produce colicin Ia that was active against methicillin-resistant *Staphylococcus aureus* (MRSA) and its methicillin-sensitive ancestor. Preclinical assays demonstrated a lack of toxic activity against *Staphylococcus epidermidis* and *Streptococcus pneumoniae*[1], indicating a high level of specificity for targeting MRSA cells.

Following the success of the first-generation pheromonicin, the team of researchers at Pheromonicin Biotech went on to broaden their technology platform[2]. "Instead of relying on naturally available pheromones and their cognate receptors found on the surface of a limited number of microorganisms, we [Qiu and the company's research and development team] successfully demonstrated that tiny antibody mimetics could also be used to guide colicin Ia to target cells."

As a result, the platform can be used to discover therapies for an array of targets beyond bacteria, ranging from cancer cells to veterinary diseases and even agricultural infections. A clear advantage of the second-generation pheromonicin platform is its versatility. Changing the target of a pheromonicin is simply a matter of identifying a new antibody mimetic sequence using the standard monoclonal antibody technology and then swapping in Pheromonicin Biotech's proprietary elements. This makes the primary rate-limiting step of pheromonicin discovery the animal immunization process, which typically takes about 4 weeks in mouse models. Once candidate monoclonal antibodies are isolated from the vast repertoire generated by the immune response, new pheromonicins can be rapidly engineered using various combinations of heavy-chain and light-chain CDRs.

A growth-inhibition or other appropriate assay is used to select the most effective combination, and the new pheromonicin is ready to be scaled-up for production. "The platform is currently capable of generating a novel lead every 4 to 6 months," according to Qiu.

Partnering opportunities

Pheromonicin Biotech is actively seeking innovative partnerships around the world to develop novel bioactive agents using the pheromonicin discovery platform. Pheromonicin Biotech's pipeline consists of more than a dozen leads for human medicine, veterinary medicine, and agricultural infection-control applications.

Of course, there is an anti-bacterial therapy in the human pipeline generated to improve upon the first MRSA-targeting pheromonicin. The research team developed a broad-acting anti-bacterial agent by conjugating colicin Ia to porin A. Bacteriocins are narrow-spectrum antibiotics, which was useful for the earlier study, but reaching a broader spectrum of infectious microbes could provide greater therapeutic benefit in the future.

Porin A is a ubiquitous molecule found on the surface of many bacteria, including certain Gram-positive strains. A prototype pheromonicin targeting *Neisseria meningitidis* using porin A (Ph-NM) demonstrated bacteriocidal activity against a wide range of infectious bacteria. Ph-NM is being investigated as a potential treatment for several bacterial infections in animals and in human patients. Preliminary results show efficacy in treating mastitis in cows, infectious serositis in farm ducks, and dermatological infection during wound healing. Ph-NM demonstrated non-inferiority compared with the other antibiotic families tested. In the United States, Pheromonicin Biotech and collaborators are planning the first primate-based preclinical study for Ph-NM as a treatment for tuberculosis in a macaque model.

At the effective dose against targeted bacteria (such as MRSA), second-generation pheromonicin Ph-NM does not kill beneficial species in the gut (such as *Lactococcus lactis* and *Bifidobacterium longum*), because they do not express the targeted type of porin. The gastrointestinal side effects of Ph-NM are therefore likely to be less severe than those of traditional antibiotics, which wipe out the gut flora indiscriminately.

The most advanced agent in the Pheromonicin Biotech pipeline is a veterinary drug undergoing a phase III clinical trial under the auspices of the National Veterinary Drug Safety Evaluation Center of China Agricultural University, Beijing, part of the Ministry of Agriculture of the People's Republic of China. A new-drug application will be filed in China before the end of this year.

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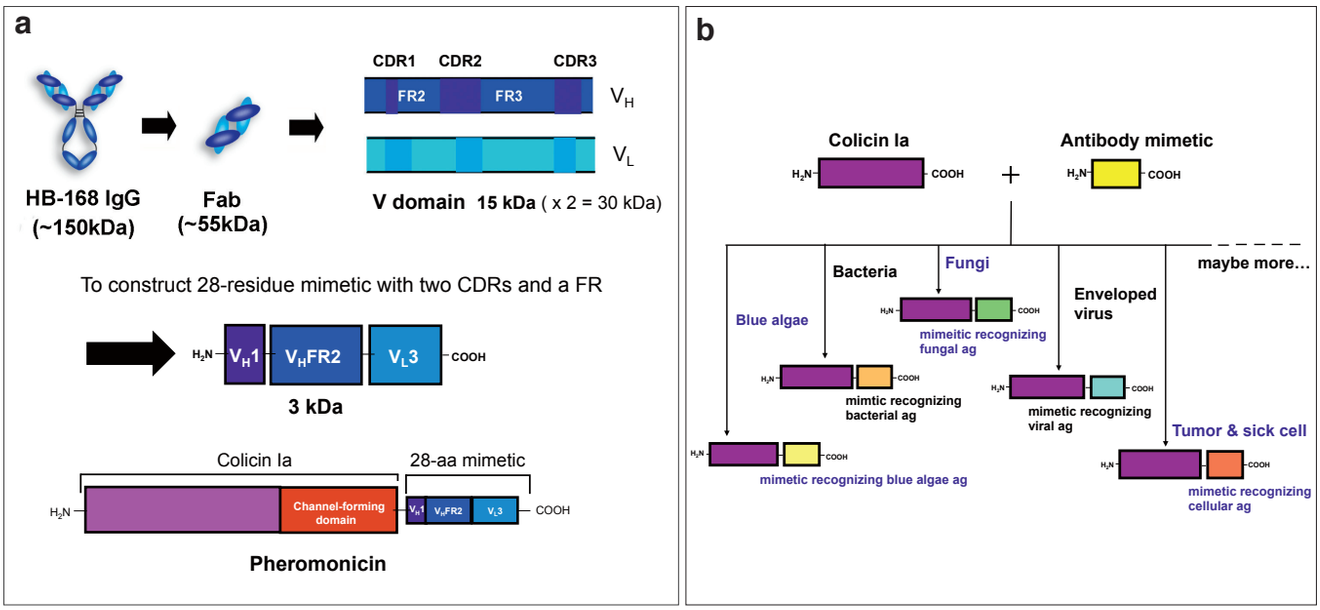

a. The *E.coli*-specific bacteriocin, known as colicin Ia, prompts voltage-activated ion channels to form when brought to in close proximity to a cell membrane. The Pheromonicin Biotechnology platform couples this potent cell-killing ability to a monoclonal antibody mimetic that is a fraction of the size of therapeutic antibodies. The mimetic (about 3 kDa) is much smaller in comparison to a multi-chain full-length immunoglobulin (150 kDa) and Fab fragment (55 kDa).

b. A diagram showing the versatility of the Pheromonicin's technology platform. The antibody mimetics can be engineered to direct colicin 1a to disease targets on lipid-bilayer membranes.

> **"Engineering target-specific pheromonicins is akin to fitting the right bit to a powerful and yet versatile drill"**

Agricultural applications

Crops are also vulnerable to pathogens, and the technology platform can generate solutions to the challenges of agricultural pathogen control. The degradable nature of recombinant proteins provides an added benefit. Residual antibiotics, pesticides, and chemical toxins can pose major food-safety concerns when used to control contamination. Genetically modified crops and animals can cause further consternation. By contrast, pheromonicins are rapidly degraded after action, as with most recombinant proteins. Pheromonicin Biotech has conducted field tests using novel anti-fungal pheromonicins to treat rice blast disease and apple canker. At the effective doses, residual pheromonicin was not detectable in the harvests, highlighting the benefits over chemical pesticide.

Pheromonicin Biotech currently produces pheromonicin compounds in 30–200–500 L fermenters with yields ranging from 50 to 500 mg/L. The goal is to reach yields of 1 g/L in late 2013, in order to produce sufficient quantities of new pheromonicins for field and preclinical investigations. The 28-residue targeting sequence is tiny with respect to the entire pheromonicin conjugate (about 70 kDa fully folded), so the scale-up production protocol requires minimal optimization for different pheromonicins.

In summary, the pheromonicin platform developed at Pheromonicin Biotech has unique advantages in terms of its versatility, short research-and-development time frames, promising safety profile, and modular design approach. Grants from the Beijing Municipal Commission of Science & Technology, National Nature and Science Foundation and Ministry of Science & Technology have provide support for the development of pheromonicins.

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2. Qui, X-Q. *et al. Nat Biotechnol.* **21**, 1480–1485 (2003).
3. Qiu, X-Q. Chapter 21, CDR-FR peptide, *Antibody Engineering Vol. 2*, Edited by R. Kontermann & S. Dubel, p267-276, Springer, 2010.

CONTACT DETAILS

Dr. Rong-Qi Li, CEO
Pheromonicin Biotech (Beijing), Ltd.
Room B106, No. 5 Kaituo Road
Biopharmaceutical Park, Haidian District
Beijing 100085, PR. China
Tel: +86-10-6245-1843, +86-10-6248-3921
Email: lirongq@tom.com