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Perspective

Peptide nucleic acid antisense oligomers open an avenue for developing novel antibacterial molecules

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Bacterial drug resistance is a growing concern around the globe. The indiscriminate use of antibiotics in many parts of the world for decades has helped bacteria to acquire resistance against commonly used antibiotics. This situation is worsened further due to transfer of resistance gene(s) within bacteria, promoting the rise of multidrug resistant bacterial strains.

Multidrug resistance in *Pseudomonas aeruginosa* is also a growing concern. This bacterium is considered as an opportunistic human pathogen and known to infect patients with suppressed immunity. *Pseudomonas* can grow in a wide variety of hosts, ranging from plants to animals [1,2]. It is also found in wetlands, soil, and marine environments, and its ability to acquire resistance against antibiotics and disinfectants makes *P. aeruginosa* different from other bacteria [3]. Several hundreds of people die each year due to *Pseudomonas* infection. In most cases, this nosocomial pathogen is responsible for the death of cystic fibrosis patients.

P. aeruginosa has a comparatively large genome of 6.3 million bases that encodes 5570 proteins [3]. Large genetic diversity enhances *P. aeruginosa* ability to gain resistance against antibiotics quickly and even during the treatment. Several *P. aeruginosa* clinical isolates are resistant to different classes of antibiotics, for example, β-lactams, fluoroquinolones, and aminoglycosides [4,5]. Resistance to β-lactam and aminoglycoside classes of antibiotics is due to the import of genetic material, whereas resistance to fluoroquinolones is linked to mutations [4]. *P. aeruginosa* acquires resistance against antibiotics either by modification of the target or overexpression of the efflux pump or alteration of the membrane permeability [6,7].

Multidrug resistant bacterial infections pose a significant challenge in the treatment of patients. Currently, we are left with few treatment options. The reduced arsenal of antibiotics is pushing us to a post-antibiotic era. The long discovery time and a huge development-associated cost limit the discovery of new antibacterial compounds. The majority of effective antibiotics are derivatives of earlier discovered antibiotics. We are in need of new classes of antibiotics with novel mechanism of action.

About one and a half decade ago, oligomers of Peptide Nucleic Acid (PNA) were first introduced as antibacterial agents, and studies showed that these antibacterial PNA molecules are capable of inhibiting the growth of outer-membrane defective *E. coli* strain [8,9]. Years later, a separate study exhibited that PNA oligomer in conjugation with KFFKFFKFFK peptide is also able to inhibit the growth of wild type *E. coli* strain [10]. Since then, numerous studies have reported the antibacterial activity of peptide-PNA conjugates in different bacterial strains, *Campylobacter jejuni*, *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Brucella suis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *E. coli*, *Shigella flexneri*, and *Streptococcus pyogenes* [11–20].

PNA is a synthetic molecule that mimics the structure of DNA. Synthesis of PNA oligomers follows a simple chemical approach, which has been well described in the literature [21]. Inside the cell, these antibacterial PNA oligomers function as antisense molecules and initiate inhibition of translation either by blocking the movement of ribosomes along the mRNA or disrupting the assembly of ribosome around the translation start site. The interaction between the PNA

oligomer and mRNA is reversible and governed by hydrogen bonds. Designing PNA oligomers is relatively straightforward, but it requires a systematic search to identify the optimal target region on the mRNA in order to achieve maximum inhibition of gene expression. Generally, the area around the translation start site −20 nucleotides up- and downstream − is optimal for designing antibacterial PNA molecules [15]. However, factors such as RNA secondary structure and association of RNA-binding proteins with RNA, could make the designing of antibacterial PNA oligomers challenging. Regions other than the one around the translation start site could also be important for antibacterial PNA design. In the study by Ghosal *et al*. [15], antibacterial molecules were designed through steps that involve identification of target, scanning of regions on mRNA, characterization of PNA oligomers length, and, lastly, validation of carrier peptides. These steps described in detail in the literature [10].

Four antibacterial peptide-PNA conjugates were discovered and developed in the study by Ghosal *et al.* [15]. These molecules efficiently inhibit *P. aeruginosa* growth at low micromolar concentrations and are effective against three strains of *P. aeruginosa*, namely, PA01 (a commonly used laboratory stain), PA14 (a highly virulent strain) and LESB58 (a clinical isolate from a cystic fibrosis patient). Two of these four molecules, target the mRNA of *acpP* gene. The target region of anti-*acpP* PNA is conserved in a range of species of *Pseudomonas*, highlighting the potential antibacterial application of anti-*acpP* conjugates in other *Pseudomonas* species.

PNA is a synthetic molecule and not found in any living systems, while number of commonly used antibiotics are natural in origin. Therefore, it is likely that in the course of evolution, bacteria may not have a chance to encounter PNA molecules, so one could assume that gaining resistance against PNA molecules will be difficult for bacteria.

Furthermore, the selective killing of pathogenic bacteria is a big challenge. The majority of antibiotics negatively affects the host-associated beneficial bacterial community. Designing of bacterial-specific antibiotics is significantly challenging while it is relatively simpler using the PNA-based approach. The delivery of antibacterial PNA molecules can be achieved by designing of bacterial-specific vehicles. Several peptides, such as $(KFF)_3K$, (R-Ahx-R)4-Ahx-βala, (R-Ahx)6-βala, YARVRRRGPRGYARVRRRGPRRC, and RFFRFFRFFRXB, are known for delivering antisense PNA or Morpholino oligomers in bacteria [10,15,22,23]. In addition to their structural and/or sequence dissimilarity, these peptides may also differ in their uptake in bacteria.

Certain modifications can also alter the entry of peptide-PNA conjugates in bacteria. For instance, the addition of a cleavable linker, -F-Gly-eg1- or -FFK-eg1-, between peptide and PNA oligomer allows PNA oligomer to separate from the conjugated peptide in the bacterial periplasm [17]. Additionally, a study on *E. coli* highlighted that the L form of $(KFF)_3K$ peptide degrades in periplasm while H-D($(KFF)_3K$), (R-Ahx-R)4-Ahx-βala and (R-Ahx)6-βala peptides remain attached to the PNA oligomer and assist delivery of PNA oligomers to the bacterial cytoplasm [17]. Moreover, the uptake of these cell permeable peptides varies among bacteria, for example, $(KFF)_3K$ peptide transports PNA oligomer efficiently in *E. coli* but not in *P. aeruginosa* [15].

It is equally important that these delivery peptides remain stable and effective in the mammalian system. It has been shown that PNA or Morpholino antisense oligomers in conjugation with $(KFF)_3K$, $(RX)_6B$-, $(RXR)_4XB$-, and $(RFR)_4XB$ peptides are able to eliminate bacterial infection in mice [18,24–26]. Furthermore, it is also possible that the peptides which efficiently transport PNA oligomers in mammalian cells may also be capable of transporting PNA oligomers in bacteria [27, 28].

Additionally, antisense PNA oligomers could also be used for studying gene function in bacteria. Following observations strongly highlight that the development of PNA antibiotics could be an alternative approach to control multidrug resistance bacterial infections.

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