Learning from cancer how to defeat bacteria

José N. Onuchic^{a,1}, Patricia A. Jennings^b, and Eshel Ben-Jacob^{a,c}

^aCenter for Theoretical Biological Physics, Rice University, Houston, TX 77005-1827;

^bDepartment of Chemistry and Biochemistry, University of California, San Diego, CA 92093;

and ^cSchool of Physics and Astronomy, Tel-Aviv University, Tel-Aviv 69978, Israel

Bacteria Strike Back

A rich variety of microorganisms are found in soil. Life in this highly competitive habitat involves both cooperative and antagonistic interactions between species. Over the course of evolution, soil bacteria have developed sophisticated strategies using a wide variety of chemical weapons to survive. These toxins provide rich sources of potential antibiotics, as well as scaffolds for the design of new antimicrobial agents. However, the massive use of antibiotics has created a major health problem worldwide: bacteria are becoming increasingly resistant to antibiotics (1). Bacteria are one of the top three killers in hospitals today. A major concern is the notorious ability of Gram-negative bacteria to rapidly develop multidrug resistance to new antibiotics.

From Natural to Synthetic Antibacterial Peptides

Clearly, novel approaches must be taken to lift this threat. A promising new direction is based on specially designed synthetic peptidomimetics based on natural antimicrobial peptides (AMPs) produced by higher animals, such as cecropins (first discovered in the hemolymph of the *Cecropia* moth but also secreted in mammalian intestine) (2). The immune system exploits AMPs as an innate response against pathogens using several mechanisms of action, including membrane permeabilization through the formation of pores and membrane thinning or micellization (2). AMPs may also target intracellular components, such as DNA, enzymes, and organelles. What makes AMPs

attractive therapeutics is their selective activity against prokaryotes. Earlier studies established that the peptide (KLAKLAK)₂ is a highly effective antibacterial agent with low toxicity (3). Particularly promising is the _D(KLAKLAK)₂ (all-D-enantiomer peptidomimetic synthetic version of the designed L- or mixed D/L-peptide), the focus of McGrath et al.'s article in PNAS (4).

Advantage of Broken Mirror Symmetry

Bacteria have impaired resistance to these man-made "mirror" (D-enantiomer) compounds because they cannot use their natural machinery, developed to degrade L-peptides. Indeed, the synthetic variation of the AMP created by substitution of the L-amino acids with their artificial D-analogs have opposite chirality (5) and high stability against proteolytic degradation. This proteolytic resistance affords high antibacterial efficacy with increased in vivo half-life, allowing low-dose administration, further reduction in toxicity, and decreased risk of the development of drug-resistant strains. The _D(KLAKLAK)₂ "killer" sequence also works in synergy with existing antibiotics against pathogenic drug-resistant bacteria and against biofilms (4).

Amphipathic Peptide and Gram-Negative Bacteria

In general, compounds that act by disrupting the prokaryotic cell membrane make the bacteria more vulnerable: disruption of the cell membrane tempts bacterial cell-cell communication essential for collective stress-response strategies and collective development of drug resistance (6). Cationic amphipathic peptides are very effective antibacterial membrane agents because they target the general properties of the lipid bilayer of the bacterial membrane and cause limited damage to the eukaryotic plasma membrane. A number of natural short amphipathic peptides with membrane-disruption activity have been identified. For example, magainin, a peptide isolated from frog with antimicrobial activity, forms transiently existing pores in the cell membrane. One proposed mechanism of membrane disruption in line with experimental studies of _D(KLAKLAK)₂

Fig. 1. Schematic description of the fundamental biochemical factors that render the _D(KLAKLAK)₂ peptidomimetic so powerful against Gram-negative bacteria and yet exhibit low toxicity against eukaryotic cells. The compound is composed of three α -amino acids with complementary functions: L, leucine, which has a hydrophobic side chain and thus provides affinity to the hydrophobic lipid component of the membrane of the bacteria; K, lysine, which is positively charged and thus provides attractive electrostatic interactions with the negatively charged headgroups on the membrane; and A, alanine, which is a neutral, helix-promoting residue and acts as a "glue" that provides structural integrity to the compound. This cationic amphipathic α -helical peptide preferentially disrupts the anionic lipid membrane of Gram-negative bacteria and has limited activity against the plasma membranes of human cells that contain zwitterionic lipids with cholesterol or ergosterole. (Right) The homing domain that is needed to cross the plasma membrane.

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See companion article on page 3477.

¹To whom correspondence should be addressed. E-mail: jonuchic@rice.edu.

proceeds as follows (5, 7, 8): (i) Peptides associate with the membrane surface in parallel orientation to the interface. (ii) Upon reaching a threshold concentration at the bilayer surface, the peptides aggregate. These aggregates facilitate formation of channels through the bilayers. In these cases, electrostatic interactions are crucial to peptide association with the membrane. A common feature of AMPs is the propensity to form helical structures in the presence of bilayers, enhancing their interaction with the membrane (Fig. 1).

From Bacteria to Cancer

The development of synthetic AMPs first began as new antitumor compounds directed against different types of cancers (5, 9), similar to the “generic cover” of natural AMPs against different pathogens. Natural antipathogenic chemical weapons secreted by bacteria are effective antitumor compounds. One example is doxycycline, a member of the tetracycline group of broad-spectrum protein-synthesis inhibitor antibiotics (secreted by soil bacteria that belong to the *Streptomyces* genus). Doxycycline gained much attention following 9/11 as a treatment and prophylaxis of anthrax (caused by the spore-forming *Bacillus anthracis* bacteria). Although the antibacterial administration of this class of antibiotics has declined as a result of the emergence of drug-resistant pathogenic bacteria, there has been a rapid new interest in doxycycline following discoveries that it is an effective antitumor compound (10). Currently several clinical studies are in progress to evaluate the effectiveness of doxycycline against various types of cancer (10). A second example is the intriguing discovery that the widely used agricultural agent salinomycin (secreted by the *Streptomyces albus* bacteria) is potent against cancer stem cells (11), subpopulations of special tumor-forming cells with the ability of differentiation into a variety of cell types. Cancer stem cells pose high risk because they cause relapse and metastasis (12). Development of specific therapies against these cancer stem cells holds hope for improvement of survival and quality of life of cancer patients.

Targeting the Mitochondria

Similar to the above examples, synthetic AMPs are often antitumorigenic by targeting the mitochondria (4, 5, 9, 13). These organelles, which are former bacteria, act as power plants of cells, and share a negatively charged membrane with similar structure to Gram-negative bacteria (Fig. 1). Thus, the synthetic AMP D-peptides effective against Gram-negative bacteria can also disrupt mitochondrial activity and initiate apoptosis once inside the cell. More specifically, the attachment of the synthetic AMP peptide

to the mitochondrial membrane opens temporary pores, leading to leakage of cytochrome *c* that in turn triggers cell apoptosis. Because the synthetic AMP cannot enter the eukaryotic cell membranes, much effort has been devoted to design special “hunter” polymers that, when coupled with the D(KLAKLAK)₂, facilitate rapid internalization and translocation toward the mitochondria (14). The challenge is to design special “homing” sequences for specific receptors enriched in tumor cells yet maintain potency against the mitochondrial membrane (see Fig. 1). This specificity is required to both protect normal cells from the synthetic AMPs and ensure drug efficacy.

Bacterial survival strategies can provide a valuable model system to study cooperative behaviors of cancer (15). Drawing upon this perspective, we propose that synthetic AMPs could temper cancer collective stress response and collective development of drug resistance. A promising direction along this line is the administration of the synthetic AMP in conjunction with targeted (biological) therapies. We expect that combining the generic nature of AMP activity along with the extra stress will inhibit the ability of the cancer cells to develop resistance to the targeted therapy.

Back to Bacteria: Targeting the Biofilm

Most wild-type bacteria thrive in multicellular communities and biofilms allow the colonization of virtually any surface and protection against most environmental stresses (16). The remarkable mechanical and chemical properties of biofilms are attributable to a subpopulation secreting an extracellular matrix, typically consisting of various proteins, exopolysaccharide, and fragments of DNA (17). Much effort has been invested in combating biofilm contamination in industrial settings, agriculture, and health. These settings are responsible for over 80% of all microbial infections in

humans. Bacteria secrete D-amino acids for biofilm disassembly (for self-control over growth). *Bacillus subtilis* produces an additional disassembly factor: the norspermidine polyamine, which together with D-amino acids breaks down existing biofilms (17). These findings might explain the results reported by McGrath et al. (4) regarding the potential of D(KLAKLAK)₂ AMP against biofilm. Their data indicate that this peptidomimetic could disrupt a 24-h-old biofilm irrespective of the growth medium. The authors propose the intriguing possibility that the synthetic AMP not only works against proliferating bacteria but also against quiescent cells. Drawing upon the way *B. subtilis* controls its own biofilm using the combined activity of natural D-peptides and norspermidine, it is likely that the synthetic AMPs will be even more effective against biofilms when administered in combination with natural polyamines.

Looking Ahead: Toward Combinatorial Treatment Using Bacterial and Man-Made Compounds

The results presented by McGrath et al. (4) show that the synthetic AMP selectivity for Gram-negative bacteria and mitochondrial membranes is largely based on cell membrane composition, polarization, and structural motifs. Comparison of the mechanisms of activity of the synthetic AMPs and those of natural antibacterial and antitumor compounds suggests that the synthetic AMPs can be most effective as part of new combinatorial treatments of bacterial infections and cancer when administered in conjunction with natural compounds.

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