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REVIEW ARTICLE

Phage Display Technique: A Novel Medicinal Approach to Overcome Antibiotic Resistance by Using Peptide-Based Inhibitors Against β-Lactamases

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Abstract: The emergence of antibiotic resistance in bacteria is a serious threat with enormous social and economic implications. The distribution of resistance genes/markers through horizontal gene transfer leads to the dissemination of resistant strains in different parts of the world. The resistant bacteria acquire the ability to overcome resistance by different modes amongst which the expression of β-lactamases is a major factor. The β-lactamase enzymes cleave the amide bond of the β-lactam antibiotics, which constitute about one-third of the antibiotics used world over. In a quest to control the spread of resistant bacteria, advanced generations of antibiotics are used either alone or in combination with inhibitors. However, these antibiotics and inhibitors also contain β-lactam ring in their structure and hence are prone to be hydrolyzed by β-lactamase enzymes in the near future. Thus, the severity of the problem is manifested due to the paucity of novel non-β-lactam core containing antibiotics in the drug development stage. One approach to overcome these shortcomings is to use peptide-based inhibitors. Here, we describe the potential use of phage display technique to screen commercially available libraries to pan against β-lactamase enzymes. The main advantage of using peptide-based inhibitors is that the bacteria will not be able to recruit pre-existing defense mechanisms and it will take a long time to evolve a new mechanism in its defense against peptide-based inhibitors.

Keywords: Antibiotic resistance, antimicrobial peptides, peptide-based inhibitors, phage display, gram-negative bacteria.

ANTIBIOTIC RESISTANCE PROBLEM

The emergence of antibiotic resistance in bacteria is a universal threat that is pushing us into pre-antibiotic era where treating simple bacterial infections would be a challenge. The antibiotic resistance phenomenon in bacteria can be described as the development of resistance against an antibiotic towards which it was earlier susceptible [1]. Over the counter sale and non-judicial consumption of antibiotics has put a selection pressure on bacteria to develop resistance against that antibiotic. An infection caused by resistant bacteria is difficult to treat which ultimately leads to an ineffective treatment, prolonged illness, elevated mortality rate and very high treatment cost [2]. Several factors contribute to the development of resistance in bacteria such as (i) mutations in the target of these drugs i.e. penicillin binding proteins involved in cell-wall biosynthesis [3], (ii) deletion and/or modification of the porin channels through which the drugs diffuse [4], (iii) expression of pumps that export the drugs out of the bacterial cells [4], and (iv) over-expression of β-lactamases in the presence of antibiotics [5, 6]. The most widespread resistance mechanism remains the expression of β-lactamase enzymes, which inactivate the β-lactam antibiotics by hydrolyzing their β-lactam ring [7].

Multidrug resistant bacterial strains such as *Klebsiella pneumoniae* and *Escherichia coli* that harbor extended spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) are most commonly reported in hospital settings [8]. ESBLs expressing bacteria are resistant towards penicillins, different generations of cephalosporins and aztreonam. Infections by ESBLs have been treated by prescribing β-lactam antibiotics in combination with mechanism-based inhibitors such as clavulanic acid and sulbactam [1]. These inhibitors compete with the antibiotics to bind at the active site of β-lactamases and thus protect them from hydrolysis [1, 9]. However, new bacterial strains have now evolved that resist these inhibitors while maintaining the ability to hydrolyze β-lactam antibiotics [10, 11]. The situation is even more complicated with the emergence of a new MBL enzyme known as New Delhi metallo-beta-lactamase 1 (NDM-1). NDM-1 was first reported in *Klebsiella*

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pneumoniae strain isolated from Swedish patient receiving treatment in India [12]. The NDM-1 carrying bacterial strain was resistant to almost all the β-lactam antibiotics including carbapenems, which are generally considered as the last resort treatment option against Gram-negative bacterial infections. Thus, the effectiveness of β-lactam antibiotics to treat resistant bacterial infections is questionable. In future, a novel β-lactam antibiotic may be developed against a resistant bacterium, but there is a high probability of resistance development in bacteria against such antibiotics also. To prevent this vicious cycle, we urgently require an alternative approach to design non-β-lactam based antibiotics to fight against bacterial infections [13]. One of such alternatives stems up from an idea of antimicrobial peptides (AMPs). The use of AMPs will be new to the bacterial defense system and hence the bacteria will take time to develop new or modify existing mechanism of resistance.

AMPs

AMPs are unique antibacterial agents that may serve as a novel therapeutic option over conventional β-lactam antibiotics to treat bacterial infections caused by multidrug resistant bacteria. AMPs are found in all living organisms as a component of innate immunity to fight against bacterial infections. They provide first-line of defense against any invading bacterial, viral, fungal pathogen as well eukaryotic parasite. AMPs are generally 12–50 amino acid residues long polypeptides that usually have a net positive charge on their surface [14]. They are diverse in their structure and sequence, containing approximately 50% hydrophobic amino acid residues. Moreover, they bind to negatively charged bacterial surface through electrostatic interaction due to their cationic and hydrophobic nature [15]. However, they cannot interact with mammalian membrane due to the presence of zwitterionic phospholipids and cholesterol in such membranes [16].

There are many different methods to identify and characterize antibacterial peptides from various sources. Some of these include crude extraction of natural antibacterial peptides, chemically synthesized antibacterial peptides using gene screening and also molecular manipulation [17-22]. Phage-displayed peptide is a powerful technique to identify novel peptides exhibiting antibacterial activity. This technique has been employed to discover peptide-based inhibitors for antimicrobial drug discovery [23, 24]. However, most of these peptides exert their effect by lysing the bacterial cell wall, and hence are non-specific in nature. The concept of screening peptides by phage display against β-lactamases to overcome antibiotic resistance has not been explored by many. Only few researchers explored the usefulness of phage display technique to screen and identify peptide inhibitors against β-lactamases. In one such study, Huang *et al.* had identified antibacterial peptides against TEM-1 β-lactamase using phage display [25]. However, there are no reports on the identification of antibacterial peptides against β-lactamases such as CTX-M-15, SHV, NDM-1, IMP, KPC, AmpC using phage-displayed peptide technique.

CLASSIFICATION OF AMPs

Antimicrobial Peptide Database (http://aps.unmc.edu/AP/database/antiB. php) has a repository of about 2521 AMPs (246 from bacteria, 2 from archaea, 7 from protists, 13 from fungi, 314 from plants, 1898 from animals and 41 synthetic peptides) that have been identified and characterized. On the basis of structural organization, AMPs can be classified into the following four classes:

α-Helical AMPs

α-helical antibacterial peptides are the most abundant and widespread peptides in nature. In aqueous solutions, α-helical peptides lack any defined structure but they rearrange themselves into α-helical structures as soon as they are exposed to organic solvents or upon contact with phospholipids of cell membrane [26]. Depending on type and concentration, they can act by forming transient pores or channels on membranes leading to membrane disruption and cellular inactivation [27, 28].

β-Sheeted AMPs

The presence of 2-3 disulphide bonds is the characteristic feature of β-sheeted antibacterial peptides. They usually adopt either a β-sheet or β-hairpin fold structure. They induce lipid flip-flop movement, undergo membrane translocation and act on intracellular targets [29].

Extended AMPs

Extended antibacterial peptides are rich in specific amino acids such as Pro, Gly, Trp, Arg or His. They lack classical secondary structures due to the presence of high Pro and/or Gly contents and form their final structures only when in contact with bacterial membrane phospholipids. Hydrogen bonds and van der Waal interactions play significant role in the interaction between extended antibacterial peptides and bacterial membrane phospholipids [30].

Looped Structured AMPs

The characteristic feature of looped AMPs is the presence of a loop structure due to the presence of a single disulphide, amide or isopeptide bond [31].

FUNCTION OF AMPs

In mammals, AMPs are expressed in a variety of cell types (monocytes or macrophages, neutrophils, epithelial cells, keratinocytes, mast cells, etc.) and act as immunomodulatory molecules in the innate immune system. In some cases, AMPs are released constitutively while in others they are induced in response to external stimuli such as inflammation or tissue injury and on exposure to microbial signature molecules. Among their diverse functions, some are: bactericidal activity [32], anti-endotoxin activity [33], chemotactic activity [34], modulation of pro-inflammatory responses [35], modulation of cell proliferation and differentiation [36], promotion of wound healing and angiogenesis [37], induction of gene expression and enhancement of protein secretion in mammalian host cells [38], initiation and polarization of adaptive immunity [39], etc.

MECHANISMS OF AMP ACTION

Generally, AMPs bind to the surface of a membrane and self-organize to form a permeation pathway when their critical threshold concentration is reached. These peptides (largely unfolded in solution) start adopting a secondary structure as soon as they come in contact with the membrane. On the membrane surface, small peptide monomers remain in equilibrium with the aggregates of small peptides. The orientation of AMPs on the membrane surface also play crucial role in determining the membrane disruption property of AMPs. At lower concentration, AMPs bind parallel to the membrane surface and above a critical peptide/lipid ratio, they adopt a perpendicular orientation to better penetrate the membrane.

It is widely believed that antibacterial peptides act as membrane disruptors. However, the possibility of their translocation into bacterial cytosol and inhibition of metabolic pathways cannot be ruled out [40]. It should be pointed out here that cationic antibacterial peptides specifically bind the negatively charged bacterial membranes and not the membranes of mammalian as they are rich in sterols and zwitterionic phospholipids with a net charge nearing neutrality. The cholesterol in mammalian cell membranes reduces the affinity of antibacterial peptides towards them and hence protects the cells from attack [16]. AMPs are particularly very effective against Gram-negative bacteria as compared to Gram-positive bacteria due to the presence of a thinner peptidoglycan layer and an outer membrane. The outer membrane of Gram-negative bacteria is highly negatively charged and thus attracts cationic antibacterial peptides through electrostatic interactions. Antibacterial peptides

disrupt the outer membrane of a bacterium by binding to the anionic lipopolysaccharides and thereby neutralizing the charge. The peptides are then inserted into or translocated across bacterial membrane lipid bilayer [14]. Various models for the mechanism of antibacterial peptides action on the bacterial membrane are discussed below.

Barrel-Stave Model

In this model, channel forming peptides form a "barrel-like" ring around an aqueous pore. The term "stave" refers to the transmembrane spokes within the barrel. The transmembrane spokes may be composed of individual peptides or peptide complexes. In this model, the hydrophilic surfaces of the peptide form the inner lining of the pore, while the hydrophobic surfaces face outwards towards acyl chains of the membrane [41, 42].

Toroidal Pore Model

It is the most well characterized model for pore formation by antibacterial peptides. It differs from "Barrel-Stave" model in that the lipids are intercalated with peptides in the transmembrane channel. This model advocates the lining of membrane-spanning pores with polar peptide groups and phospholipid head groups both. This has been proposed to be the mechanism by which AMPs enter the cytosol of microbes to target intracellular organelles and proteins [43].

Carpet Model

It is a non-specific membrane permeabilization method in which AMPs act against micro-organisms in a relatively diffuse manner. No specific quaternary structure has been reported in this model. This model suggests that a large quantity of peptides accumulate on the surface of the membrane leading to displacement of phospholipids and change in the membrane fluidity, which ultimately leads to membrane disruption [15].

PHAGE DISPLAY TECHNIQUE

Phage display, a relatively new biotechnological technique which came to limelight in 1980s, can be used to screen protein interactions [44]. It has been used extensively in making discoveries and understanding the protein-protein interactions in the fields of cell biology, microbiology, immunology, pharmacology, and drug discovery. In the past few decades, phage display has been extensively used in drug design and discovery, drug target validation, vaccines development, selection of new antibodies, analysis of protein-protein interaction, epitope mapping, identification of novel enzyme substrates and inhibitors, designing abzymes and novel enzymes. Commonly, filamentous phage particles (Ff) such as M13, f1, Fd and ft are used to display a protein on its coat surface. The single stranded DNA of M13 codes for a total of 10 genes which are named using Roman numerals I through X (Fig. **1**). The major and minor structural proteins of this bacteriophage are coded by gene VIII and III, respectively. The gene for the protein to be displayed is first inserted into the N-terminus of the phage coat protein III or VIII gene and its subsequent expression and display on phage surface results in connection of the genotype with the phenotype [45].The libraries based on coat protein pIII can display 3-5 copies of the peptide, whereas the pVIII libraries can display upto 2700 copies of the short peptides. As per the classification of Smith, type 3 system represents a phage display system where all 5 copies of pIII are transcribed from a phage vector carrying a single fusion gene. When the phage vector carries both the recombinant and wild type pIII genes, the system is known as 33 system. A 3+3 system is defined as the pIII phagemid system which carries two different forms of pIII (recombinant peptide-pIII fusion genes are encoded in phagemids, while wild type pIII gene is encoded by a helper phage). Similarly, pVIII display systems are referred as 8, 88 and 8+8 systems [45].

In principle, the gene of interest containing multiple cloning sites is ligated with coat protein gene of phage. This is followed by transduction which ensures that our gene of interest is delivered inside the host bacterium too. When a phagemid vector is used, at least some of the mature virions are assembled with our protein of interest present in their outer protein coat. The phage has displayed the protein and the resultant phage display library can be screened for binding to target protein or DNA partners by the process called 'Biopanning' [46]. Biopanning is the most crucial step in setting up the phage display experiment. It is based on the affinity of the displayed peptide against a desired target molecule, which can be controlled by manipulating the selection and screening conditions. During biopanning, the target protein or DNA is immobilized to microtitre plate while the phage display library is added and incubated with it [46]. The unbound fraction is washed away whereas the bound phages are eluted and enriched. Three to four rounds of selection are generally carried out in a biopanning experiment. Finally, the DNA within the interacting phage is sequenced to identify the interaction protein. A two-step selection can also be applied by first using biotinylated target immobilized on streptavidin coated magnetic beads. After the phage display library is sufficiently incubated with target to ensure binding, the unbound fraction can be washed away and the bound fraction can be eluted. This fraction can be amplified and selection can be repeated next using panning [46].

The main advantage of this technique is the ability to construct a library containing as many as 10^{10} variants with differently displayed peptide simultaneously. Moreover, phage particles remain stable in the extremes of pH and temperatures without affecting their bacterial infectivity. It allows experimentalists to use protocols with low pH and high urea concentration to isolate phage particles that are bound to the target for the next round of biopanning.

APPLICATIONS OF PHAGE DISPLAY TECHNIQUE

Phage display can be put to variety of significant uses. It can produce monoclonal antibodies of virtually any specificity and hence be put to a therapeutic use in human beings. Moreover, as the genetic makeup of the antibody is known, with the gene of expressed antibody being present within the phage, the genetic and functional aspects of antigen specific monoclonal antibodies can be better understood. This can lead to better understanding of immunological processes and autoimmunity [47, 48]. Also, the monoclonal antibodies produced using phage display can be easily purified using different protein tags [49].

Phage-display has become a powerful tool for selecting novel peptides exhibiting affinity towards a protein or cell membrane. Sainath Rao S *et al.* have used phage-displayed random peptide library to identify peptides binding to the cell surface of *Escherichia coli* [50]. They found that a peptide with sequence RLLFRKIRRLKR showed high affinity towards cell surface of *E. coli* [50]. This peptide can permeabilize the outer membrane of *E. coli* as well as *Pseudomonas aeruginosa*, thereby causing rapid depolarization of the cytoplasmic membrane and killing of the cells [50]. Using phage display technique, Desimmie *et al.* have identified cyclic peptides that inhibit the interaction of LEDGF/p75 with HIV integrase (IN) [51]. As LEDGF/p75-IN interaction is crucial for the replication of HIV, a potential method to prevent AIDS has been envisioned [51]. Short bactericidal peptides that bind to cell surface of virulent strains *Haemophilus influenza* and *Campylobacter jejuni* have been isolated by subtractive phage display approach [52-54]. Phage-displayed peptide library has also been used to screen against bacterial magnetic particles isolated from *Magnetospirillum magneticum* coated with a lipid bilayer membrane. The isolated peptide was reported to exclusively inhibit *Bacillus subtilis* [54]. Moreover, a combination of panning strategies against the hemagglutinin (HA) of H5N1 have been used to identify and isolate peptides displaying affinity towards more conserved regions of HA

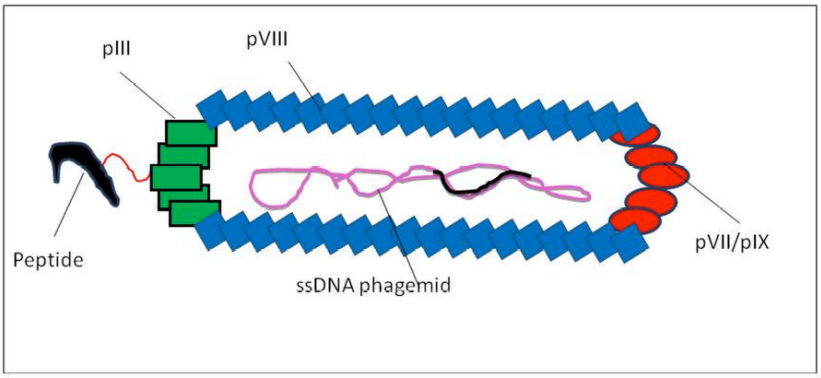

Fig. (1). Phage Display by M13 bacteriophage. A phage particle displays a peptide fused to the N-terminus of the minor coat protein, pIII, through a short peptide linker. The major coat protein, pVIII, encapsulates the single-stranded DNA (ssDNA) viral genome that encodes the genes for the displayed peptide as well as pIII.

[55]. Peptide inhibitors for an important enzyme in the biosynthesis of bacterial cell wall, MurA has been reported by panning against the NEB PhD-12 and PhD-C7C libraries using *Pseudomonas aeruginosa* MurA enzyme [56]. A peptide with consensus sequence Cys-Val-His-Ser-Pro-Asn-Arg-Glu-Cys has been identified by Sanschagrin and co-workers as an inhibitor of MBL (L-1) hydrolytic activity using phage display and competitive biopanning assay [57]. Broad spectrum antibacterial peptide inhibitor of TEM-1 β-lactamase has also been reported using phage display and peptide arrays [58].

Fusion peptides displayed by phage have been recently shown to differentiate between receptors that are expressed by different types of cancer cell lines. This can be an all new approach to treat cancer, because the heterogeneous tumor population has long posed a problem in proper utilization of targeted anti-cancer drugs [59]. Phage display technique can also be used to modify different types of nanomedicine structures, whether they are liposomal, micellar or nanorods and help in specific delivery of anti cancer drugs [60-62]. *In vivo* phage display has been utilized for even targeting the drugs that are systematically given due to the presence of disease and organ specific molecular signatures on the blood vessels [63]. In fact, the vascular diversity can be explored without any prejudice by employing the *in vivo* phage display. The significance of this cannot be more emphasized as the protein based drugs can be easily screened in an *in vivo* environment [64].

SIGNIFICANCE AND FUTURE PROSPECTS

Since the discovery of penicillin by Fleming in 1929, β-lactam containing antibiotics have been widely used world over to treat bacterial infections [65]. However, the prolonged, extensive and non-judicial use of β-lactam antibiotics has given an opportunity to bacteria to develop resistance. The selection and adaptation of resistant bacterial strains represent an efficient system that can hydrolyze any β-lactam core containing antibiotics. Thus, the need of the hour is to look for non-β-lactam molecules and develop them as efficient weapon against resistant bacterial pathogens. The screening of peptide based inhibitors (antibacterial peptides) by phage display represents one such alternative approach. The advantage of using antibacterial peptides against resistant bacteria is that the bacteria will be unable to recruit pre-existing resistance mechanisms.

The prerequisite of a molecule to be defined as drug is its ability to bind its molecular target with a reasonable affinity. However, more than 60% of the candidate drug molecules discovered by medicinal chemistry approach do not qualify in the 'hit-to-lead' process either due to poor affinity towards their target or the failure of the finding a druggable biological target. Phage display is advantageous in facilitating the screening of diverse targets to discover candidate drug molecules with high potency. It may also be proved useful to screen peptide inhibitors against β-lactamases that are otherwise resistant to β-lactam core containing antibiotics. The antibacterial peptides may be used as scaffold for further modification and production of peptides with better efficacy. Moreover, these molecules will be novel to the bacterial defense system and hence bacteria will take time to develop new or modify existing mechanisms of resistance. Moreover, since all β-lactamases share a common three-dimensional structure, it is expected that the antibacterial peptide screened against β-lactamases (binding to the active site) of one group will be effective against the enzymes of other groups as well.

LIST OF ABBREVIATIONS

AMPs	=	Antimicrobial peptides
ESBL	=	Extended spectrum β-lactamase
MBL	=	Metallo-β-lactamase
NDM-1	=	New Delhi metallo-beta-lactamase 1

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Bush, K. The impact of beta-lactamases on the development of novel antimicrobial agents. *Curr. Opin. Investig. Drugs (London, England: 2000)*, **2002**, *3*, (9), 1284-1290.

[2] Faheem, M.; Rehman, M.T.; Danishuddin, M.; Khan, A.U. Biochemical Characterization of CTX-M-15 from Enterobacter cloacae and Designing a Novel Non-β-Lactam-β-Lactamase Inhibitor. *PLoS ONE*, **2013**, *8*, (2).

[3] Chambers, H.F., Penicillin-binding protein-mediated resistance in pneumococci and staphylococci. *J. Infect. Dis.*, **1999**, *179 Suppl 2*, S353-359.

[4] Li, X.-Z.; Zhang, L.; Nikaido, H. Efflux pump-mediated intrinsic drug resistance in Mycobacterium smegmatis. *Antimicrob. Agents Chemother.*, **2004**, *48*, (7), 2415-2423.

[5] Thiolas, A.; Bornet, C.; Davin-Régli, A.; Pagès, J.-M.; Bollet, C. Resistance to imipenem, cefepime, and cefpirome associated with mutation in Omp36 osmoporin of Enterobacter aerogenes. *Biochem. Biophys. Res. Commun.*, **2004**, *317*, (3), 851-856.

[6] Tondi, D.; Morandi, F.; Bonnet, R.; Costi, M.P.; Shoichet, B.K. Structure-based optimization of a non-beta-lactam lead results in inhibitors that do not up-regulate beta-lactamase expression in cell culture. *J. Am. Chem. Soc.*, **2005**, *127*, (13), 4632-4639.

[7] Bennett, P.M.; Chopra, I. Molecular basis of beta-lactamase induction in bacteria. *Antimicrob. Agents Chemother.*, **1993**, *37*, (2), 153-158.

[8] Alekshun, M.N.; Levy, S.B. Molecular mechanisms of antibacterial multidrug resistance. *Cell*, **2007**, *128*, (6), 1037-1050.

[9] Charnas, R.L.; Knowles, J.R. Inhibition of the RTEM beta-lactamase from Escherichia coli. Interaction of enzyme with derivatives of olivanic acid. *Biochemistry*, **1981**, *20*, (10), 2732-2737.

[10] Henquell, C.; Chanal, C.; Sirot, D.; Labia, R.; Sirot, J. Molecular characterization of nine different types of mutants among 107 inhibitor-resistant TEM beta-lactamases from clinical isolates of Escherichia coli. *Antimicrob. Agents Chemother.*, **1995**, *39*, (2), 427-430.

[11] Imtiaz, U.; Billings, E.M.; Knox, J.R.; Mobashery, S. A structure-based analysis of the inhibition of class A beta-lactamases by sulbactam. *Biochemistry*, **1994**, *33*, (19), 5728-5738.

[12] Yong, D.; Toleman, M.A.; Giske, C.G.; Cho, H.S.; Sundman, K.; Lee, K.; Walsh, T.R. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob. Agents Chemother.*, **2009**, *53*, (12), 5046-5054.

[13] Matsuzaki, S.; Rashel, M.; Uchiyama, J.; Sakurai, S.; Ujihara, T.; Kuroda, M.; Ikeuchi, M.; Tani, T.; Fujieda, M.; Wakiguchi, H.; Imai, S. Bacteriophage therapy: A revitalized therapy against bacterial infectious diseases. *J. Infect. Chemother.*, **2005**, *11*, (5), 211-219.

[14] Hancock, R.E. Cationic peptides: Effectors in innate immunity and novel antimicrobials. *The Lancet. Infectious Diseases*, **2001**, *1*, (3), 156-164.

[15] Yeaman, M.R.; Yount, N.Y. Mechanisms of antimicrobial peptide action and resistance. *Pharmacol. Rev.*, **2003**, *55*, (1), 27-55.

[16] Giuliani, A.; Pirri, G.; Nicoletto, S.F. Antimicrobial peptides: An overview of a promising class of therapeutics. *Cent. Eur. J. Biol*, **2007**, *2*, (1), 1-33.

[17] Aliahmadi, A.; Roghanian, R.; Emtiazi, G.; Ghassempour, A. A simple method for primary screening of antibacterial peptides in plant seeds. *Iran J. Microbiol*, **2011**, *3*, (2), 104-108.

[18] Battison, A.L.; Summerfield, R.; Patrzykat, A. Isolation and characterisation of two antimicrobial peptides from haemocytes of the American lobster Homarus americanus. *Fish Shellfish Immunol.*, **2008**, *25*, (1-2), 181-187.

[19] Treffers, C.; Chen, L.; Anderson, R.C.; Yu, P.-L. Isolation and characterisation of antimicrobial peptides from deer neutrophils. *Int. J. Antimicrob. Agents*, **2005**, *26*, (2), 165-169.

[20] Wang, X.; Song, Y.; Li, J.; Liu, H.; Xu, X.; Lai, R.; Zhang, K. A new family of antimicrobial peptides from skin secretions of Rana pleuraden. *Peptides*, **2007**, *28*, (10), 2069-2074.

[21] Wang, H.; Yu, Z.; Hu, Y.; Li, F.; Liu, L.; Zheng, H.; Meng, H.; Yang, S.; Yang, X.; Liu, J. Novel antimicrobial peptides isolated from the skin secretions of Hainan odorous frog, Odorrana hainanensis. *Peptides*, **2012**, *35*, (2), 285-290.

[22] Zhong, J.; Wang, W.; Yang, X.; Yan, X.; Liu, R. A novel cysteine-rich antimicrobial peptide from the mucus of the snail of Achatina fulica. *Peptides*, **2013**, *39*, 1-5.

[23] Hamill, P.; Brown, K.; Jenssen, H.; Hancock, R.E.W. Novel anti-infectives: Is host defence the answer? *Curr. Opin. Biotechnol.*, **2008**, *19*, (6), 628-636.

[24] Hancock, R.E.W.; Sahl, H.G. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat. Biotechnol.*, **2006**, *24*, (12), 1551-1557.

[25] Huang, W.; Beharry, Z.; Zhang, Z.; Palzkill, T. A broad-spectrum peptide inhibitor of beta-lactamase identified using phage display and peptide arrays. *Protein Eng.*, **2003**, *16*, (11), 853-860.

[26] Zhang, G.; Lin, X.; Long, Y.; Wang, Y.; Zhang, Y.; Mi, H.; Yan, H. A peptide fragment derived from the T-cell antigen receptor protein α-chain adopts β-sheet structure and shows potent antimicrobial activity. *Peptides*, **2009**, *30*, (4), 647-653.

[27] Oren, Z.; Shai, Y. Selective lysis of bacteria but not mammalian cells by diastereomers of melittin: structure-function study. *Biochemistry*, **1997**, *36*, (7), 1826-1835.

[28] Matsuzaki, K. Magainins as paradigm for the mode of action of pore forming polypeptides. *Biochim. Biophys. Acta*, **1998**, *1376*, (3), 391-400.

[29] Sahl, H.-G.; Pag, U.; Bonness, S.; Wagner, S.; Antcheva, N.; Tossi, A. Mammalian defensins: structures and mechanism of antibiotic activity. *J. Leukoc. Biol.*, **2005**, *77*, (4), 466-475.

[30] Powers, J.P.S.; Hancock, R.E.W. The relationship between peptide structure and antibacterial activity. *Peptides*, **2003**, *24*, (11), 1681-1691.

[31] Structural, functional and evolutionary studies of antimicrobial peptides. https://lup.lub.lu.se/search/publication/1470860

[32] Bowdish, D.M.E.; Davidson, D.J.; Lau, Y.E.; Lee, K.; Scott, M.G.; Hancock, R.E.W. Impact of LL-37 on anti-infective immunity. *J. Leukoc. Biol.*, **2005**, *77*, (4), 451-459.

[33] Scott, M.G.; Davidson, D.J.; Gold, M.R.; Bowdish, D.; Hancock, R.E.W. The human antimicrobial peptide LL-37 is a multifunctional modulator of innate immune responses. *J. Immunol.*, **2002**, *169*, (7), 3883-3891.

[34] Tjabringa, G.S.; Ninaber, D.K.; Drijfhout, J.W.; Rabe, K.F.; Hiemstra, P.S. Human cathelicidin LL-37 is a chemoattractant for eosinophils and neutrophils that acts via formyl-peptide receptors. *Int. Arch. Allergy Immunol.*, **2006**, *140*, (2), 103-112.

[35] Mookherjee, N.; Brown, K.L.; Bowdish, D.M.E.; Doria, S.; Falsafi, R.; Hokamp, K.; Roche, F.M.; Mu, R.; Doho, G.H.; Pistolic, J.; Powers, J.P.; Bryan, J.; Brinkman, F.S.L.; Hancock, R.E.W. Modulation of the TLR-mediated inflammatory response by the endogenous human host defense peptide LL-37. *J. Immunol.*, **2006**, *176*, (4), 2455-2464.

[36] Davidson, D.J.; Currie, A.J.; Reid, G.S.D.; Bowdish, D.M.E.; MacDonald, K.L.; Ma, R.C.; Hancock, R.E.W.; Speert, D.P. The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization. *J. Immunol.*, **2004**, *172*, (2), 1146-1156.

[37] Koczulla, R.; von Degenfeld, G.; Kupatt, C.; Krötz, F.; Zahler, S.; Gloe, T.; Issbrücker, K.; Unterberger, P.; Zaiou, M.; Lebherz, C.; Karl, A.; Raake, P.; Pfosser, A.; Boekstegers, P.; Welsch, U.; Hiemstra, P.S.; Vogelmeier, C.; Gallo, R.L.; Clauss, M.; Bals, R. An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. *J. Clin. Invest.*, **2003**, *111*, (11), 1665-1672.

[38] Bowdish, D.M.E.; Davidson, D.J.; Speert, D.P.; Hancock, R.E.W. The human cationic peptide LL-37 induces activation of the extracellular signal-regulated kinase and p38 kinase pathways in primary human monocytes. *J. Immunol.*, **2004**, *172*, (6), 3758-3765.

[39] Yang, D.; Biragyn, A.; Kwak, L.W.; Oppenheim, J.J. Mammalian defensins in immunity: more than just microbicidal. *Trends Immunol.*, **2002**, *23*, (6), 291-296.

[40] Hale, J.D.F.; Hancock, R.E.W. Alternative mechanisms of action of cationic antimicrobial peptides on bacteria. *Expert Review of Anti-Infective Therapy*, **2007**, *5*, (6), 951-959.

[41] Ehrenstein, G.; Lecar, H. Electrically gated ionic channels in lipid bilayers. *Q. Rev. Biophys.*, **1977**, *10*, (1), 1-34.

[42] Breukink, E.; de Kruijff, B. The lantibiotic nisin, a special case or not? *Biochim. Biophys. Acta*, **1999**, *1462*, (1-2), 223-234.

[43] Uematsu, N.; Matsuzaki, K. Polar angle as a determinant of amphipathic alpha-helix-lipid interactions: A model peptide study. *Biophys. J.*, **2000**, *79*, (4), 2075-2083.

[44] Smith, G.P. Filamentous fusion phage: Novel expression vectors that display cloned antigens on the virion surface. *Science*, **1985**, *228*, (4705), 1315-1317.

[45] Molek, P.; Strukelj, B.; Bratkovic, T. Peptide phage display as a tool for drug discovery: Targeting membrane receptors. *Molecules (Basel, Switzerland)*, **2011**, *16*, (1), 857-887.

[46] Huang, J.X.; Bishop-Hurley, S.L.; Cooper, M.A. Development of anti-infectives using phage display: Biological agents against bacteria, viruses, and parasites. *Antimicrob. Agents Chemother.*, **2012**, *56*, (9), 4569-4582.

[47] Lee, C.M.Y.; Iorno, N.; Sierro, F.; Christ, D. Selection of human antibody fragments by phage display. *Nature Protocols*, **2007**, *2*, (11), 3001-3008.

[48] Schüller, C.; Wiebe, J.C.; Pegel, A.; Kramer, K.; Skerra, A.; Hock, B. A system for repertoire cloning and phage display of murine and leporid antibody fragments. *J. AOAC Int.*, **2010**, *93*, (1), 66-79.

[49] Phage Display: A Laboratory Manual.

[50] Sainath Rao, S.; Mohan, K.V.K.; Atreya, C.D. A peptide derived from phage display library exhibits antibacterial activity against E. coli and Pseudomonas aeruginosa. *PLoS ONE*, **2013**, *8*, (2), e56081.

[51] Desimmie, B.A.; Humbert, M.; Lescrinier, E.; Hendrix, J.; Vets, S.; Gijsbers, R.; Ruprecht, R.M.; Dietrich, U.; Debyser, Z.; Christ, F. Phage display-directed discovery of LEDGF/p75 binding cyclic peptide inhibitors of HIV replication. *Mol. Ther.*, **2012**, *20*, (11), 2064-2075.

[52] Bishop-Hurley, S.L.; Schmidt, F.J.; Erwin, A.L.; Smith, A.L. Peptides selected for binding to a virulent strain of Haemophilus influenzae by phage display are bactericidal. *Antimicrob. Agents Chemother.*, **2005**, *49*, (7), 2972-2978.

[53] Bishop-Hurley, S.L.; Rea, P.J.; McSweeney, C.S. Phage-displayed peptides selected for binding to Campylobacter jejuni are antimicrobial. *Protein engineering, design & selection: PEDS*, **2010**, *23*, (10), 751-757.

[54] Tanaka, T.; Kokuryu, Y.; Matsunaga, T. Novel method for selection of antimicrobial peptides from a phage display library by use of bacterial magnetic particles. *Appl. Environ. Microbiol.,* **2008**, *74*, (24), 7600-7606.

[55] Lim, A.P.C.; Chan, C.E.Z.; Wong, S.K.K.; Chan, A.H.Y.; Ooi, E.E.; Hanson, B.J. Neutralizing human monoclonal antibody against H5N1 influenza HA selected from a Fab-phage display library. *Virology Journal*, **2008**, *5*, 130.

[56] Molina-López, J.; Sanschagrin, F.; Levesque, R.C. A peptide inhibitor of MurA UDP-N-acetylglucosamine enolpyruvyl transferase: The first committed step in peptidoglycan biosynthesis. *Peptides*, **2006**, *27*, (12), 3115-3121.

[57] Sanschagrin, F.; Levesque, R.C. A specific peptide inhibitor of the class B metallo-beta-lactamase L-1 from Stenotrophomonas maltophilia identified using phage display. *J. Antimicrob. Chemother.*, **2005**, *55*, (2), 252-255.

[58] Huang, J.F.; Xu, Y.M.; Hao, D.M.; Huang, Y.B.; Liu, Y.; Chen, Y. Structure-guided de novo design of α-helical antimicrobial peptide with enhanced specificity. *Pure Appl. Chem.*, **2010**, *82*, (1), 243-257.

[59] Gross, A.L.; Gillespie, J.W.; Petrenko, V.A. Promiscuous tumor targeting phage proteins. *Protein engineering, design & selection: PEDS*, **2016**.

[60] Wang, T.; D'Souza, G.G.M.; Bedi, D.; Fagbohun, O.A.; Potturi, L.P.; Papahadjopoulos-Sternberg, B.; Petrenko, V.A.; Torchilin, V.P. Enhanced binding and killing of target tumor cells by drug-loaded liposomes modified with tumor-specific phage fusion coat protein. *Nanomedicine (London, England)*, **2010**, *5*, (4), 563-574.

[61] Wang, T.; Petrenko, V.A.; Torchilin, V.P. Paclitaxel-loaded polymeric micelles modified with MCF-7 cell-specific phage protein: enhanced binding to target cancer cells and increased cytotoxicity. *Mol. Pharm.*, **2010**, *7*, (4), 1007-1014.

[62] Wang, F.; Liu, P.; Sun, L.; Li, C.; Petrenko, V.A.; Liu, A. Biomimetic nanostructure self-assembled from Au@Ag heterogeneous nanorods and phage fusion proteins for targeted tumor optical detection and photothermal therapy. *Scientific Reports*, **2014**, *4*, 6808.

[63] Ruoslahti, E.; Bhatia, S.N.; Sailor, M.J. Targeting of drugs and nanoparticles to tumors. *The Journal of Cell Biology*, **2010**, *188*, (6), 759-768.

[64] Teesalu, T.; Sugahara, K.N.; Ruoslahti, E. Mapping of vascular ZIP codes by phage display. *Methods Enzymol.*, **2012**, *503*, 35-56.

[65] Fleming, A. On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of B. influenzæ. *Br. J. Exp. Pathol.*, **1929**, *10*, (3), 226-236.

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