Review Article

Investigación en Discapacidad
Vol. 5, Núm. 2
Mayo-Agosto 2016
pp 96-115

# Properties and design of antimicrobial peptides as potential tools against pathogens and malignant cells

## *Propiedades y diseño de péptidos antimicrobianos como herramientas potenciales contra patógenos y células malignas*

**Jazmín Huerta-Cantillo,* Fernando Navarro-García***

* Department of Cell Biology, Centro de Investigación y de Estudios Avanzados del IPN, CINVESTAV-IPN, México D.F.

Mailing address:
Fernando Navarro-García
Department of Cell Biology, CINVESTAV-IPN.
Ap. Postal 14-740, 07000, México City, México.
Tel: (52-55)57473990
Fax: (52-55)57473393
E-mail: fnavarro@cell.investav.mx

Recibido: 08 de marzo de 2016.
Aceptado: 30 de marzo de 2016.

Este artículo puede ser consultado en versión completa en:
http://**www.medigraphic.com/rid**

**Key words:** Infection, cancer, peptides, molecular structure, anticancer activity, bacteriocins, tumor cell, chemical modifications, clinical studies, designed peptides.

***Palabras clave:*** *Infección, cáncer, péptidos, estructura molecular, actividad anticancerígena, bacteriocinas, células tumorales, modificaciones químicas, estudios clínicos, diseño de péptidos.*

**Abstract**

In the last years, the indiscriminate use of conventional antibiotics has generated a worrisome increase of resistant pathogens. Antimicrobial peptides (AMPs) are considered a plausible alternative therapy against pathogens due to their structural and functional characteristics, as well as their low toxicity against eukaryotic cells and their broad spectrum of action against different pathogens, including Gram-negative and Gram-positive bacteria, fungi, parasite and virus. Interestingly, AMPs also have the capability to recognize certain types of plasma membranes, and this selectivity allows differential recognition of normal cells, non-malignant tumor cells and malignant tumor cells; thereby the use of these AMPs could be a viable alternative for cancer treatment. These peptides can be isolated from different organisms, such as microorganisms, plants and animals. Such peptides are amphipathic and cationic molecules of low molecular weight and they have a low probability to generate resistance. Therefore these natural peptides have been utilized as the base for synthetizing new analog peptides with chemical or structural modifications for improving their antimicrobial stability and efficiency. In this review, we focused on an overview of the AMPs: properties, mechanisms of action, and their different applications for combating pathogens in diverse fields, as well as their use due to the anticancer activity. We also focused on some strategies for the design of new peptides, and finally, we discussed some drawbacks to overcome their use as therapeutic agents.

***Resumen***

*En los últimos años, el uso indiscriminado de antibióticos convencionales ha generado un aumento inquietante de patógenos resistentes. Los péptidos antimicrobianos (PAMs) se consideran una terapia alternativa plausible contra los patógenos debido a sus características estructurales y funcionales, así como a su baja toxicidad frente a células eucariotas y a su amplio espectro de acción contra diferentes patógenos, incluyendo bacterias Gram-negativas y Gram-positivas, hongos, parásitos y virus. Curiosamente, los PAMs también tienen la capacidad de reconocer ciertos tipos de membranas plasmáticas, y esta selectividad permite el reconocimiento de células normales, células tumorales no malignas y células tumorales malignas; así el uso de estos PAMs podría ser una alternativa viable para el tratamiento del cáncer. Estos péptidos se pueden aislar de diferentes organismos, tales como microorganismos, plantas y animales. Tales péptidos son moléculas anfipáticas y catiónicas de bajo peso molecular y tienen baja probabilidad de generar resistencia. Por lo tanto estos péptidos naturales se han utilizado como base para la síntesis de nuevos péptidos análogos con modificaciones químicas o estructurales para mejorar su estabilidad y su eficacia antimicrobiana. En esta revisión, nos centramos en una visión general de los PAMs: propiedades, mecanismos de acción, y sus diferentes aplicaciones para combatir a los patógenos en diversos campos, así como su uso derivado de su actividad contra el cáncer. También nos enfocamos en algunas estrategias para el diseño de nuevos péptidos, y por último, discutimos algunos inconvenientes a superar para su uso como agentes terapéuticos.*

## Introduction

AMPs have been isolated from a great variety of organisms, including animals, plants, insects, bacteria, fungi, and even from viruses.[1] These peptides are part of the innate immunity that is an ancestral defense mechanism used by organisms for controlling the natural flora and for combating pathogens.[2] These peptides are the first in recognizing foreign particles and they show specificity to components of bacterial cell wall such as lipopolysaccharide (LPS) from Gram-negative bacteria, lipoteichoic acid from Gram-positive bacteria, glycolipids from mycobacteria, β-glycan from yeast and RNA strands from viruses; they are able to recognize to most of the microorganisms.[2-4]

Peptides are of low molecular weight (2-50 kDa), although some of them can be heavier. The main characteristics of the AMPs are based on what they are (i) net positive charge molecules (generally +2 to +9) due to their high amount of positive amino acids (aa) such as Lys and Arg, (ii) well-defined amphipathic molecules (with ≥ 30% of hydrophobic residues), stable in aqueous and hydrophobic solutions, (iii) they can undergo post-translational modifications.[5,6]

AMPs are mainly coded in the genome as one or several copies. All AMPs are derived from larger precursors, including a signal sequence. Post-translational modifications include proteolytic processing, and in some cases glycosylation, carboxyl-terminal amidation and amino-acid isomerization, halogenations and cyclization. Some peptides are derived by proteolysis from larger proteins, such as buforin II from histone 2A and lactoferricin from lactoferrin.[5]

The AMPs show different secondary structures, but most of them fall into four types *(Figure 1)*: (i) α-helix, (ii) β-pleated sheet, due to two or more disulfide bridges, (iii) mix of β-sheet and α-helix, due to a disulfide bridge and/or peptide circularization, and (iv) lineal or random.[7] Most of these peptides do not show a particular structure when they are free in solution and they are folded to their final conformation when directly bind to the membrane.[8]

The AMPs are generally synthesized in exposed tissues, such as skin, intestine, lungs and red blood cells. These peptides are synthesized 100 times more rapid than an immunoglobulin and at low metabolic cost, but they can also be stored as a reserve into cells and be released when cells are stimulated by the contact with pathogens.[9,10] These peptides are a fast non-specific way to combat a broad spectrum of microorganisms and it has been discovered in the last decade that these AMPs are efficient to combat tumor cells as well.[4,11]

## The role of the AMPs on the innate immune response

AMPs exist in virtually all multicellular organisms and have been studied in insects and other invertebrate organism that lack an adaptive immune system. AMPs also play an important role in the immune system of mammals, including humans. AMPs are present at all human body sites normally exposed to microbes such as the skin and mucosa.[12]

AMPs are part of the innate immune response with a relevant role in the first line of defense against microorganisms. Peptides can be inducible or constitutive, they are nonspecific and respond before the acquired immune response had been initiated, they do not generate immune memory, they are not catalytic but provide economy to cells by being small effector molecules.[9,10,12]

In addition to their direct antimicrobial activity, some AMPs possess different immunomodulatory functions.[12,13] Some of the most relevant are: 1) Chemotactic activity: in a direct manner, AMPs act as a chemo-attractant capable of recruit immune cells to the site of infection. In indirect manner, AMPs induce the expression of a broad range of chemokines such as

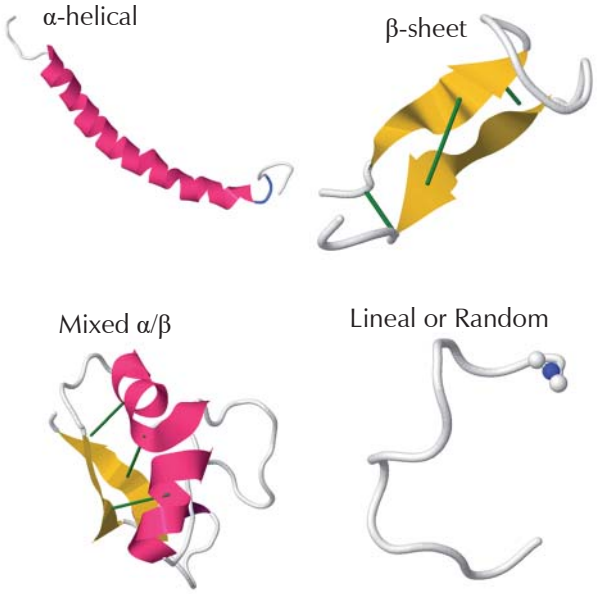


**Figure 1.** Representative secondary structure of AMPs. α-helix (Moricin), β-sheet (Hepcidin-20), mixed α-helix/β-sheet (Hydramacin-1) and lineal or random (Lf11). α-helix structures are shown in magenta, β-sheet in yellow, and green lines represent the disulfide bridges.

CXCL8/IL8, CCL2/MCP1 by neutrophils, monocytes and other immune cells.[14] 2) Anti-endotoxin activity: AMPs possess the capacity to dampen production of endotoxin-induced pro-inflammatory mediators such as tumor necrosis factor alpha (TNF-α) by blocking or modulating toll like receptor (TLR) signaling pathways.[15] 3) Immune cell differentiation: AMPs appear to have a direct link inducing cell differentiation and activation, bridging the innate and adaptive immune responses.[16] 4) Wound healing and angiogenesis: wound healing involves the re-growth of epithelial layers and the formation of new blood vessels (angiogenesis). AMPs act directly on epithelial and endothelial cells, inducing promoting re-epithelialization and angiogenesis. AMPs also induce wound healing indirectly through their chemotactic effects.[17] Due to these immunomodulatory and antibacterial properties, AMPs are excellent candidates for infections treatment since they can control inflammation in the infection site.

## Distribution of AMPs

AMPs are conserved from primitive organisms to mammals as humans with a phylogenetic relation relatively close indicating their presence since thousands of years in the earth, without suffering marked changes during evolution. Since the first AMP was discovered, more than 1400 AMPs have been isolated from bacteria, plants, insects and other invertebrates, including vertebrates such as amphibians, birds, fishes and mammals.[18]

**Bacteria.** Bacteria produce some AMPs as strategy for maintaining the control of population and for combating other microorganisms competing by space and nutrients of their environment. AMPs produced by bacteria are known as bacteriocins.[19] Bacteriocins produced by Gram-negative bacteria are classified in two large groups: 1) Colicins, which are high molecular weight molecules (25-80 kDa), encoded on plasmids and possessing three functional domains: a translocation amino-terminal domain, a central domain for receptor binding and a cytotoxic carboxyl-terminal domain (possessing the antibacterial activity). Colicins are simultaneously synthesized with specialized proteins to maintain them inactive and in that way avoid the damage to their own producer bacteria. Colicins are divided in three categories depending on their mechanism of action: (i) pore-forming colicins: they form pores in the plasma membrane of the target cell causing ions and cytoplasmic components loss, (ii) nuclease type colicins: DNAases, rRNAases 16s, tRNAases and for unspecific digestion of DNA and RNA, and (iii) peptidoglycanase type colicins: they digest the peptidoglycan precursors.[20] 2) The other group is that of the microcins, which are molecules of low molecular weight (< 10 kDa) produced as precursor peptides (amino-terminal signal peptide and core peptide) and are secreted to the extracellular milieu through the type I secretion system. Microcins are divided in two categories depending on their molecular weight and their post-translational modifications: (i) Class I microcins: they are of < 5 kDa and are post-translational modified, and (ii) Class II microcins: they are of 5-10 kDa and can or can not have post-translational modifications. The mechanism of action of microcins is varied and includes pore formation, nuclease activity, interference with energetic processes and inhibition of protein synthesis, and DNA replication.[21]

The bacteriocins produced by Gram-positive bacteria have a major diversity of characteristics regarding to size, structure and physicochemical properties. These bacteriocins are divided in three classes: (i) Class I: they are post-translational modified peptides, lantibiotics (contain lanthionine and dehydrated amino acids), of < 5 kDa and can be lineal o globular; lineal peptides are membrane disruptors and the globular peptides have enzymatic activity. (ii) Class II: they are unmodified peptides, without lanthionine, of < 10 kDa, positively charged and with great heat resistance. (iii) Class III: they are high molecular weight peptides (> 30 kDa), very sensible to heat and can or not have bacteriolytic function.[19]

**Plants.** Plants produce small cysteine-rich AMPs and are produced in all organs, but they are more abundant in the outer layer. Most of plant AMPs have a molecular weight between 2-10 kDa and are classified based on the identity of their amino acid sequence and the number and position of cysteines forming disulfide bonds.[22] There are twelve families described for plant AMPs: (i) thionins are synthesized in leafs, flowers and seeds and have activity against fungi and bacteria, (ii) denfesins are synthesized in seeds during germination and have activity against fungi and bacteria, (iii) cyclotides are macrocyclic peptides, resistant to protease degradation and have activity against bacteria, virus and insects, (iv-vi) knottin-like, Ib-AMPs and Hevein-like are effective only against Gram-positive bacteria and fungi, (vii-xi) shepherdins, MBP-1, LTP, Snakins and 2S albumins have antibacterial and antifungal activity and (xii) β-barrelins are only actives against fungi. Thionins, defensins and cyclotides have been shown to have anticancer activity.[3,22,23]

Additionally, there are peptides in plants whose main function is the inhibition of proteases, such AMPs

are found in tubers and seeds and they are stored as energy source and as defense mechanism. Tomatoes produce inhibitors of trypsin and chymotrypsin and potatoes produce inhibitors of serine-proteases and carboxypeptidases; these inhibitors are involved in the response induced by wounds and UV radiations. The importance of these inhibitor peptides is due to they can be used in a safe way in humans because they are not toxic and can avoid the growth of tumor cells, helping to reduce the food ingest and to inhibit most of the intestinal proteases; however, their specificity and potency depend on the inhibition sites and on the organism from the target proteinase was derived.[4]

## Invertebrates

**Insects.** AMPs are synthesized in insect fat bodies and constitutive AMPs are accumulated in blood cells and salivary glands, in the presence of microbes the AMPs are secreted into the hemolymph. Induced AMPs are synthesized after the microbial infection takes place.[24] Some of the peptides produced by insects are: (i) Cecropins are linear α-helical peptides of 3-4 kDa, which have a high content of proline but no cysteine. Cecropins have activity against Gram-negative bacteria, fungi, viruses and also have insecticidal and antitumor activities. (ii) Insect defensins are cyclopeptides of 4-5 kDa with three disulfide bridges and they can control most Gram-positive bacteria and a few fungi and yeast. (iii) Drasomycin is cyclopeptide of 5 kDa which has a strong antifungal activity. (iv) Thanatin is a cyclopeptide of 2.5 kDa with one disulfide bridge and has antimicrobial activity against multidrug resistant clinical isolates of *Enterobacter aerogenes* and *Klebsiella pneumoniae*. (v) Apidaecin and abaecin from bees are peptides of 4 kDa and have a high content of proline and are effective mainly against Gram-negative bacteria. (vi) Metchnikowin is a proline-rich peptide of 2 kDa and has activity against Gram-positive bacteria, fungi and insects. (vii) Formaecin, drosocin, pyrrhocoricin and lebocin are proline-rich peptides (2-3.5 kDa), which have activity against Gram-negative bacteria. (viii) Attacin, gloverin, diptericin, hymenoptaecin, coleoptericin and hemiptericin are glyicine-rich peptides of 8-30 kDa and these peptides are mostly active against Gram-negative bacteria. (ix) Attacin and sarcotoxin also have insecticidal activity.[3,24,25]

**Marine invertebrates.** AMPs are important for survival of marine invertebrates because they lack acquired immune response mediated by B and T lymphocytes.[26] Several AMPs have been detected in marine invertebrates and mainly possess antibacterial and antifungal activity. These peptides are: (i) Porifera (sponges): discodermins, halicylindramides, theonellamides, cyclolithistides and phoriospongins; (ii) Cnidaria (anemones, corals and medusas): aurelins, hydralysins, styholysins; (iii) Mollusca (snails, clams, mussels, squids and octopi): mytilus, mytilins and myticins; (iv) Annelida (segmented worms): hedistins, arenicins, theromyzins and theromacins; (v) Arthropoda (crustaceous): penaedins, callinectins, astacidins, thachyplesin and tachystatins; and (vi) Echinodermata (sea stars): strongyolocins.[26,27] The peptides derived from marine organism have been shown to have inherent ability to sustain under high salt concentrations, so they may have high probability of success under physiological salt concentrations.[26,28]

## Vertebrates

**Amphibians.** The amphibian skin is characterized by its remarkable cutaneous exocrine apparatus with numerous granular and mucous glands.[29] Additionally, amphibian skin possesses the greater variety and concentration of AMPs that can be found in a vertebrate or invertebrate, allowing them a great capability to heal wounds and prevent infections. Frogs and toads (Anura) skins produce different AMPs, which present a broad spectrum of action against Gram-positive and Gram-negative bacteria, fungi and protozoa.[30] Some examples of the families of peptides in Anura are: brevinins, esculetins, japonicins, mellitin-like peptides, nigrocins, palustrins, ranacyclins, ranatuerins, temporins, aureins, caerins, citropins, dermasepins, phylloseptins, plasticins, phylloxins, alyteserins, bombinins, magainins and signiferins.[31]

**Birds.** For avian species, three main classes of AMPs have been described. These are β-defensins, cathelicidins (CATH) and liver-expressed antimicrobial peptide-2 (LEAP-2). These peptides protect the avian embryo and young hatchling from infections. Post-hatch, AMPs protect chickens, turkeys, ostriches, ducks, gooses, quails and penguins from Gram-negative and Gram-positive bacteria, mycoplasm and fungi infections.[32]

**Mammals.** Within mammalian species, defensins and cathelicidins are the two principal antimicrobial peptide families. Defensins are 18-45 amino acid long (2-4.5 kDa), cysteine-rich, cationic peptides characterized by three conserved disulfide bridges without glycosyl- or acyl- side-chain modification, a β-sheet structure and both hydrophobic and cationic amino acids. Based on the site of expression, size, structure and pattern of disulphide bridge, the defensin family

can be subdivided into three main groups: the α, β and θ-defensins.[33,34]

α-defensins are 29-35 residues long with a disulfide alignment pattern of 1-6, 2-4 and 3-5 and only present in mammalian species. α-defensins are either predominantly synthesized in neutrophils at high concentration and in a constitutive way in its active form or stored as propeptides in paneth cells.[35] β-defensins are 36-42 residues long with a disulfide alignment pattern of 1-5, 2-4 and 3-6 and can be found in all vertebrate species. β-defensins are mainly produced by epithelial cells, at low concentration and are generally produced in an inducible way.[36,37] The θ-defensins are 18 residues cyclic defensins with a disulfide alignment patter of 1-6, 2-5 and 3-4 and they are formed by post-translational ligation of two 9-residue sequences derived by heterodimeric splicing of α-defensin-related precursors. θ-defensins are found in neutrophils and leukocytes from certain non-human primates, whereas only pseudogenes for θ-defensins are present in the human genome.[38] Since human do not produce θ-defensins, the peptides corresponding to pseudogenes have been recreated using solid-phase synthetic approaches and are called retrocyclins.[39] Retrocyclins and other θ-defensins are also known as minidefensins.[40,41]

Cathelicidins form the second largest group of AMPs produced by mammals, and are characterized by far N-terminal end named cathelin domain, a central conserved region and a variable C-terminal region.[42] Like defensins, cathelicidins are synthesized as propeptides, which are cleaved in a two-step process to release the active peptides. To date, cathelicidins have been found in fish, birds, snakes and mammals. There is only one type of cathelicidin in humans (hCAP18/LL37).[18]

The human skin also produces a great amount of cathelicidin and defensins, which are effective against Gram-positive and Gram-negative bacteria, as well as against yeasts. The intestine produces β-defensins that control the enteric flora and pathogens, while granules from leucocytes contain a high concentration of α-defensins that control Gram-positive and Gram-negative bacteria, filamentous fungi, enveloped viruses and some mycobacteria.[18,43,44]

Unlike humans, which have α- and β-defensins genes, the bovine genome contains only the β-defensin subfamily. Cattle have the most diverse repertoire of β-defensin genes so far identified and they are expressed mainly in epithelial mucosa but also in neutrophils and macrophages. Bovine β-defensin are often related to the reproductive success and immunity of cattle to diverse diseases.[45] Mouth, trachea, lungs, bronchus, colon and rectus of bovine produce β-defensins with activity against *E. coli*, *K. pneumoniae*, *S. aureus*, *P. aerugionosa* and *C. albicans*.[43]

## Mechanism of action

The classification of the mechanisms of action of the AMPs can be divided in two classes: membrane disruptors and non-membrane disruptors; however, the initial common target for both is their interaction with the plasma membrane through electrostatic charges. Membrane disruptor peptides are the most predominant and much of them have α-helix structures, which directly act at the plasma membrane level, altering the cell permeability or lysing cells through pores formation. Membrane disruptor peptides can be subclassi-

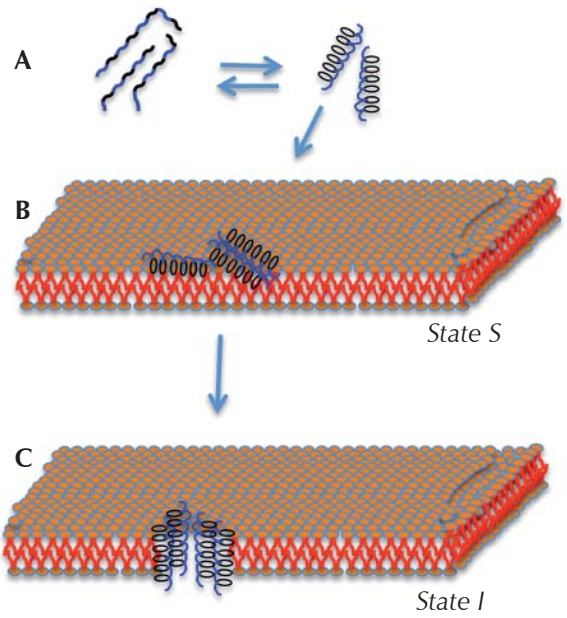


**Figure 2.** Schematic model of action mechanism of α-helix AMPs. **A.** Peptides in aqueous solution have lineal conformation. **B.** At the contact with the membrane, the peptides acquire a helix structure, where are polarized the hydrophobic and hydrophilic amino acids; the electrostatic interaction between the phospholipids of the membrane and the cationic peptides allows that peptides get a parallel position respect to the membrane (state S). **C.** At high concentration, the peptides are perpendicularly inserted into the membrane (state I) forming pores. Hydrophobic regions are represented in black and the hydrophilic regions in blue.

fied in two groups based on their biological activity: (i) selective, which are active against bacteria but inactive against mammalian cells, and (ii) non selective, which have activity against both type of cells.[46] The mechanism of AMPs with α-helix structure is given by the following steps *(Figure 2)*: (i) binding of AMPs to acid phospholipids through electrostatic forces. Monomers are accommodated by their amphipathic regions in a way that their positive amino acids match with the negative heads of phospholipids, when the local relative concentration of the peptide is low, these are parallel oriented on the membrane and they stay inactive in a state known as state S «surface»; (ii) when the local relative concentration of the peptide increases, it tends to take a perpendicular orientation respect to the membrane, in that way it becomes active and is inserted into the membrane, known as state I «inserted». The different susceptibility grades of the membrane depend on their composition and properties, which confer determined acceptation of the peptide; (iii) finally, the insertion of peptides alters the permeability of the lipid bilayer or the cell lysis occurs by the pore formation.[47] Actually, three models have been proposed by which the peptides are inserted in the membrane by *(Figure 3)*: type barrel-stave, toroidal pore and type carpet or detergent.[3,11,47]

Non-membrane disruptor AMPs use some of the following mechanisms of action: (i) binding to nucleic acids, (ii) interference with the synthesis of nucleic acids, (iii) inhibition of the synthesis of proteins, (iv) inhibition of the enzymatic activity, (v) inhibition of the synthesis of cell wall, (vi) cell damage by the accumulation of peptides inside the cell, (vii) flocculation of intracellular components, (viii) alteration of the septum formation, and (ix) blocking some virulence factors, such as flagella, proteases, secretion systems, effector proteins, etc. The blocking of these factors represses the bacterium motility or reduces its infectivity, which allows to the host controlling the infection.[47-49]

## Importance and specific applications of AMPs

Due to their structural and functional characteristics, as well as by their low toxicity to eukaryotic cells, their broad spectrum of action against different pathogens and their immumodulatory properties, the AMPs are being evaluated as therapeutic and prophylactic agents.[13]

The main interest of the use of AMPs in clinical applications is based on the great concern on resistance and multi-drug resistance of pathogens to antibiotics, which has increased dramatically over the past few years. A promising solution is the production of new antibiotics, which must be completely different to those that have loss the effectively and whose resistance has

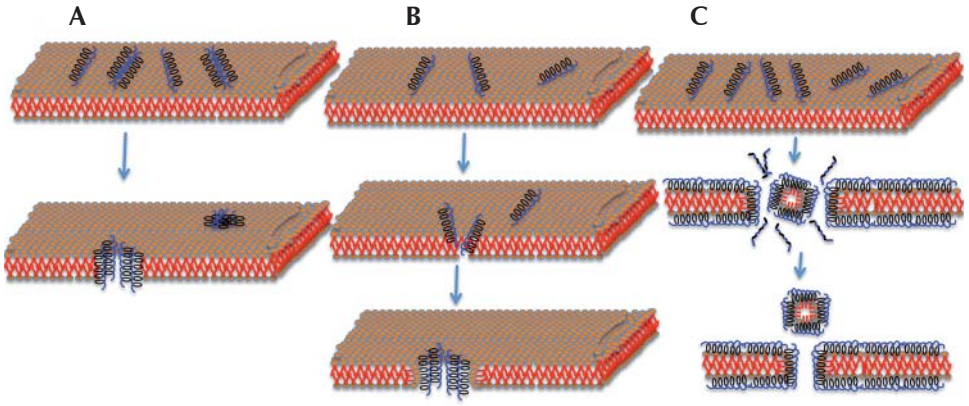




**Figure 3.** Schematic models of action mechanisms of membrane disruption by AMPs. **A.** Barrel stave pore. Peptides on the membrane surface are aggregated and inserted inside the membrane; the hydrophobic regions are aligned with the central lipid region of the bilayer, while the hydrophilic regions form the pore interior. **B.** Toroidal pore. Peptides are aggregated and induce that the lipid monolayer are continuously curved forming a pore; the pore interior is covered of peptides and also of polar heads of lipids. **C.** Carpet or detergent type. Peptides cover the membrane surface forming a carpet; toroidal pore are transitorily formed, which permit the entry of peptides and the membrane is starting to be disaggregated by the formation of micelles. Hydrophobic regions are represented in black and the hydrophilic in blue.

not been easy to reach. Unlike the current antibiotics, the action of AMPs depends not only on the neutralization of the chemical agent but also on the alteration of the membrane or blocking of a cellular component that is not possible to mutate or replace. Peptides have the advantage that there is not possibility of resistance transmission by horizontal transfer or in an interspecies way. Additionally, unlike the traditional antibiotics, these peptides, used in a therapeutic way, will not affect to the natural flora of patients during the treatment of an infection.

Currently, there is a wide field of applications for the AMPs. In the treatment of diseases associated to pathogens, bacteriocins have been used to inhibit important pathogens of animals, humans and plants, such as Shiga-toxin producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococci* (VRE), *Agrobacterium* and *Brenneria spp.*[19,50]

In the combat to sexually transmitted diseases, defensins, minidefensins and cathelicidins have been studied as peptides with activity against the human immunodeficiency virus (HIV). Some of these anti-viral peptides have been tested as topical microbicides against other sexually transmitted diseases.[39,41,51,52] Dermaseptins and magainins, which are AMPs from the frog skin, have spermicide activity and can be used as intravaginal contraceptives, in the same way, they are being studied as candidates to protect against HIV and other sexually transmitted pathogens.[53] Subtilosin has demonstrated being effective for combating bacterial vaginitis caused by multi-drug resistant *Gardnerella vaginalis* without affecting lactobacilli of the normal vaginal microbiota. Subtilosin is safe, remains viable for long periods and also completely eliminates the spermatozoa motility in a dose dependent way.[54]

In the development of resistant plants to several pathogens, an ecologically responsible alternative could be the genetic introduction of AMPs that serve for protecting plants from pests and pathogen microorganisms. This approach could reduce the use of harmful pesticides and could improve the production of vegetable species with major resistance to pathogens.[4] In the production of marine species of commercial interest, the study of defense mechanisms in commercial species of crab, white shrimp and mussel has opened the possibility of the manipulation of genes that encode for AMPs allowing to obtain cultivable organisms more resistant to diseases.[3] In the food industry, bacteriocins are widely used to avoid contamination by microorganisms and then to extend the food preservation. Bacteriocins are considered as natural innocuous preservatives since due to their sensibility to protease, once inside the gastrointestinal tract, they can be degraded to small peptides or to amino acids. In the livestock trade, diverse colicins have been used as food additives for pigs and chicken to fight infections and reducing the use of antibiotics in animals.[50,55]

## Peptides with anticancer activity

Currently, the cancer is a serious health problem worldwide. According to the World Health Organization (WHO) there were 8.2 millions of deaths caused by cancer and 14.5 millions of new cases worldwide in 2012.[56] In spite of many investigations on cancer, it is not completely understood due to the complexity of its origins, which makes more difficult to find alternatives for its treatment. The feature to all types of cancer is the abnormal growth of cells, which results from different genetic or external factors. The current treatment against located cancers remains surgery and/or chemotherapy, which are associated with severe damage to surrounding normal cells; furthermore, the number of cancers resistant to chemotherapy has increased.[57] For these reasons, it is needed to develop a new class of anticancer drugs, which do not cause toxicity, with low risk for inducing multi-drug resistance but being more effective and specific to attack cancer cells. In last years, the research on the use of AMPs as an alternative for cancer treatment has started and until today great advances have been achieved against cancer in different systems, *in vivo* and *in vitro*.[11,22,58]

The basis of this application lies in the capability of these peptides to selectively recognize the membranes with certain characteristics. Such selectivity depends on the difference in the membrane composition, and on the electric charge in the extracellular side of the membrane. The cationic AMPs are selectively attracted to the cell membrane depending on their negative charge; a reason why which the peptides have a strong affinity for bacterial membranes, since they have a large amount of negative phospholipids on their exterior.[5,59] The same selectivity allows recognizing the difference among normal cells, non-malignant tumor cells and malignant tumor cells, since the plasma membrane characteristics of the cells change as the cancer progresses. In a natural way, normal mammalian cells have mostly zwitterionic phospholipids in the external side of the plasma membrane, thus they have a neutral net charge. As the neoplasm advances, these phospholipids acquire a net negative charge, like normal bacteria have; this change in charge makes them more

susceptible for the attack by some AMPs and anticancer agents.[60] Differences in the membrane composition, fluidity, charge and surface area among the cell types of cancer affect the efficiency of each anticancer peptide. Other changes in the membrane composition, during a cancerous process that favors its susceptibility to the peptides, are a variation in glycosylation patterns, the amount of cholesterol and the amount and type of proteins present in the membrane.[61] Drosocin is a potential anticancer peptide since it highly recognizes glycosylated membranes, which are present in breast cancer.[62] Anticancer peptides have a broad spectrum of action, ability to rapidly kill cancer cells, to destroy primary tumors, to prevent metastasis, and at adequate concentrations do not damage normal cells or vital organs.[58,63] Antitumor AMPs effects may generally occur either by membranolytic or non-membranolytic mechanisms. Beside of the membrane disruption, membranolytic events involve the permeation and swelling of mitochondria with release of cytochrome c, apoptosis events or also acting by necrotic pathways. Non-membranolytic activities involve the perturbation of angiogenesis process.[64]

Nowadays, there are different peptides with tested anticancer activity.[1,11,22,64,65] In fact, there are various examples of anticancer peptides from different organisms, some examples are:

**Bacteria.** Some bacteriocins have activity against different tumor cells. Colicins A and E1 form pores and inhibit the growth of 11 tumor cell lines, while colicins D, E2 and E3 inhibit the viability of leukemia cells. Nisin is a lantibiotic that induce DNA fragmentation and apoptosis in three tumor cell lines of head and neck squamous cell carcinomas (HNSCC). Lantibiotics and colicins could be an alternative therapy for the treatment against cancer, since they are safe due to the properties of these bacteriocins.[19,66] The AMPs m2163 and m2386 from a lactic acid bacterium *Lactobacillus casei* induce apoptosis in human colorectal cancer cell by both extrinsic and intrinsic apoptosis pathways.[67]

**Plants.** Of the 12 plant AMP families, 3 contain members with cytotoxic and anticancer properties: the thionins, defensins, and cyclotides.[22] Unfortunately, most of the thionins and cyclotides with anticancer activity are unselective or un-tested in their ability to damage normal cells; nevertheless they could be use for the design of new anticancer molecules. Defensin from the purple pole bean inhibit the proliferation of four cancer cell lines and do not affect human embryonic liver cells or human erythrocytes under de same conditions.[68] Coccinin, a peptide purified from the seeds of large scarlet runner beans and phaseococcin from the seeds of small scarlet runner beans, both inhibit proliferation in the leukemia cell lines and does not affect the proliferation of mouse splenocytes.[69,70] Cycloviolacin O2 (CyO2), a cyclotide from *Viola odorata* has antitumor effects and causes cell death by membrane permeabilization in breast cancer cells, but it does not produce significant membrane disruption in primary human brain endothelial cells. It also can work as chemosensitizing agent against drug resistant breast cancer cells.[71] Cn-AMP2 from green coconut water is an anionic peptide with anti-proliferative activity against 1321N1 and U87MG human glioma cell lines.[72] Cyclosaplin from somatic seedlings of sandalwood has antiproliferative activity against human breast cancer cells but not against normal fibroblast cells by inducing apoptosis.[73]

**Insects.** Cecropins A and B exert selective cytotoxic and antiproliferative efficacy in bladder cancer cells without damaging the normal human fibroblast by inducing an increase in LDH release that decompensate and kill the cells.[74] CB1a, a cecropin derived peptide, show high cytotoxic activity against leukemia and stomach carcinoma with low hemolysis.[75] CecropinXJ belongs to cecropin-B family; it could kill cancer cells but have no toxicity to some normal cells. The mechanism of action occurs by inducing damage to microtubules and actin of human esophageal carcinoma cells, and inhibits migration and invasion of tumor cells.[76] CopA3, an analog of coprisin, an insect defensin from dung beetle, shows cytotoxicity against three human leukemia cell lines by induction of a caspase-independent signaling pathway mediated by apoptosis inducing factor (AIF). CopA3 also causes necrosis of gastric cancer cells, probably through interactions with phosphatidylserine.[77,78] HaA4, the synthetic analogue of harmoniasin from ladybug, is a homodimeric peptide, which has cytotoxic effect against two human leukemia cell types by induction of necrosis and apoptosis.[79] Anoplin is a recently discovered AMP isolated from the venom sac of a spider wasp; it could inhibit the proliferation of murine erythroleukemia cells via disrupting the integrity of cell membrane and additionally arrests the cell cycle in the $G_0/G_1$ phase.[80] MP1-1 is an analog of the AMP polybia-MPI, a peptide isolated from the venom of wasp; it binds to human prostate and liver cancer cells causing injury, swelling, bursting, and final cell death by necrosis.[81]

**Myriapods.** It is recently reported that the AMP colopendrasin VII derived from a centipede has anticancer activity against leukemia cell lines by decreasing their viability and inducing necrosis mediated by specific

interaction with phosphatidylserine, which is enriched in the membrane of cancer cells.[82]

**Arachnids.** MAP-04-03, which is derived from Ixosin-B, an AMP found in the salivary glands of the hard tick shows antiproliferative effects on breast cancer cells and also inhibits cancer cells migration.[83]

**Marine invertebrates.** Tachyplesin I restricts prostate cancer cells by activating the classical complement pathway and cell lysis mediated by tachyplesin also activates the alternative complement pathway; all these events provoke a decrease of the proliferative capacity of several types of cancerous cells.[84] Penaeidin-2 (Pen-2) is a derived-AMP from the Pacific white shrimp that induce apoptosis specifically on kidney cancer cell.[85]

**Amphibians.** Magainins and other peptides derived from amphibian have cytotoxic activity in cell lines of bladder cancer, permeabilize membranes from cervical cancer lines, and induce apoptosis by releasing cytochrome c from the mitochondria of leukemia cells. Magainins have been intratumorally injected and are able to completely eradicate the melanoma growth.[11] MG2A (magainin II conjugated to the N-terminus of the cell-penetrating peptide [CPP]) causes enhanced cytotoxicity against tumor cells, at least 30 times higher than mangainin unconjugated and it is about three to five times less cytotoxic to normal cells.[86] Temporin-1CEa is an antimicrobial peptide isolated from the skin secretions of the Chinese brown frog; this AMP triggers a rapid cytotoxicity in breast cancer cells through membrane-destruction and intracellular mechanisms involving mitochondria, such as intracellular calcium release and ROS over production.[87] Dermaseptin B2 is found in skin secretions of the Amazonian tree frog, inhibits the proliferation of various human tumor cell types *in vitro* and inhibit tumor growth of the human prostate adenocarcinoma cell line in a mice model *in vivo* inducing necrosis by increasing lactate dehydrogenase (LDH) release.[88]

**Birds.** GLI13-8, one of AMP from linear avian β-defensin-4 (RL38) analogs, has cytotoxicity in three human carcinoma cells (HepG2, SGC7901, and A375) in a dose-dependent manner. GLI13-8 induces apoptosis and loss of mitochondrial membrane potential in HepG2 cells, and in the appropriate doses it had no toxicity towards the normal human fibroblasts.[89]

**Fishes.** Currently, there are several studies on AMPs from fishes with anticancer properties. Tilapia hepcidin TH2-3 specifically inhibits human fibrosarcoma cell proliferation and migration in a concentration-dependent manner causing lethal membrane disruption.[90] NRC-03 and NRC-07 are two peptides from the AMP pleurocidin family from flatfish with activity against human breast cancer cells including drug-resistant variants and with decreased affinity toward human healthy cells even by intratumoral administration.[91] Piscidin-1, isolated from mast cells of a hybrid striped bass, at low doses induces both apoptosis, necrosis and also inhibits the migration of fibrosarcoma cells.[92] Pardaxin, another fish AMP exhibits antitumor activity toward murine fibrosarcoma *in vitro* and *in vivo*; it inhibits the proliferation of MN-11 cells and induces apoptosis through the death receptor/nuclear factor (NF)-κB signaling pathway in tumor-bearing mice.[93]

**Mammals.** BMAP-27 and BMAP-28 that are derived of bovine cathelicidin have cytotoxic effects against different leukemia cells by altering the influx of $Ca^{2+}$, DNA fragmentation and reducing the mitochondrial potential provoking a permeabilization that kills the cancer cells without having hemolytic activity.[65] Defensins have cytotoxic effects on different human and mouse cancer cell lines from lymphomas, oral carcinoma, teratocarcinoma and renal cancers through an interaction with the membrane.[11] In fact, the β-defensin 2 (BD2) has been proved in studies of cancer gene therapy, BD2 exhibits chemotactic activity in dendritic cells (DCs) both *in vitro* and *in vivo* models. Recruitment and activation of DCs in tumor niches result in significant tumor growth inhibition.[94] Bovine lactoferricin (LfcinB), like defensins, has a cytotoxic activity on leukemia, fibrosarcoma, neuroblastoma and breast carcinoma cells and its activity involves the sequential generation of reactive oxygen species, loss of mitochondrial potential and activation of the caspase cascade, culminating in cell death by apoptosis.[95] LL-37/hCAP-18 is a cathelicidin that induces DNA break and mitochondrial damage in leukemia cells and inhibits proliferation and induces $G_0/G_1$ phase cell cycle arrest in gastric cancer cells.[96] An analog of the LL-37 peptide, FF/CAP18, induces apoptotic cell death, via mitochondrial membrane depolarization and DNA fragmentation, in the oral squamous cell carcinoma cells and in human colon carcinoma cells. FF/CAP18 induces apoptotic cell death via changes in the metabolic profile in colon cancer cells.[97] In fact, LL37 is one of few AMPs with anticancer properties that has currently been approved to start clinic phase I and II assays. The goal of this clinical research study is to find the appropriate dose of LL37 that can be given to patients with melanoma by intra-tumoral injections and also learn if LL37 can stimulate the immune system to help control cancer (NCT02225366).



## Challenges to overcome about the AMPs

In spite of the great potential of the AMPs, it is still needed to overcome several challenges and to improve

several factors to obtain safe and functional products capable to hit the market. These challenges consist on finding optimal doses and routes of administration to achieve the high concentrations required so the peptides can work, to reduce their hemolytic activity to avoid any kind of toxicity, to improve their specificity to avoid their broad spectrum of action, which affect the normal flora and to improve their stability for reducing their lability to proteases *in vivo*. Additionally, it is important to improve other factors as the efficiency of synthesis, and production costs for that the AMPs can really be a viable alternative for their use as drugs.[98] There are many theoretical advantages and disadvantages of development of peptides as anti-infective drugs, such as those mentioned above and in other reviews.[99]

Currently, it has been envisaged different strategies to overcome some of these disadvantages. Since anticancer peptides require constant administration for maintaining determined systemic levels for their effectiveness, an alternative can be the introduction of genes encoding for the peptides directly to the cancer cells. To reduce the toxicity of the peptides, it has been proposed optimizing their sequences using computational approaches for decreasing their hemolytic activity but keeping the antibacterial activity.[98] To diminish the susceptibility to peptide proteolysis, the use of D-amino acids for their synthesis has been proposed.[46] Finally, to improve the specificity of the peptides, it has been proposed to design peptides directed to a specific binding sequence of factors of pathogen agents.[100,101]

Regarding to the economic viability, the main problem is the high cost of the synthesis for producing the AMPs. It is possible to synthesize most of the peptides including defensins with their complex disulfide bridges, but the cost is very high, which prevents their production at large scale. To avoid this problem, it has been currently developed biological expression systems, as recombinant systems in baculovirus expressing active defensins, which are pure, structurally correct and in milligram amounts. The AMPs can be produced in transgenic plants, in bacteria and in milk from transgenic cows; however, it is still needed to improve the purification process to avoid the contamination by host proteins, which could induce an immune response in the patient.[102]

## Design of novel peptides

The design of novel peptides is looking for more efficient peptides and stable in different environments and conditions but mostly, looking to find particular results of interest and clinical importance. The design of novel peptides is based on conserving the characteristics and fundamental properties of the AMPs, such as their cationic character, amphipathic region and tridimensional structure, which are crucial characteristics that determine their antimicrobial activity.[103] In the same way, it is known that the hemolytic activity and therefore their toxicity correlate with the high hydrophobicity, amphipathicity and helicity of the peptides. The electrostatic interactions are responsible for inducing a stable secondary structure, so the binding process requires that the peptide conformation is adapted in response to these interactions to maximize the attraction and minimize the repulsion forces.[46]

Peptides can also be designed base on the previous knowledge of proteins and types of membranes; for instance all D-amino acid analogues are highly resistant to proteolytic degradation without negative effects in the peptide or protein functionality; also by knowing that the chemical composition of different types of membranes contributes to the selectivity of the AMPs, which confers the specificity for attacking to a determined type of pathogen or certain type of cells.[11,46]

The following strategies are used for the design of these novel peptides:

(i) **Synthesis *de novo* by the selective incorporation of unusual or non-natural amino acids.** The incorporation of D-amino acids with different hydrophobicity can reduce the resultant hemolytic activity, but it can also modify their microbicidal activity by favoring the attack to some types of pathogens but decreasing the affinity by others. This strategy consists in deleting or inserting natural amino acids or exchanging them for unusual amino acids having distinct functional groups to the originals. This change generates larger or smaller spaces within the amino acid sequence provoking a change in the tertiary structure of peptides. Distinct parameters such as the hemolytic activity, selectivity, microbicidal activity or resistance to proteases can be evaluated or improved by changing the tertiary structure.[46]

Synthetic anticancer diastereoisomer peptides have been designed, with the ability to permeabilize only membranes with phospholipids with negative charges as the D-$K_4R_2L_9$, that has a third of its sequence composed by D-amino acids. This peptide binds and lyses cells from melanoma, and when intravenously injected prevents the formation of lung tumors. The D-$K_6L_9$ diastereoisomer, containing D-Lys and D-Leu in a third of its sequence, has anticancer activity and synergically acts with other drugs, it has the capacity

to stop, reduce and inclusive disappear tumors *in vivo*. The D-$K_6L_9$ diastereoisomer avoids the metastasis dissemination in immunosuppressed mice, and it is resistant to the degradation by proteases, while the L-$K_6L_9$ (that does not contain D-amino acids) does not have activity and it is degraded by proteases once it is inoculated to mice.[104]

In control of plant pathogens, an alternative to the currently used pesticides, AMPs with non-natural amino acids such as triazolyl, biaryl and D-amino acids into their sequence with activity against plant pathogenic bacteria and fungi are a great promise.[105]

(ii) **Design by quantitative structure-activity relationship (QSAR).** This strategy is used starting from a model of proven effectiveness, from which the peptide analogues are generated by making small modifications in the sequence. As in the previous strategy, the modifications provoke significant structural and biophysical changes that correspond to very marked effects in the antimicrobicidal activity and the toxicity of the peptides. Thus, a peptide with proven effectiveness is used as a base sequence, then a library of variants or analogues can be created by making point mutations and combination of two or three particular mutations, such mutations are introduced at different positions along the base sequence, and finally the microbicide potential for each analog is evaluated. Based on certain number of analogues and their corresponding activity, a quantitative model is developed making a structure-activity relationship (QSAR), which is used for predicting variants with improved activity. The construction of the QSAR model requires a set of surface descriptors as: surface and volume (VolSurf) and the charged partial surface area (CPSA). The VolSurf descriptor is based on interaction regions specifically designed for the optimization of the pharmacokinetic properties of the protein, while CPSA descriptor is based on the partial charges of the proteins according to the field force used. In the QSAR model, these descriptors mimic the interaction of the peptide with the membrane, as well as the surrounding environmental; finally the reliability of the model is validated by predicting and testing the new peptides.[106]

The knowledge that the net positive charge of peptides must be maintained during the design was obtained from this type of model, and that a big polar area will not provide a significant increase in their potency. The hydrophobic area is extremely needed, and the increased hydrophobicity allows a major insertion within the membrane, but causes a loss of selectivity. Consequently, a balance between charges and the hydrophobic amino acids is required and to be careful with the insertion positions, since they can dramatically affect the biological activity. The same methodology can be used to test the toxicity in erythrocytes varying the type of descriptors used to build the QSAR model.[106-108] Several studies of QSAR have been done using different descriptors and models to find lactoferricin derivatives, protegrin analogues, bactenecin-derived peptides or similar de novo peptides.[109-111] QSAR modeling of AMPs to date has been based on predicting differences between peptides that are highly similar. The mathematical models used to relate the QSAR descriptors to biological activity have been linear models such as principle component analysis or multivariate linear regression. However, with the development of high-throughput peptide synthesis and an antibacterial activity assay, the numbers of peptides and sequence diversity able to be studied have increased dramatically. Also, «inductive» QSAR descriptors have been recently developed to accurately distinguish active from inactive drug-like activity in small compounds. «Inductive» QSAR in combination with more complex mathematical modeling algorithms such as artificial neural networks (ANNs) may yield powerful new methods for *in silico* identification of novel AMPs.[112,113]

(iii) **Design of selective peptides for Gram-negative bacteria.** Gram-negative bacteria as *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and enterobacteria are the main agents causing hospital-acquired infections.[114] Gram-negative bacteria have as a particular feature the presence of lipopolysaccharides (LPS) on their cell surface, which are responsible for septic shock in the patients; for this reason, it has been proposed the design of peptides that specifically recognize to Gram-negative bacteria. Cyclic peptides mimicking the binding sites of LPS-binding proteins have been designed and they show 200 times more activity than on Gram-positive bacteria.[115] In fact, some derivatives of cathelicidins, polymixins and bactericidal/permeability-increasing protein (BPI) display an ability to strongly bind LPS and break its aggregates, but they have also endotoxin-neutralizing activity which can potentially suppress LPS-induced hyper-inflammatory responses that leads to multiple organ failure and lethality.[116-118] Similarly, peptides, having other cellular components of Gram-negative bacteria as target, can be designed.

(iv) **Design of specifically targeted AMPs (STAMP).** One of the complications of AMPs is that they must reach certain concentrations on the membrane to become actives, and thereby it is needed that peptides reach and accumulate on their target sites. To help on this, a technology to smartly design selec-

tive peptides has been developed, using a technology known as specifically targeted AMPs (STAMP). This technology involves the construction of a peptide for binding to two functionally independent components: a region for target localization and an antimicrobial region; both joined by a short and flexible connector. The target localization region provides selectivity to the antimicrobial region since it specifically binds to determinants of the pathogen. This binding increases the selective accumulation of the peptide and significantly increases its local concentration, thus the antimicrobial region can eliminate the pathogen. The STAMP technology is an effective tool for eradicating pathogens without affecting the normal flora, and thereby preventing secondary infections.[100,101]

The STAMP C16G2 was developed to target the cariogenic oral pathogen *Streptococcus mutans*. C16G2 specifically kills *S. mutans* in a mixed-culture environment, having minimal effects on noncariogenic oral flora, which is beneficial to prevent exogenous *S. mutants* colonization. C16G2 has a truncated version of the *S. mutans* competence stimulating peptide ($CPS_{C16}$) pheromone, as the STAMP targeting domain for effective accumulation on the *S. mutants* cell surface.[119,120] Other new STAMP recently reported is CDAK, which is composed of a cyclic *iso*DGR motif, which is used as a targeted delivery tool because it binds to $\alpha_v\beta_3$, a type of integrin that overexpresses in tumor cells and of an AMP region. CDAK shows cytotoxic activity in $CD13^-/\alpha_v\beta_3$ breast cancer cells *in vitro* and *in vivo*, whereas normal cells are less sensitive to this peptide.[121] Thus, peptides specifically directed against overexpressed compounds in tumor cells are good candidates for being used as anticancer agents since they would have little chance of affecting healthy tissue.

(v) **Design of hybrid peptides.** This is another strategy that consists in the fusion of two peptides with tested activity but directed to a different target, enhancing its action spectrum.[122] To date, several new peptides has been designed using this strategy: human β-defensins have different intensities of antibacterial activity; human β-defensin-3 (HBD3) kills *Staphylococcus aureus* with a 4- to 8-fold higher efficiency compared to human β-defensin-2 (HBD2) and both has the same activity against *E. coli* but one HBD2/HBD3-chimera kill both *S. aureus* and *E. coli* with an even higher efficacy compared to the wild type molecules.[123] Hybrid peptides design has also been used to reduce the hemolytic activity of the original peptides; cecropin-melittin hybrids (CAM and CBM) have strong antibacterial abilities against Gram-positive and negative bacteria and fungi as the original peptides, but they had neither hemolytic, nor melittin toxicity in *in vitro* and *in vivo* experiments.[124] The hybrid peptides melittin-LL37 combining the hydrophobic N-terminal fragment of melittin with the core antibacterial fragment of LL37, had an even more potent antibacterial activity again bacteria than melittin and LL37 alone, whereas did not exhibit hemolytic activity to sheep erythrocytes.[125] Hybrid design also helps to achieve short length peptides with improved potency and selectivity; a novel short peptide CS-1a (14 residues) was derived using a sequence hybridization approach on sarcotoxin I (39 residues) and cecropin B (35 residues). CS-1a shows a good antibacterial activity against susceptible as well as drug-resistant bacterial strains with non-hemolytic activity against human red blood cells even at very high concentration.[126] In the same manner, hybrid peptide design also can be used to develop salt resistant peptides. The human β-defensin-1 (HBD-1) antibacterial activity is considerably impaired by elevated ionic strength meanwhile retrocyclins (synthetic human θ-defensins) are salt resistant, non-hemolytic and non-cytotoxic *in vitro*. The hybrid peptide of the C-terminal region of HBD-1 and the nonapeptide sequence of a retrocyclin exhibits enhanced antimicrobial activity against bacteria, fungi and clinical isolated of eye infections and the peptide retained activity in the presence of NaCl and serum and is non-hemolytic *in vitro*.[127] Finally, hybrid peptides could serve as enhancer of activity. Lysozyme is highly active against Gram-positive bacteria but alone is inactive against Gram-negative bacteria because it cannot reach the peptidoglycan layer and cecropin may disrupt the outer membrane. Novel hybrid protein combining cecropin with human lysozyme shows improved *in vitro* antimicrobial activity and action spectrum being that cecropin disrupts the Gram-negative bacteria outer membrane, giving the enzyme access to the peptidoglycan in cell wall.[128]

(vi) **Peptidomimetics.** There is a new class of designed AMPs that can mimic the structure, function and mode of action of native AMPs, while being stable to enzymatic degradation and conceivably exhibit better pharmacological properties. Peptidomimetics can display antibacterial activity against drug-resistant strains and are less susceptible to resistance development in bacteria.[129,130] Peptidomimetics have been prepared by cyclization of linear peptides and/or coupling of stable unnatural amino acids. Unnatural amino acids can be generated modifying the native ones by different ways such as: amine alkylation, side chain substitution, structural bond extension, cyclization and isosteric

replacement within the amino acid backbone. Isosteric replacements within a peptide backbone result in improved pharmacokinetic properties. Backbone modifications of amino acids can be categorized as follows: (i) changing the amino functionality; (ii) replacement of α-carbon; (iii) extension of the backbone by one or two atoms and (iv) atom modification of the carbonyl function.[131] Some peptidomimetic types are β-peptides, peptoids, arylmamide oliogmers, β-turn mimetics and AA peptides (which contains N-acylated-N-aminoethyl amino acids).[130] It has been shown that peptidomimetics have more dihedral angles compared to canonical peptides, and this molecular design induces high flexibility. The presence of backbones with certain flexibility leads to a potent and broad-spectrum of antimicrobial activity; this is more important than the secondary structures.[130,132]

(vii) **Design of peptides sensible to the environment.** The pathogen organisms frequently cause drastic changes in the surrounding environment as a result of their growth and metabolic activities. Thereby, a strategy consists on designing peptides sensible to the changes in the environment, such as the pH. Peptides highly sensitive to pH changes in their environment have been designed, these peptides have not activity at normal physiological pH of 7.5 but they become active at pH of 5.5. Therefore, they will work only in where they find an acid environment generated by a pathogen.[133] This strategy has also been used to obtain an anticancer peptide with less toxicity, since this peptide only can be activated by tumor acidity and it is not active or less active under physiological conditions. AMitP is an activatable heterodimer formed by the AMP mitoparan (MitP) fused to its anionic binding partner peptide ($MitP_E$) (which contains glutamic acids and histidines). AMitP remains inactivated at normal pH values; MitP has no lytic activity because is binding to $MitP_E$ by electrostatic attraction. In contrast, MitP dissociates from $MitP_E$ and recovers its lytic activity when the anionic charges of glutamate residues in MitPE are neutralized at acidic pH values.[134]

## Developments in clinic phase and commercial application of AMPs

There are different strategies of therapeutic application for AMPs: (i) as simple anti-infective agents, (ii) in combination with conventional antibiotics or antivirus to promote an additive or synergistic effect, (iii) as immunostimulatory agents to improve the innate immune response, and (iv) as neutralizing agents of endotoxins to prevent complications associated with virulence factors.[99] Many peptides have entered into clinical trials, with varying degrees of success.[13,18,135] In *Table I* are shown some peptides that have reached a clinical phase and obtained good results.

Regarding to the peptides that failed, the mayor problems are poor distribution, frequent toxicity and presence of adverse events, fast descomposition or half-life, or that induce a high level of humoral response and antibody production.[18] However, there are some peptides that do not present these inconvenient but they have been rejected because they have not demonstrated any significant advantage over some approved drugs. Based upon the data obtained from clinical trials, no data exist regarding peptides for development of immune response when they are applied topically.

In spite of there currently are not drugs based on approved antimicrobial peptides, in the food industry have had better advances regarding the use and commercialization of AMPs. Nisin is a bacteriocin produced by *Lactococcus lactis* and the first approved lantibiotics as food preservative and it is commercialized in more than 45 countries (Nisaplin®). The bacteriocin, pediocin PA-1, inhibits *Listeria monocytogenes* growth in meat products and it is commercialized as Alta® 2341. For inhibiting contamination, it has also been used the enterocin AS-48 in ciders, fruit juices and canned vegetables, while the enterocin CCM4231 has been used in soy milk and the enterocin EJ97 in puree.[136]

Although hundreds of AMPs have been identified, only some ones have entered to clinic phase assays and only a few ones have obtained favorable results and no one has been approved yet for clinical use. Thereby, it is necessary to continue with more research to improve AMPs to reach clinic phases. However, we can recognize that in the last years great advances have been done and the future appears promising for developing and use of AMPs mainly for those that will be used in topical way.

## Conclusions and perspectives

The AMPs have a completely different mechanism of action to common antibiotics, and during the evolution they have been functioning as a chemical shield of defense over millions of years in plants and animals, thereby they could become in an alternative for combating diseases, mainly by the increase of multi-drug resistant pathogens or superbugs. The mechanism by which these peptides act, make them very difficult to generate bacterial resistance, becoming a promising future as more durable therapeutic agents. Besides, knowing the role of peptides as modulator of the in-

**Table I.** Antimicrobial peptides tested in clinical studies.

| Peptide name/ phase/company | Parent name | Indication | Outcome | Method of application | References |
|---|---|---|---|---|---|
| Antimicrobial/immunomodulatory | | | | | |
| **hLF1-11** Phase I *AM-Pharma* | Lactoferrin | Study on the tolerability and early efficacy of hLF1-11 in patients with proven candidaemia | Safety in humans established | Intravenous | NCT00509834 [138] |
| **hLF1-11** Phase I/II *AM-Pharma* | Lactoferrin | Safety, tolerability and prevention of fungal infections in autologous hematopoietic stem cell transplant recipients | Safety in humans established | Intravenous | NCT00509938 [138] |
| **P-113** Phase I/II | Histatins | Treatment of experimental gingivitis | Safety and reduces plaque, gingivitis and gingival bleeding | Mouthrinse/ topical gel | [139,140] |
| **Talactoferrin** Phase II *Agennix* | Lactoferrin | Treatment of severe sepsis | Reduce 28-day all-cause mortality in patients with severe sepsis. This reduction was sustained at six months | Oral solution | NCT00630656 [7] |
| **eBPI$_{21}$** Phase III *XOMA* | BPI (Bactericidal/permeability-increasing protein) | Treatment of meningitis | Reduce serious complications, such as amputations | Intravenous | [141,142] |
| **MSI-78 (Pexiganan)** Phase III *MacroChem Corporation* | Magainins | Treatment of diabetic foot ulcers | Effective treating diabetic patients with a mildly infected foot ulcer and might reduce the risk of selecting antimicrobial-resistant bacteria | Topical cream | NCT00563394 NCT00563433 [143] |
| **Pexiganan** Phase III *Dipexium pharmaceuticals* | Magainins | Treatment of mildly infected diabetic foot ulcers | Recruiting | Topical cream | NCT01590758 NCT01594762 |
| **Omiganan (MBI 594AN)** Phase IIa/IIb *Cutanea Life Sciences, Inc.* | Indolicidin | Treatment of acne | Diminish the severity of the disease in subjects with mild to moderate acne/reduce all types of acne lesions | Topical solution | NCT02066545 [12,144] |

**Table I.** Antimicrobial peptides tested in clinical studies.

| Peptide name/ phase/company | Parent name | Indication | Outcome | Method of application | References |
|---|---|---|---|---|---|
| **Omiganan (MX-226, CLS001)** Phase III *Mallinckrodt* | Indolicidin | Prevention of catheter-related infections | Reduce catheter colonization and catheter-related local site infections | Topical gel | NCT00231153 [12,144] |
| **Omiganan** Phase III *Mallinckrodt* | Indolicidin | For topical skin antisepsis | Effective for killing bacteria (germs) that live on the surface of the skin | Topical gel | NCT00608959 |
| **Omiganan (CLS001)** Phase II *Cutanea Life Sciences, Inc.* | Indolicidin | Safety and efficacy in subjects with Rosacea | Reduce lesion count with once-daily at nine weeks of treatment | Topical gel | NCT01784133 |
| **LTX-109** Phase I/IIa *Lytix Biopharma AS* | Peptidomi-metic | Treatment of nasal colonization with *Staphylococcus aureus* (MRSA/MSSA) | Reduce the bacterial load already after a single day of treatment | Intranasal topical gel | NCT01158235 [145] |
| **Brilacidin (PMX-30063)** Phase II *Cellceutix Corporation* | Peptidomi-metic | Treatment of acute skin infec-tions | Decrease of lesion area, and this effect is sustained at least until 28 days | Intravenous infusion | NCT01211470 NCT02052388 [130] |
| Anticancer | | | | | |
| **LL37** Phase I/II M.D. Anderson Cancer Center | Cathelicidin | Treatment of melanoma | Approved, no yet recruiting | Intratumoral Injections | NCT02225366 |
| **Talactoferrin** Phase I *Agennix* | Lactoferrin | Treatment of advanced renal cell carcinoma | Completed, no results posted | Oral solution | NCT00095186 |

nate immune response, they can be helpful as guide for developing immunomodulatory therapies. AMPs fulfill many of the requirements demanded by the pharmaceutical industry, agriculture, aquaculture, and the food production in general. The manipulation of the chemical structure and fundamental characteristics of AMPs, will allow the creation of synthetic peptides specifically designed to find responses to different problems affecting health.

Due to that the AMPs differentially interact with diverse types of membranes, small changes, carefully selected, in the structural and physicochemical properties of the amino acids can allow major changes in the potency and selectivity of a particular peptide for a specific pathogen or cell. Nowadays, some AMPs are being optimized for a more effective anticancer activity as well as for a better therapeutic alternative and economically more viable for generating chemotherapeutic drugs in a close future.

In spite of the great potential of the AMPs, out of thousands of potential synthetic peptides, only a reduced number has been thoroughly studied and tested. Even though promising data have been obtained, it is still premature to conclude whether AMPs can clinically be used as therapeutic agents since the field is still young. Many AMPs have demonstrated antimicrobial activity *in vitro*, under controlled conditions, but these data have not been successfully brought to therapeutics in clinical trials and some of them have failed in these clinical trials.[137] Another important challenge to overcome in the next years will be developing new potential delivery systems for AMPs; however nanotechnology approaches have emerged as important tools and could improve the stability and delivery of these AMPs.

In spite of all the inconveniences that must be overcome to develop a product with the feasibility to hit the market, there are different strategies to reduce costs of production and to increase the peptides functionality, such as *in silico* approaches. The combinations of the last approaches with QSAR could facilitate the development of AMPs more effective and safer, and at lower cost than the currents. Similarly, enzymes involved in the lantibiotics synthesis could be used for the production of peptides with unusual amino acids and complex modifications, which would represent an alternative to the complex chemical synthesis of peptides that may further make cheaper the process. There is also the possibility in the future to use gene therapy using coding genes for antimicrobial or anticancer peptides.

There is still much to learn from the antibacterial peptides, mainly *in vivo*, but there are encouraging data that allow anticipating that over the next decade some of these results will be used by the industry and will contribute to the society in the fight against pathogens.

**Acknowledgment**

We thank Secretaría de Ciencia, Tecnología e Innovación de la Ciudad de México for a grant to FNG (PICSA 12-231), and Alejandra Ochoa and Roberto Vidal for their invaluable comments to improve this work.

## Bibliography

1. Pushpanathan M, Gunasekaran P, Rajendhran J. Antimicrobial peptides: versatile biological properties. Int J Pept. 2013; 2013: 675391.
2. Boman HG. Innate immunity and the normal microflora. Immunol Rev. 2000; 173: 5-16.
3. Montano-Perez K, Vargas-Albores F. Péptidos antimicrobianos: un mecanismo de defensa ancestral con mucho futuro. Interciencia. 2002; 27: 21-27.
4. Kim JY, Park SC, Hwang I et al. Protease inhibitors from plants with antimicrobial activity. Int J Mol Sci. 2009; 10 (6): 2860-2872.
5. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature. 2002; 415 (6870): 389-395.
6. Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol. 2006; 24 (12): 1551-1557.
7. Guntupalli K, Dean N, Morris PE et al. A phase 2 randomized, double-blind, placebo-controlled study of the safety and efficacy of talactoferrin in patients with severe sepsis. Crit Care Med. 2013; 41 (3): 706-716.
8. Zhang M, Zhao J, Zheng J. Molecular understanding of a potential functional link between antimicrobial and amyloid peptides. Soft Matter. 2014; 10 (38): 7425-7451.
9. Rao AG. Antimicrobial peptides. Mol Plant Microbe Interact. 1995; 8 (1): 6-13.
10. Nissen-Meyer J, Nes IF. Ribosomally synthesized antimicrobial peptides: their function, structure, biogenesis, and mechanism of action. Arch Microbiol. 1997; 167 (2-3): 67-77.
11. Hoskin DW, Ramamoorthy A. Studies on anticancer activities of antimicrobial peptides. Biochim Biophys Acta. 2008; 1778 (2): 357-375.
12. Wiesner J, Vilcinskas A. Antimicrobial peptides: the ancient arm of the human immune system. Virulence. 2010; 1 (5): 440-464.
13. Afacan NJ, Yeung AT, Pena OM et al. Therapeutic potential of host defense peptides in antibiotic-resistant infections. Curr Pharm Des. 2012; 18 (6): 807-819.
14. Mookherjee N, Hancock RE. Cationic host defence peptides: innate immune regulatory peptides as a novel approach for treating infections. Cell Mol Life Sci. 2007; 64 (7-8): 922-933.
15. Bowdish DM, Hancock RE. Anti-endotoxin properties of cationic host defence peptides and proteins. J Endotoxin Res. 2005; 11 (4): 230-236.

16. Davidson DJ, Currie AJ, Reid GS et al. The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization. J Immunol. 2004; 172 (2): 1146-1156.
17. Wu WK, Wong CC, Li ZJ et al. Cathelicidins in inflammation and tissue repair: Potential therapeutic applications for gastrointestinal disorders. Acta Pharmacol Sin. 2010; 31 (9): 1118-1122.
18. Pasupuleti M, Schmidtchen A, Malmsten M. Antimicrobial peptides: key components of the innate immune system. Crit Rev Biotechnol. 2012; 32 (2): 143-171.
19. Yang SC, Lin CH, Sung CT et al. Corrigendum: antibacterial activities of bacteriocins: application in foods and pharmaceuticals. Front Microbiol. 2014; 5: 683.
20. Cascales E, Buchanan SK, Duche D et al. Colicin biology. Microbiol Mol Biol Rev. 2007; 71 (1): 158-229.
21. Duquesne S, Petit V, Peduzzi J et al. Structural and functional diversity of microcins, gene-encoded antibacterial peptides from enterobacteria. J Mol Microbiol Biotechnol. 2007; 13 (4): 200-209.
22. Guzman-Rodriguez JJ, Ochoa-Zarzosa A, Lopez-Gomez R et al. Plant antimicrobial peptides as potential anticancer agents. Biomed Res Int. 2015; 2015: 735087.
23. Lacerda AF, Vasconcelos EA, Pelegrini PB et al. Antifungal defensins and their role in plant defense. Front Microbiol. 2014; 5: 116.
24. Ezzati-Tabrizi R, Farrokhi N, Talaei-Hassanloui R et al. Insect inducible antimicrobial peptides and their applications. Curr Protein Pept Sci. 2013; 14 (8): 698-710.
25. Otvos L, Jr. Antibacterial peptides isolated from insects. J Pept Sci. 2000; 6 (10): 497-511.
26. Ponnappan N, Budagavi DP, Yadav BK et al. Membrane-active peptides from marine organisms-antimicrobials, cell-penetrating peptides and Peptide toxins: applications and prospects. Probiotics Antimicrob Proteins. 2015; 7 (1): 75-89.
27. Otero-Gonzalez AJ, Magalhaes BS, Garcia-Villarino M et al. Antimicrobial peptides from marine invertebrates as a new frontier for microbial infection control. FASEB J. 2010; 24 (5): 1320-1334.
28. Sperstad SV, Haug T, Blencke HM et al. Antimicrobial peptides from marine invertebrates: challenges and perspectives in marine antimicrobial peptide discovery. Biotechnol Adv. 2011; 29 (5): 519-530.
29. Wells KD. The ecology and behaviour of amphibians. Chicago: University of Chicago Press 2007.
30. Kreil G. Antimicrobial peptides from amphibian skin: an overview. Ciba Found Symp. 1994; 186: 77-85; discussion 85-90.
31. Konig E, Bininda-Emonds OR, Shaw C. The diversity and evolution of anuran skin peptides. Peptides. 2015; 63: 96-117.
32. Cuperus T, Coorens M, van Dijk A et al. Avian host defense peptides. Dev Comp Immunol. 2013; 41 (3): 352-369.
33. Selsted ME, Ouellette AJ. Mammalian defensins in the antimicrobial immune response. Nat Immunol. 2005; 6 (6): 551-557.
34. Jarczak J, Kosciuczuk EM, Lisowski P et al. Defensins: natural component of human innate immunity. Hum Immunol. 2013; 74 (9): 1069-1079.
35. Lehrer RI, Lu W. alpha-Defensins in human innate immunity. Immunol Rev. 2012; 245 (1): 84-112.
36. Joly S, Maze C, McCray PB, Jr. et al. Human beta-defensins 2 and 3 demonstrate strain-selective activity against oral microorganisms. J Clin Microbiol. 2004; 42 (3): 1024-1029.
37. Ganz T. Defensins and other antimicrobial peptides: a historical perspective and an update. Comb Chem High Throughput Screen. 2005; 8 (3): 209-217.
38. Lehrer RI, Cole AM, Selsted ME. theta-Defensins: cyclic peptides with endless potential. J Biol Chem. 2012; 287 (32): 27014-27019.
39. Penberthy WT, Chari S, Cole AL et al. Retrocyclins and their activity against HIV-1. Cell Mol Life Sci. 2011; 68 (13): 2231-2242.
40. Leonova L, Kokryakov VN, Aleshina G et al. Circular minidefensins and posttranslational generation of molecular diversity. J Leukoc Biol. 2001; 70 (3): 461-464.
41. Cole AM, Lehrer RI. Minidefensins: antimicrobial peptides with activity against HIV-1. Curr Pharm Des. 2003; 9 (18): 1463-1473.
42. Kosciuczuk EM, Lisowski P, Jarczak J et al. Cathelicidins: family of antimicrobial peptides. A review. Mol Biol Rep. 2012; 39 (12): 10957-10970.
43. Schroder JM. Epithelial peptide antibiotics. Biochem Pharmacol. 1999; 57 (2): 121-134.
44. Sorensen OE, Borregaard N, Cole AM. Antimicrobial peptides in innate immune responses. Contrib Microbiol. 2008; 15: 61-77.
45. Meade KG, Cormican P, Narciandi F et al. Bovine beta-defensin gene family: opportunities to improve animal health? Physiol Genomics. 2014; 46 (1): 17-28.
46. Hicks RP, Bhonsle JB, Venugopal D et al. De novo design of selective antibiotic peptides by incorporation of unnatural amino acids. J Med Chem. 2007; 50 (13): 3026-3036.
47. Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol. 2005; 3 (3): 238-250.
48. Dubin A, Mak P, Dubin G et al. New generation of peptide antibiotics. Acta Biochim Pol. 2005; 52 (3): 633-638.
49. Nguyen LT, Haney EF, Vogel HJ. The expanding scope of antimicrobial peptide structures and their modes of action. Trends Biotechnol. 2011; 29 (9): 464-472.
50. Cotter PD, Ross RP, Hill C. Bacteriocins - a viable alternative to antibiotics? Nat Rev Microbiol. 2013; 11 (2): 95-105.
51. Cole AM. Minidefensins and other antimicrobial peptides: candidate anti-HIV microbicides. Expert Opin Ther Targets. 2003; 7 (3): 329-341.
52. Barlow PG, Findlay EG, Currie SM et al. Antiviral potential of cathelicidins. Future Microbiol. 2014; 9 (1): 55-73.
53. Zairi A, Tangy F, Bouassida K et al. Dermaseptins and magainins: antimicrobial peptides from frogs' skin-new sources for a promising spermicides microbicides-a mini review. J Biomed Biotechnol. 2009; 2009: 452567.

54. Sutyak KE, Anderson RA, Dover SE et al. Spermicidal activity of the safe natural antimicrobial peptide subtilosin. Infect Dis Obstet Gynecol. 2008; 2008: 540758.
55. Ghrairi T, Chaftar N, Hani K. Bacteriocins: recent advances and opportunities. In: Preservation PiF, ed. Bhat R, Karim Alias A, Paliyath G Oxford, UK: Wiley-Blackwell 2012. pp. 485-511.
56. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin. 2013; 63 (1): 11-30.
57. Gatti L, Zunino F. Overview of tumor cell chemoresistance mechanisms. Methods Mol Med. 2005; 111: 127-148.
58. Leuschner C, Hansel W. Membrane disrupting lytic peptides for cancer treatments. Curr Pharm Des. 2004; 10 (19): 2299-2310.
59. Glukhov E, Stark M, Burrows LL et al. Basis for selectivity of cationic antimicrobial peptides for bacterial versus mammalian membranes. J Biol Chem. 2005; 280 (40): 33960-33967.
60. Dobrzynska I, Szachowicz-Petelska B, Sulkowski S et al. Changes in electric charge and phospholipids composition in human colorectal cancer cells. Mol Cell Biochem. 2005; 276 (1-2): 113-119.
61. Yoon WH, Park HD, Lim K et al. Effect of O-glycosylated mucin on invasion and metastasis of HM7 human colon cancer cells. Biochem Biophys Res Commun. 1996; 222 (3): 694-699.
62. Boland MP, Separovic F. Membrane interactions of antimicrobial peptides from Australian tree frogs. Biochim Biophys Acta. 2006; 1758 (9): 1178-1183.
63. Mader JS, Hoskin DW. Cationic antimicrobial peptides as novel cytotoxic agents for cancer treatment. Expert Opin Investig Drugs. 2006; 15 (8): 933-946.
64. Gaspar D, Veiga AS, Castanho MA. From antimicrobial to anticancer peptides. A review. Front Microbiol. 2013; 4: 294.
65. Mulder KC, Lima LA, Miranda VJ et al. Current scenario of peptide-based drugs: the key roles of cationic antitumor and antiviral peptides. Front Microbiol. 2013; 4: 321.
66. Joo NE, Ritchie K, Kamarajan P et al. Nisin, an apoptogenic bacteriocin and food preservative, attenuates HNSCC tumorigenesis via CHAC1. Cancer Med. 2012; 1 (3): 295-305.
67. Tsai TL, Li AC, Chen YC et al. Antimicrobial peptide m2163 or m2386 identified from Lactobacillus casei ATCC 334 can trigger apoptosis in the human colorectal cancer cell line SW480. Tumour Biol. 2015; 36 (5): 3775-3789.
68. Lin P, Wong JH, Ng TB. A defensin with highly potent antipathogenic activities from the seeds of purple pole bean. Biosci Rep. 2009; 30 (2): 101-109.
69. Ngai PH, Ng TB. Coccinin, an antifungal peptide with antiproliferative and HIV-1 reverse transcriptase inhibitory activities from large scarlet runner beans. Peptides. 2004; 25 (12): 2063-2068.
70. Ngai PH, Ng TB. Phaseococcin, an antifungal protein with antiproliferative and anti-HIV-1 reverse transcriptase activities from small scarlet runner beans. Biochem Cell Biol. 2005; 83 (2): 212-220.
71. Gerlach SL, Rathinakumar R, Chakravarty G et al. Anticancer and chemosensitizing abilities of cycloviolacin 02 from Viola odorata and psyle cyclotides from *Psychotria leptothyrsa*. Biopolymers. 2010; 94 (5): 617-625.
72. Prabhu S, Dennison SR, Mura M et al. Cn-AMP2 from green coconut water is an anionic anticancer peptide. J Pept Sci. 2014; 20 (12): 909-915.
73. Mishra A, Gauri SS, Mukhopadhyay SK et al. Identification and structural characterization of a new pro-apoptotic cyclic octapeptide cyclosaplin from somatic seedlings of *Santalum album L*. Peptides. 2014; 54: 148-158.
74. Suttmann H, Retz M, Paulsen F et al. Antimicrobial peptides of the Cecropin-family show potent antitumor activity against bladder cancer cells. BMC Urol. 2008; 8: 5.
75. Wu JM, Jan PS, Yu HC et al. Structure and function of a custom anticancer peptide, CB1a. Peptides. 2009; 30 (5): 839-848.
76. Xia L, Wu Y, Kang S et al. CecropinXJ, a silkworm antimicrobial peptide, induces cytoskeleton disruption in esophageal carcinoma cells. Acta Biochim Biophys Sin (Shanghai). 2014; 46 (10): 867-876.
77. Kang BR, Kim H, Nam SH et al. CopA3 peptide from Copris tripartitus induces apoptosis in human leukemia cells via a caspase-independent pathway. BMB Rep. 2012; 45 (2): 85-90.
78. Lee JH, Kim IW, Kim SH et al. Anticancer activity of CopA3 dimer peptide in human gastric cancer cells. BMB Rep. 2014.
79. Kim IW, Lee JH, Kwon YN et al. Anticancer activity of a synthetic peptide derived from harmoniasin, an antibacterial peptide from the ladybug *Harmonia axyridis*. Int J Oncol. 2013; 43 (2): 622-628.
80. Zhu LN, Fu CY, Zhang SF et al. Novel cytotoxic exhibition mode of antimicrobial peptide anoplin in MEL cells, the cell line of murine Friend leukemia virus-induced leukemic cells. J Pept Sci. 2013; 19 (9): 566-574.
81. Zhang W, Li J, Liu LW et al. A novel analog of antimicrobial peptide Polybia-MPI, with thioamide bond substitution, exhibits increased therapeutic efficacy against cancer and diminished toxicity in mice. Peptides. 2010; 31 (10): 1832-1838.
82. Lee JH, Kim IW, Kim SH et al. Anticancer activity of the antimicrobial peptide scolopendrasin VII derived from the centipede, *Scolopendra subspinipes* mutilans. J Microbiol Biotechnol. 2015.
83. Hsiao YC, Wang KS, Tsai SH et al. Anticancer activities of an antimicrobial peptide derivative of Ixosin-B amide. Bioorg Med Chem Lett. 2013; 23 (20): 5744-5747.
84. Chen J, Xu XM, Underhill CB et al. Tachyplesin activates the classic complement pathway to kill tumor cells. Cancer Res. 2005; 65 (11): 4614-4622.
85. Meng MX, Ning JF, Yu JY et al. Antitumor activity of recombinant antimicrobial peptide penaeidin-2 against kidney cancer cells. J Huazhong Univ Sci Technolog Med Sci. 2014; 34 (4): 529-534.

86. Liu S, Yang H, Wan L et al. Penetratin-mediated delivery enhances the antitumor activity of the cationic antimicrobial peptide Magainin II. Cancer Biother Radiopharm. 2013; 28 (4): 289-297.
87. Wang C, Zhou Y, Li S et al. Anticancer mechanisms of temporin-1CEa, an amphipathic alpha-helical antimicrobial peptide, in Bcap-37 human breast cancer cells. Life Sci. 2013; 92 (20-21): 1004-1014.
88. van Zoggel H, Carpentier G, Dos Santos C et al. Antitumor and angiostatic activities of the antimicrobial peptide dermaseptin B2. PLoS One. 2012; 7 (9): e44351.
89. Gu Y, Dong N, Shan A et al. Antitumor effect of the antimicrobial peptide GLI13-8 derived from domain of the avian beta-defensin-4. Acta Biochim Biophys Sin (Shanghai). 2013; 45 (11): 904-911.
90. Chen JY, Lin WJ, Lin TL. A fish antimicrobial peptide, tilapia hepcidin TH2-3, shows potent antitumor activity against human fibrosarcoma cells. Peptides. 2009; 30 (9): 1636-1642.
91. Hilchie AL, Doucette CD, Pinto DM et al. Pleurocidin-family cationic antimicrobial peptides are cytolytic for breast carcinoma cells and prevent growth of tumor xenografts. Breast Cancer Res. 2011; 13 (5): R102.
92. Lin HJ, Huang TC, Muthusamy S et al. Piscidin-1, an antimicrobial peptide from fish (hybrid striped bass morone saxatilis x M. chrysops), induces apoptotic and necrotic activity in HT1080 cells. Zoolog Sci. 2012; 29 (5): 327-332.
93. Wu SP, Huang TC, Lin CC et al. Pardaxin, a fish antimicrobial peptide, exhibits antitumor activity toward murine fibrosarcoma in vitro and *in vivo*. Mar Drugs. 2012; 10 (8): 1852-1872.
94. Li D, Wang W, Shi HS et al. Gene therapy with beta-defensin 2 induces antitumor immunity and enhances local antitumor effects. Hum Gene Ther. 2014; 25 (1): 63-72.
95. Furlong SJ, Ridgway ND, Hoskin DW. Modulation of ceramide metabolism in T-leukemia cell lines potentiates apoptosis induced by the cationic antimicrobial peptide bovine lactoferricin. Int J Oncol. 2008; 32 (3): 537-544.
96. Chow JY, Li ZJ, Wu WK et al. Cathelicidin a potential therapeutic peptide for gastrointestinal inflammation and cancer. World J Gastroenterol. 2013; 19 (18): 2731-2735.
97. Kuroda K, Fukuda T, Isogai H et al. Antimicrobial peptide FF/CAP18 induces apoptotic cell death in HCT116 colon cancer cells via changes in the metabolic profile. Int J Oncol. 2015; 46 (4): 1516-1526.
98. Aoki W, Kuroda K, Ueda M. Next generation of antimicrobial peptides as molecular targeted medicines. J Biosci Bioeng. 2012; 114 (4): 365-370.
99. Gordon YJ, Romanowski EG, McDermott AM. A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs. Curr Eye Res. 2005; 30 (7): 505-515.
100. He J, Yarbrough DK, Kreth J et al. Systematic approach to optimizing specifically targeted antimicrobial peptides against *Streptococcus mutans*. Antimicrob Agents Chemother. 2010; 54 (5): 2143-2151.
101. Eckert R, Qi F, Yarbrough DK et al. Adding selectivity to antimicrobial peptides: rational design of a multidomain peptide against *Pseudomonas spp*. Antimicrob Agents Chemother. 2006; 50 (4): 1480-1488.
102. Villarruel-Franco R, Huizar-Lopez R, Corrales M et al. Péptidos naturales antimicrobianos: escudo esencial de la respuesta inmune. Investigación en Salud. 2004; 6 (3): 170-179.
103. Fjell CD, Hiss JA, Hancock RE et al. Designing antimicrobial peptides: form follows function. Nat Rev Drug Discov. 2012; 11 (1): 37-51.
104. Papo N, Shahar M, Eisenbach L et al. A novel lytic peptide composed of DL-amino acids selectively kills cancer cells in culture and in mice. J Biol Chem. 2003; 278 (23): 21018-21023.
105. Ng-Choi I, Soler M, Guell I et al. Antimicrobial peptides incorporating non-natural amino acids as agents for plant protection. Protein Pept Lett. 2014; 21 (4): 357-367.
106. Taboureau O, Olsen OH, Nielsen JD et al. Design of novispirin antimicrobial peptides by quantitative structure-activity relationship. Chem Biol Drug Des. 2006; 68 (1): 48-57.
107. Juretic D, Vukicevic D, Ilic N et al. Computational design of highly selective antimicrobial peptides. J Chem Inf Model. 2009; 49 (12): 2873-2882.
108. Juretic D, Vukicevic D, Petrov D et al. Knowledge-based computational methods for identifying or designing novel, non-homologous antimicrobial peptides. Eur Biophys J. 2011; 40 (4): 371-385.
109. Jenssen H, Gutteberg TJ, Lejon T. Modelling of anti-HSV activity of lactoferricin analogues using amino acid descriptors. J Pept Sci. 2005; 11 (2): 97-103.
110. Hilpert K, Elliott MR, Volkmer-Engert R et al. Sequence requirements and an optimization strategy for short antimicrobial peptides. Chem Biol. 2006; 13 (10): 1101-1107.
111. Langham AA, Khandelia H, Schuster B et al. Correlation between simulated physicochemical properties and hemolycity of protegrin-like antimicrobial peptides: predicting experimental toxicity. Peptides. 2008; 29 (7): 1085-1093.
112. Cherkasov A. Inductive QSAR descriptors. Distinguishing compounds with antibacterial activity by Artificial Neural Networks. Int J Mol Sci. 2005; 6: 63-86
113. Hilpert K, Fjell CD, Cherkasov A. Short linear cationic antimicrobial peptides: screening, optimizing, and prediction. Methods Mol Biol. 2008; 494: 127-159.
114. Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. N Engl J Med. 2010; 362 (19): 1804-1813.
115. Muhle SA, Tam JP. Design of Gram-negative selective antimicrobial peptides. Biochemistry. 2001; 40 (19): 5777-5785.
116. Giuliani A, Pirri G, Rinaldi AC. Antimicrobial peptides: the LPS connection. Methods Mol Biol. 2010; 618: 137-154.
117. Bhattacharjya S. De novo designed lipopolysaccharide binding peptides: structure based development of antiendotoxic and antimicrobial drugs. Curr Med Chem. 2010; 17 (27): 3080-3093.

118. David SA. Antimicrobial peptides for gram-negative sepsis: a case for the polymyxins. Front Immunol. 2012; 3: 252.
119. Li LN, Guo LH, Lux R et al. Targeted antimicrobial therapy against *Streptococcus mutans* establishes protective non-cariogenic oral biofilms and reduces subsequent infection. Int J Oral Sci. 2010; 2 (2): 66-73.
120. Kaplan CW, Sim JH, Shah KR et al. Selective membrane disruption: mode of action of C16G2, a specifically targeted antimicrobial peptide. Antimicrob Agents Chemother. 2011; 55 (7): 3446-3452.
121. Hou L, Zhao X, Wang P et al. Antitumor activity of antimicrobial peptides containing CisoDGRC in CD13 negative breast cancer cells. PLoS One. 2013; 8 (1): e53491.
122. Acuna L, Picariello G, Sesma F et al. A new hybrid bacteriocin, Ent35-MccV, displays antimicrobial activity against pathogenic Gram-positive and Gram-negative bacteria. FEBS Open Bio. 2012; 2: 12-19.
123. Spudy B, Sonnichsen FD, Waetzig GH et al. Identification of structural traits that increase the antimicrobial activity of a chimeric peptide of human beta-defensins 2 and 3. Biochem Biophys Res Commun. 2012; 427 (1): 207-211.
124. Cao Y, Yu RQ, Liu Y et al. Design, recombinant expression, and antibacterial activity of the cecropins-melittin hybrid antimicrobial peptides. Curr Microbiol. 2010; 61 (3): 169-175.
125. Wu R, Wang Q, Zheng Z et al. Design, characterization and expression of a novel hybrid peptides melittin (1-13)-LL37 (17-30). Mol Biol Rep. 2014; 41 (7): 4163-4169.
126. Joshi S, Bisht GS, Rawat DS et al. Comparative mode of action of novel hybrid peptide CS-1a and its rearranged amphipathic analogue CS-2a. FEBS J. 2012; 279 (20): 3776-3790.
127. Olli S, Nagaraj R, Motukupally SR. A hybrid cationic peptide composed of human beta-defensin-1 and humanized theta-defensin sequences exhibits salt-resistant antimicrobial activity. Antimicrob Agents Chemother. 2015; 59 (1): 217-225.
128. Lu XM, Jin XB, Zhu JY et al. Expression of the antimicrobial peptide cecropin fused with human lysozyme in *Escherichia coli*. Appl Microbiol Biotechnol. 2010; 87 (6): 2169-2176.
129. Lohan S, Bisht GS. Recent approaches in design of peptidomimetics for antimicrobial drug discovery research. Mini Rev Med Chem. 2013; 13 (7): 1073-1088.
130. Mendez-Samperio P. Peptidomimetics as a new generation of antimicrobial agents: current progress. Infect Drug Resist. 2014; 7: 229-237.
131. Avan I, Hall CD, Katritzky AR. Peptidomimetics via modifications of amino acids and peptide bonds. Chem Soc Rev. 2014; 43 (10): 3575-3594.
132. Niu Y, Wu H, Li Y et al. AApeptides as a new class of antimicrobial agents. Org Biomol Chem. 2013; 11 (26): 4283-4290.
133. Li L, He J, Eckert R et al. Design and characterization of an acid-activated antimicrobial peptide. Chem Biol Drug Des. 2010; 75 (1): 127-132.
134. Song J, Zhang W, Kai M et al. Design of an acid-activated antimicrobial peptide for tumor therapy. Mol Pharm. 2013; 10 (8): 2934-2941.
135. Fernebro J. Fighting bacterial infections-future treatment options. Drug Resist Updat. 2011; 14 (2): 125-139.
136. Settanni L, Corsetti A. Application of bacteriocins in vegetable food biopreservation. Int J Food Microbiol. 2008; 121 (2): 123-138.
137. Midura-Nowaczek K, Markowska A. Antimicrobial peptides and their analogs: searching for new potential therapeutics. Perspect Medicin Chem. 2014; 6: 73-80.
138. Velden WJ, van Iersel TM, Blijlevens NM et al. Safety and tolerability of the antimicrobial peptide human lactoferrin 1-11 (hLF1-11). BMC Med. 2009; 7: 44.
139. Mickels N, McManus C, Massaro J et al. Clinical and microbial evaluation of a histatin-containing mouthrinse in humans with experimental gingivitis. J Clin Periodontol. 2001; 28 (5): 404-410.
140. Paquette DW, Simpson DM, Friden P et al. Safety and clinical effects of topical histatin gels in humans with experimental gingivitis. J Clin Periodontol. 2002; 29 (12): 1051-1058.
141. Levin M, Quint PA, Goldstein B et al. Recombinant bactericidal/permeability-increasing protein (rBPI21) as adjunctive treatment for children with severe meningococcal sepsis: a randomised trial. rBPI21 Meningococcal Sepsis Study Group. Lancet. 2000; 356 (9234): 961-967.
142. Domingues MM, Santos NC, Castanho MA. Antimicrobial peptide rBPI21: a translational overview from bench to clinical studies. Curr Protein Pept Sci. 2012; 13 (7): 611-619.
143. Lipsky BA, Holroyd KJ, Zasloff M. Topical versus systemic antimicrobial therapy for treating mildly infected diabetic foot ulcers: a randomized, controlled, double-blinded, multicenter trial of pexiganan cream. Clin Infect Dis. 2008; 47 (12): 1537-1545.
144. Melo MN, Dugourd D, Castanho MA. Omiganan pentahydrochloride in the front line of clinical applications of antimicrobial peptides. Recent Pat Antiinfect Drug Discov. 2006; 1 (2): 201-207.
145. Nilsson AC, Janson H, Wold H et al. LTX-109 is a novel agent for nasal decolonization of methicillin-resistant and -sensitive *Staphylococcus aureus*. Antimicrob Agents Chemother. 2015; 59 (1): 145-151.