

Anticancer Mechanisms and Potential Anticancer Applications of Antimicrobial Peptides and Their Nano Agents

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Abstract: Traditional chemotherapy is one of the main methods of cancer treatment, which is largely limited by severe side effects and frequent development of multi-drug resistance by cancer cells. Antimicrobial peptides (AMPs) with high efficiency and low toxicity, as one of the most promising new drugs to replace chemoradiotherapy, have become a current research hotspot, attracting the attention of worldwide researchers. AMPs are natural-source small peptides from the innate immune system, and certain AMPs can selectively kill a broad spectrum of cancer cells while exhibiting less damage to normal cells. Although it involves intracellular mechanisms, AMPs exert their anti-cancer effects mainly through membrane destruction effect; thus, AMPs also hold unique advantages in fighting drug-resistant cancer cells. However, the poor stability and hemolytic toxicity of peptides limit their clinical application. Fortunately, functionalized nanoparticles have many possibilities in overcoming the shortcomings of AMPs, which provides a huge prospect for better application of AMPs. In this paper, we briefly introduce the characteristics and different sources of AMPs, review and summarize the mechanisms of action and the research status of AMPs used as an anticancer therapy, and finally focus on the further use of AMPs nano agents in the anti-cancer direction.

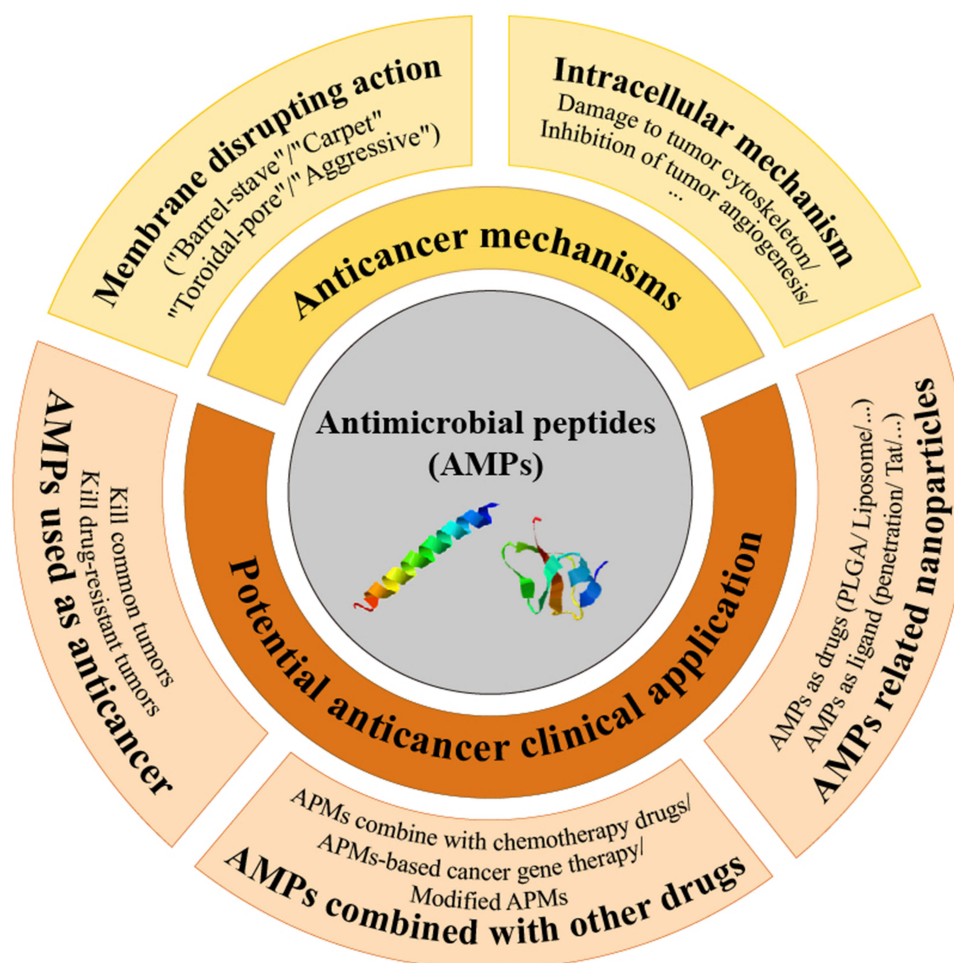
Keywords: antimicrobial peptides, anticancer mechanisms, drug combination, anticancer application, peptide nano agents

Introduction

Cancer, as one of the most lethal diseases in the world, is still a serious threat to human survival.¹ Chemotherapy alone or combined with surgery/radiotherapy plays an important role in improving the life expectancy of cancer patients. However, due to the lack of specificity of many chemotherapeutic drugs for cancer cells, they can also kill healthy cells that are in rapid proliferation phase while killing cancer cells, which may lead to serious side effects.^{2,3} Additionally, cancer cells are prone to develop resistance to chemotherapeutic drugs resulting from drug inactivation or efflux, target protein alteration, DNA damage repair, and signaling cascade alteration, which greatly limits the efficacy of such drugs.^{4,5} Therefore, it is urgent to find new potent drugs with high tumor selectivity.

In recent years, antimicrobial peptides (AMPs) have attracted the attention of researchers. AMPs are small-molecule peptides with positive charges and amphipathic properties.⁶ They are part of the innate immune defense system of organisms with multiple biological activities such as antibacterial activity, antifungal activity, anticancer activity, and immune system regulation.^{7,8} On the one hand, due to their positive charges and amphiphilic nature, AMPs can selectively bind to negatively charged cancer cell membranes through electrostatic attraction and form a transmembrane channel, destroying the integrity of the cell membrane, thereby killing cancer cells.^{9,10} Besides, AMPs can target various cellular processes within cancer cells, such as DNA and protein synthesis, protein folding,

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enzymatic activity, and cell wall synthesis.^{11,12} On the other hand, AMPs can be internalized into cancer cells via endocytosis mechanisms, leading to apoptosis and the inhibition of autophagy.¹³

At the same time, due to their unique membrane mechanism, AMPs do not easily induce drug resistance in tumor cells.¹⁴ Tumor multidrug resistance (MDR) is the main cause of tumor chemotherapy failure of tumor chemotherapy. The overexpression of P-glycoprotein (P-gp) in the tumor cell membrane is one of the main reasons for the formation of MDR.¹⁵ Fitly, P-gp participates in the formation of cell membranes and increases the negative charges on the surface of the tumor cell membrane, which is conducive to the combination of cationic AMPs and drug-resistant tumor cell membranes, thereby better exerting anti-cancer activity.^{16,17} Therefore, AMPs have great prospects for the treatment of drug-resistant tumors. In recent years, the use of AMPs alone or in combination with other drugs for cancer treatment has attracted extensive attention.^{18–20}

Characteristics and Sources of Antimicrobial Peptides

Antimicrobial peptides (AMPs) are also called host defense peptides or antibiotic peptides. They are a type of biologically active peptides that exist widely in the biological world. AMPs are an important part of the innate immune defense system of almost every organism including bacteria, plants, insects, fish, amphibians, birds, and mammals.^{21,22} Representative AMP sequences from different sources are shown in Table 1.

Table 1 AMP Sequences from Different Sources

Sources		AMP Name	Sequence	Refs
Insect	<i>Hyalophora cecropia</i>	Cecropin A	KWKLFKKIEKVGQNIRDGIIKAGPAVAVVGQATQIAK	[23,24]
	<i>Anopheles gambiae</i>	Defensin I	ATCDLASGFGVSSSLCAAHCIAARRYRGGYCNSKAVCVCRN	[25]
Mammals	<i>Homo sapiens</i>	LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLPRTES	[26,27]
	<i>Bos taurus</i>	Indolicidin	ILPWKWPWWPWRR-NH ₂	[28,29]
Amphibian	<i>Sus scrofa</i> (Pig)	Protegrin I	RGGRLCYCRRRFCVCVGR-NH ₂	[30]
	<i>Xenopus laevis</i>	Magainin-2	GIGKFLHSAKKFGKAFVGEIMNS	[31,32]
Marine animal	<i>Rana temporaria</i>	Temporin L	FVQWFSKFLGRIL-NH ₂	[33,34]
	<i>Pseudopleuronectes americanus</i>	Pleurocidins	GWKKWFKKATHVGKHVGKAALDAYL	[35–37]
Plant	<i>Litopenaeus vannamei</i>	Penaeidins	YRGGYTGPPIRPPPIGRPLRLVVCACYRLSVSDARNCCIKFGSCCHLVK	[38,39]
	<i>Mytilus edulis</i>	Myticin B	SCASRCKGHCRARRCGYYVSVLYRGRICYCKCLRC	[40]
	<i>Arabidopsis thaliana</i> (L.) Heynh.	Thionin-2.1	KICCPNQARNGYSVCRIRFSKGRCMQVSGCQNSDTCPRGWVN	[41,42]
	<i>Solanum tuberosum</i> L.	Snakin-2 (StSN2)	IQTDQVTSNAISEAA	[43,44]
Microorganism	<i>Bacillus brevis</i>	Gramicidin S	Cyclo-(L-Val-Orn-L-Leu-D-Phe-L-Pro) ₂	[45,46]
	<i>Streptococcus lactis</i>	Lantibiotic Nisin-A	ITSISLCTPGCKTGALMGCMKMTATCHCSIHVSK	[47,48]

AMPs are small peptides that are usually composed of 10–100 amino acid residues, most of which are cationic.^{49,50} Therefore, AMPs are often referred to as cationic antimicrobial peptides (CAPs). The primary structure of most AMPs shares the following characteristics: N-terminal is rich in lysine, arginine, and other cationic amino acids; C-terminal is rich in alanine, valine, glycine, and other hydrophobic amino acids, taking up to 50% of the entire amino acid sequence.⁵¹ In addition, AMPs mainly have the following four secondary structures^{52,53} (as shown in Figure 1): 1. α -helical structure, such as as Melittin,⁵⁴ Magainin,⁵⁵ and Cecropins;⁵⁶ 3. Extended helices structure, which usually occurs in a linear peptide that is rich in proline and arginine or tryptophan residues, such as bovine-derived AMP (indolicidin)²⁸ and Drosophila-derived AMP (drosocin);⁵⁷ 4. Loop structure, with which AMPs often contain an intramolecular disulfide bond at the C-terminus to form a loop, while the N-terminus is a linear structure, forming a cyclic chain structure, such as bovine neutrophils-derived AMP (bactenecin)⁵⁸ and frog skin-derived AMP (brevinins).⁵⁹ Among them, α -helical AMPs are the most studied type of AMPs. The α -helical structure is a nearly perfect hydrophobic amphiphilic structure, that is, one side of the longitudinal axis of the helix is a positively charged hydrophilic region, and the opposite side is a hydrophobic region. This amphiphilic structure is the key to the activity of AMPs.^{60,61}

Although AMPs have the above-mentioned common features, their sequences and activities are different. These peptides are produced in large quantities at the infection and/or inflammation sites of the organism, and can rapidly and broadly kill Gram-positive and Gram-negative bacteria, fungi, parasites, and certain viruses.^{62,63} In addition,

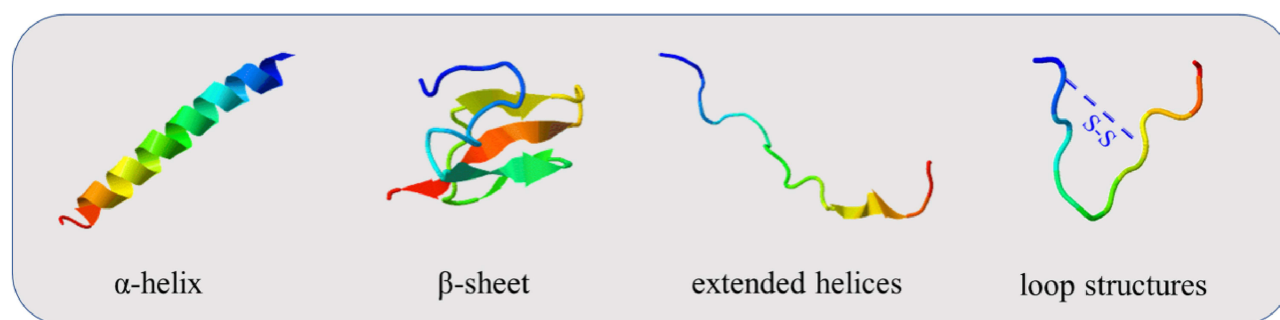
**Figure 1** Four secondary structures of antimicrobial peptides.

Table 2 Representative Antimicrobial Peptides with Different Activities

Antimicrobial Peptide Name	Main Activity	Refs
Defensins, indolicidin, dermaseptins	Broad-spectrum antimicrobial/anticancer activity	[70–72]
Indolicidin, histatins, protegrin	Antifungal activity	[73–75]
LL-37	Anti-endotoxin/anticancer activity	[76]
Indolicidin, defensins, protegrin	Antiviral activity	[77–79]
Indolicidin, defensins	Antiparasitic activity	[80]
Indolicidin, defensins, dermaseptins, cecropins	Anticancer activity	[81]
Defensins, PR39	Wound healing activity	[82,83]

AMPs also play an important role in the inflammatory response, immune activation, and wound healing.^{64,65} It is worth noting that increased studies have shown that many AMPs have significant selective anti-cancer activity^{66–68} and such AMPs can also be called anti-cancer peptides (ACPs). Table 2⁶⁹ gives examples of AMPs with different activities.

Anticancer Mechanisms of AMPs

The anti-cancer mechanism of AMPs can be divided into two types:⁸⁴ selective membrane disruption action and non-membranolytic actions.^{85,86} The selective membrane disruption action is similar to its bactericidal action: the cationic AMPs directly interact with the cancer cell membrane through electrostatic attraction to form transient pores and destroy the integrity of the cell membrane and finally cause cell death.⁸⁷ Besides, non-membranolytic actions of AMPs include the destruction of the cytoskeleton in cancer cells, inhibition of DNA and protein synthesis, inhibition of tumor angiogenesis, regulation of immunity, and induction of tumor cell apoptosis or necrosis. Each mechanism is not entirely separate, AMPs may show a complex anti-tumor mechanism. AMPs may first interact with the tumor cell membrane, enter into the cells and then act on the intracellular targets, or induce cell apoptosis or necrosis.^{88,89}

Basis for Anticancer Action of AMPs: The Particularities of Cancer Cell Membranes

Similar to its antibacterial mechanism, almost all the AMPs can initially interact with the negatively charged cancer cell membranes, and the disruption of cancer cell membranes is considered to be the primary mechanism for the anticancer effect of AMPs, which determines the broad-spectrum anticancer effect of AMPs.⁹⁰ Compared to normal cell membranes, the structural characteristics of tumor cell membranes are more conducive to AMPs to exert their anticancer effects. First of all, cancer cells, unlike healthy eukaryotic cells that contain zwitterionic phosphatidylcholine and sphingomyelin in their membranes and therefore overall neutrally charged,⁹¹ carry a net negative charge due to the elevated expression of anionic molecules such as phosphatidylserine (PS) and O-glycosylated mucins in their outer membrane leaflet,^{92,93} which results in a greatly enhanced capacity for electrostatic interactions with AMPs. The binding affinity of some AMPs to tumor cell membranes is 10 times higher than that of normal cells, showing selective tumor cytotoxicity.^{94,95} Furthermore, tumor cells have more abundant microvilli on their cell surface in comparison with normal cells, and the increased overall surface area of tumor cells allows a greater number of AMP molecules to interact with the surface of cancer cells, thereby promoting AMP-mediated cytotoxicity.^{96,97} Collectively, the aforementioned characteristics account for the ability of some AMPs to selectively kill tumor cells without damaging healthy cells.

In addition, many AMPs exhibit strong cytotoxicity on MDR tumor cell lines. Also, certain AMPs can reverse the drug-resistance of tumor cells, thereby increasing tumor sensitivity to chemotherapeutic drugs. One of the main reasons causing tumor MDR is the over-expression of P-gp. The over-expressed P-gp could increase the negative charge on the MDR tumor cell membrane, thus enhancing AMP binding by electrostatic attraction.^{98,99} Then, the hydrophobic C-terminal of AMP may insert into the hydrophobic region of the cell membrane, which may change the conformation

of the cell membrane and reduce the activity of P-gp by inhibiting the efflux of chemotherapy drugs, increasing the intracellular drug concentration, and reversing drug resistance of MDR tumor cells.¹⁰⁰

Recognized Models of Selective Membrane-Disrupting Mechanism

At present, scholars have proposed several models to explain the interaction between AMPs and tumor cell membranes from different perspectives (as shown in Figure 2) including “Barrel-stave” model, “Carpet” model, “Toroidal-pore” model, and “Aggregate” model. These models are not completely independent, that is, AMPs under different concentrations and different conditions may function through complex mechanisms.

“Barrel-Stave” Model

In the “Barrel-stave” model (Figure 2A), the monomers of α -helix cationic AMPs (such as cecropin B¹⁰¹ and bee venom peptide¹⁰²) first bind to the tumor cell membrane by electrostatic attraction. Then, the conformation of AMPs changes, that is, AMPs perpendicularly insert their hydrophobic region into the hydrophobic core of the cell membrane and form a position like “staves” in a barrel. Thereafter, the AMP monomers will self-aggregate into the α -helix polymer with their hydrophilic side constituting the barrel-like hollow channel and insert into the hydrophobic membrane cores more deeply, forming transient transmembrane pores which may disrupt the ion gradients and transmembrane potential and eventually lead to cell death.¹⁰³ It is worth noting that the “barrel-stave” model cannot explain the membrane disruption effect of AMPs with fewer than 23 amino acids as AMPs need to be long enough to pass through the cell membrane in this model.¹⁰⁴

“Carpet” Model

In the carpet model (Figure 2B), the AMPs initially interact with the tumor cell membrane through electrostatic attraction and arrange parallel to the cell surface in a “carpet”-like manner, which interfered with the accumulation of phospholipids. Subsequently, the local membrane is destabilized due to the change in curvature stress and internal osmotic

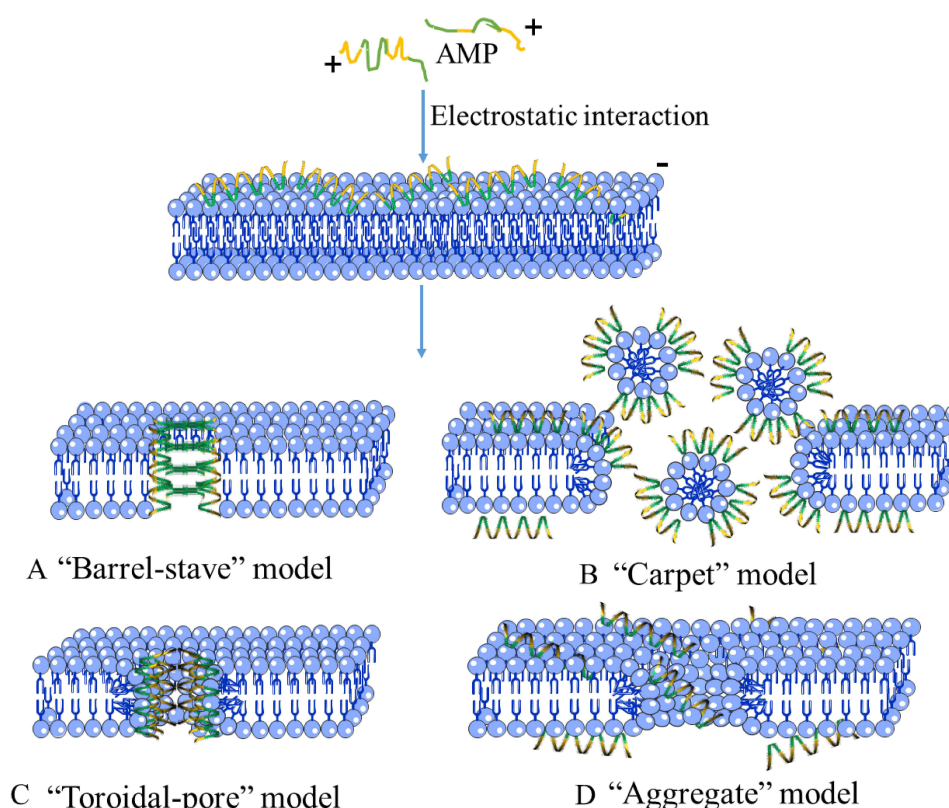


Figure 2 Schematic diagram of four widely recognized models of the membrane disruption mechanisms of antimicrobial peptides. (A) “Barrel-stave” model, (B) “Carpet” model, (C) “Toroidal-pore” model, (D) “Aggregate” model.

pressure. Finally, a circular gap will be formed, and the cell membrane will be disintegrated. During this process, the AMPs insert into the hydrophobic core of the lipid bilayer.⁹¹ Studies suggested that the AMP magainin worked through the “carpet” model at low concentrations, while worked through a “barrel plate” model at high concentrations.^{105,106}

“Toroidal-Pore” Model

In the “Toroidal-pore” model (Figure 2C), AMPs also originally bind to the cell membrane through electrostatic attraction and then induce continuous bending of the lipid monolayer with the hydrophilic group of AMPs interacting with the hydrophilic head of the cell membrane and then spiral insertion into the cell membrane, forming transmembrane pores.¹⁰⁷ Different from the “barrel-stave” model, the hydrophilic parts of AMPs and hydrophilic heads of the upper and lower lipids always together face to the center of the pores and the AMPs are unnecessary to pass through the whole phospholipid bilayer; thus, this model can explain the effect of some short AMPs.¹⁰⁴ Studies have shown that AMPs such as melittin^{103,108} and protegrin-1¹⁰⁹ work through the “Toroidal-pore” model.

“Aggregate” Model

Similar to the “Toroidal-pore” model, in the “Aggregate” model (Figure 2D), the AMPs are first electrostatically attracted to the tumor cell membrane. Then, the AMPs combine with lipids to form micelle complexes, and the aggregates will insert into the cell membrane, forming dynamic transmembrane pores. The difference with the “Toroidal-pore” model is that the AMPs working through the “Aggregate” model have no specific orientation and there is usually only one AMP located in the center of the aggregate.^{110,111}

Non-Membrane Disrupting Mechanisms of AMPs

Although the electrostatic interaction with tumor cell membrane and further disrupting action is the key of AMPs to kill the tumor cells, certain AMPs are accompanied by other mechanisms, such as inhibiting tumor DNA synthesis, destroying tumor cytoskeleton, inhibiting tumor angiogenesis, destroying tumor mitochondria, inducing tumor cell apoptosis or necrosis, and regulating immunity, as shown in Figure 3.

Inhibition and Destruction of Chromosomes and DNA in Cancer Cells

Some AMPs may accumulate in the nucleus area of cancer cells and kill cancer cells by breaking the tumor DNA, thus playing an anti-cancer role.¹¹² For example, the AMP named Cecropin-XJ isolated from Xinjiang silkworm was found to have the ability to combine with the groove of tumor cell DNA and then insert into the DNA sequence, resulting in the disordered arrangement of base pairs and looseness of DNA double helix structure, leading to DNA damage and cell death.¹¹³ In addition, using a single-cell gel electrophoresis technique, Wang et al¹¹⁴ found that the CM4-component of the AMP from *Bombyx mori* could selectively break the DNA of K562 cancer cells and kill cells without affecting the nuclear chromatin DNA of normal human leukocytes.

Damage to Cytoskeleton in Cancer Cells

The cell cytoskeleton is a fine network structure that can not only maintain cell morphology and maintain the order of cell internal structure but also participate in important life activities such as cell movement, material transportation, information transmission, cell division, and gene expression. There is a highly developed cytoskeleton system in eukaryotic cells with microfilaments and microtubules as important components of the cytoskeleton. However, the cytoskeleton of cancer cells is underdeveloped with a reduced number of microfilaments and microtubules.¹¹⁵ Tubulin is the target of many anti-cancer drugs, and the cytoskeleton damage induced by AMPs can occur in both cancer cells and normal cells. Normal cells can self-heal due to their complete cytoskeleton system after being treated by AMPs, while tumor cells cytoskeleton is incomplete and cannot be repaired in time after the action of AMPs, resulting in cell death. Studies have shown that the AMP, cecropins B3, can disintegrate microtubules, disrupt the normal function of microtubules, and affect the integrity of the tumor cytoskeleton, thereby killing tumor cells.¹¹⁶

Inhibition of Tumor Angiogenesis

Certain AMPs have been found to inhibit tumor cell growth by inhibiting tumor angiogenesis. For example, the AMP called K6L9 can kill tumor cells through anti-angiogenesis and metastasis inhibition.¹¹⁷ Another example is that the

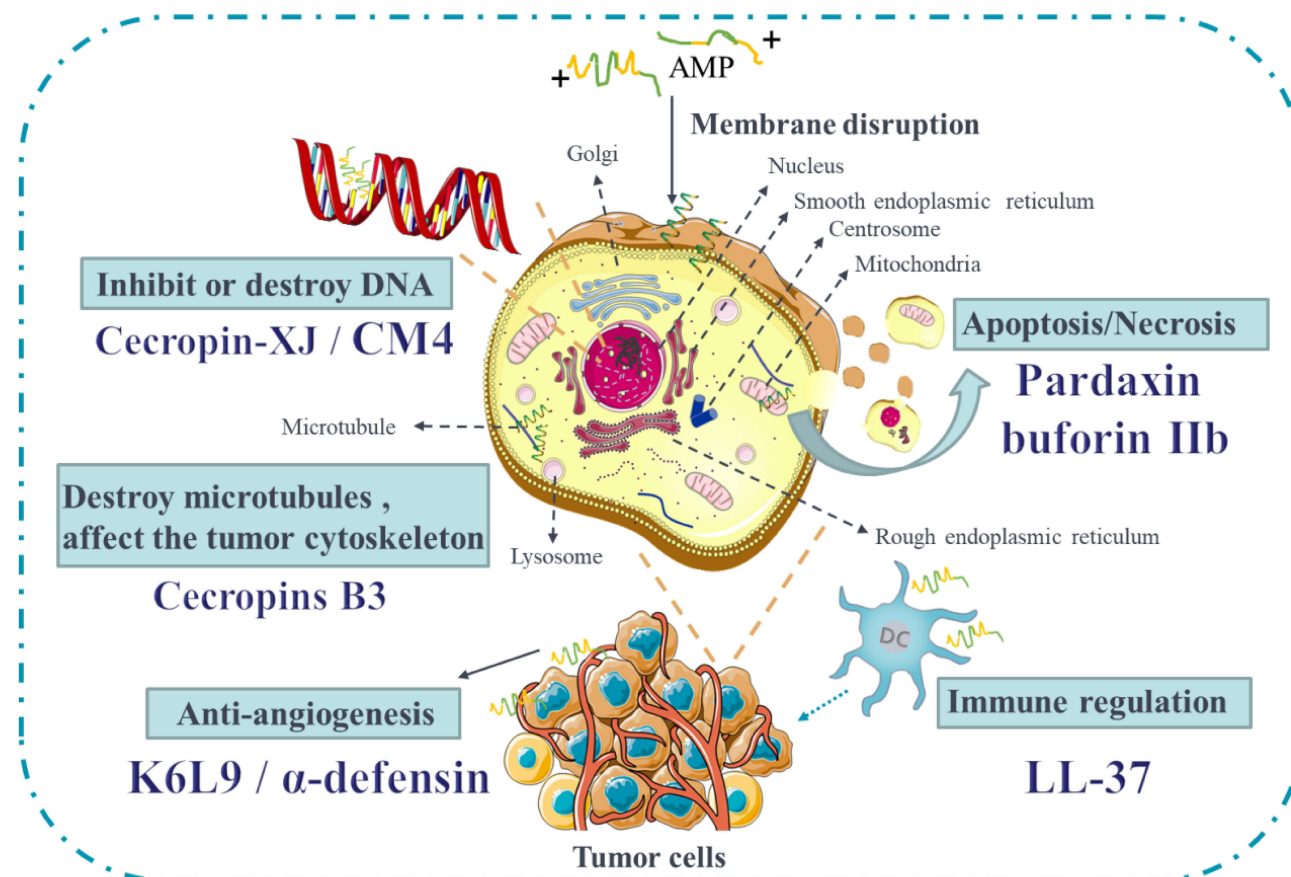


Figure 3 Schematic diagram of the intracellular mechanisms of AMPs on cancer cells. Although interacting with the negatively charged cancer cell membranes is the key to the anti-cancer effects of AMPs, many AMPs can also act on the intracellular structures or molecules, including inhibiting tumor DNA synthesis (Cecropins B3); destroying Cytoskeleton (Cecropin-XJ and CM4); inhibiting tumor angiogenesis (K6L9 and α -defensin); destroying tumor mitochondria and inducing tumor cell apoptosis or necrosis (Pardaxin and buforin IIb); regulating the body immunity (LL-37).

AMPs, human α -defensins, can specifically bind to fibronectin (FN) to block the adhesion and transfer of integrin $\alpha 5 \beta 1$ mediating endothelial cells to fibronectin, and block the proliferation and apoptosis of endothelial cells mediated by vascular endothelial growth factor (VEGF), that is, human α -defensins inhibit tumor angiogenesis in many ways, thereby exerting its anti-cancer effect.¹¹⁸

Attack the Mitochondria Leading to Cancer Cell Apoptosis or Necrosis

Many AMPs can also induce apoptosis or necrosis of tumor cells in a variety of ways. It was found that pardaxin, a marine antimicrobial peptide, can act on the mitochondrial membrane, reduce the potential of mitochondrial membrane, release cytochrome c (cyt c) in the cytoplasm, and release a large amount of reactive oxygen species (ROS). Finally, the pardaxin activates the apoptosis proteins caspases 3 and 7, and induces apoptosis in the mitochondrial pathway, resulting in cell death.⁹⁷ In addition, buforin II B, an AMP derived from histone H2A, can pass through the tumor cell membrane without damage, act on mitochondria, release cytochrome c, and activate the apoptosis protein caspase-9, and induce tumor cell apoptosis in the mitochondrial pathway.¹¹⁹

Regulation of Immunity

Antimicrobial peptides are a part of the body's immune system, so many antimicrobial peptides can play their anti-tumor effects through immunomodulation. Some AMPs can chemoattract monocytes, dendritic cells (DC), T cells and neutrophils, etc, promoting the uptake and presentation of tumor cells by antigen-presenting cells and enhancing the immunogenicity of tumors.^{120,121} As an example, the AMP LL-37 can inhibit tumor growth by improving the phagocytic capacity of DC cells, increasing the expression and strengthening the function of phagocytic receptors, enhancing the

expression of co-stimulatory molecules, promoting the immune response of Th1 cells, and increasing the secretion of various cytokines.¹²² Another example is Cecropin XJ. Cecropin XJ can induce hepatocellular carcinoma cell apoptosis by activating caspase-3 and poly(ADP-ribose) polymerase, downregulating the expression of B-cell lymphoma 2 (Bcl-2), and upregulating the expression of Bcl-2-associated death promoter and Bcl-2-associated X protein.¹²³

Potential Clinical Application of AMPs as Antitumor Agents

Although the research on AMPs mainly focuses on their antimicrobial properties, more and more studies have shown that many AMPs possess outstanding antitumor activities *in vitro* and *in vivo*, especially in breast cancer, cervical cancer, lung cancer, etc. AMPs are natural drugs with exciting potential as a new kind of anticancer drugs, and AMPs with excellent antitumor effects are often called anticancer peptides (ACP).¹²⁴ Traditional chemotherapeutics have shortcomings such as lack of tumor cell selectivity, which can easily cause serious side effects, and they also easily induce tumor cell resistance. In contrast, AMPs have significant advantages in anti-tumor as they offer a wide range of antimicrobial and anticancer spectrum, high selectivity to cancer cells, harmlessness to normal cells and important organs, and low propensity to develop drug resistance. Furthermore, AMPs also have the ability to quickly kill tumor cells and destroy and prevent primary tumor metastasis. As AMPs can act directly on tumor cell membranes, they also have significant anti-proliferation effects on drug-resistant tumors.¹²⁵ Therefore, AMPs have great application prospects in cancer treatment with the following potential anti-tumor strategies:^{126,127} (1) AMPs used alone as anticancer agents; (2) AMPs combined with other drugs; (3) AMPs combined with nanotechnology.

Anti-Cancer Application of AMPs Alone

Antimicrobial Peptides Used Alone to Kill Common Tumors

AMPs with anticancer activities (anticancer peptides, ACP) are mainly α -helix and β -sheet peptides. The ACPs mainly include the following four antimicrobial peptide families:⁶⁷ Defensins, Lactoferrins, Cecropins, and Magainins. Also, there are many types of ACPs,⁸⁴ including Melitins, Cathelicidins, Dermaseptins, Temporins, Tachyplesin, etc.

ACPs usually have broad-spectrum and excellent *in vitro* antitumor activities, and many of them also have anti-tumor activity *in vivo*. For example, magainin 2 was found to have selective cytotoxicity to tumor cells, which can kill a variety of tumor cells at a peptide concentration of 5–10 times lower than the peptide concentration required to kill normal cells.¹²⁸ *In vivo* studies have shown that administration of magainin 2 and its derivatives (MSI-136 and MSI-238) could reduce the invasion of tumor cells into soft tissue and increase the survival rate of leukemia cells in P388 mice.¹²⁹ Another example is Cecropin A and B. They can lyse a variety of tumor cells at a concentration that is non-toxic to normal eukaryotic cells, and Cecropin B possesses an antitumor effect on mice bearing ascites colon adenocarcinoma cells.^{130,131} Table 3 summarizes the antitumor activity of AMPs from different AMP families and their antitumor mechanism.

Antimicrobial Peptides Used Alone to Kill Drug-Resistant Tumors

The chemotherapy resistance of cancer cells is one of the main reasons that limits the efficacy of chemotherapy drugs, and it seriously affects the prognosis of advanced cancer patients and the prognosis of patients with advanced cancer. Traditional anti-tumor drugs need to enter the cells to take effect, and if the uptake of drugs in the tumor tissue decreases or the drug efflux increases, the emergence of drug resistance in the tumor will occur. In contrast, many AMPs are less active in cells but act directly on tumor cell membranes, making them more effective in drug-resistant tumor cells. The most researched mechanism for the generation of MDR of tumor cells is the overexpression of the cell membrane transporter P-gp. P-gp participates in the formation of tumor cell membranes and can increase the net negative charge of the cell surface, so it is helpful for AMPs that possess positive charges to combine with the drug-resistant tumor cell membrane under electrostatic attraction.¹⁷ As a result, it is difficult for drug-resistant tumor cells to resist the action of AMPs. In recent years, a large number of studies have shown that many AMPs can kill a variety of drug-resistant tumors, and certain AMPs are more effective on drug-resistant tumor cells than normal tumor cells,¹⁶² which means that AMPs are of great value for the treatment of advanced and refractory cancers.

Table 3 Targeted Tumors and Relevant Antitumor Mechanism of Different AMPs

Peptide Name	Secondary Structure/Sequence	Targeted Tumors	Mechanisms	Refs
Defensins HNP-1	β-sheet ACYCRIPACIAGERRYGTCTIYQGRLWAFCC	Blood system tumors and solid tumors, such as colon cancer, pancreatic cancer	Membrane disruption - Cell cycle arrest - Cell apoptosis induction - Inhibition of cell invasion and migration	[132–135]
hBD-1	DHYNCVSSGGQCLYSACPIFTKIQGTCTYRGKAKCCK	A variety of hematologic tumors and solid tumors, such as melanoma, breast cancer	Membrane disruption - Antitumor angiogenesis - Cell apoptosis induction	[136–138]
Lactoferricin LfcinB	β-sheet FKCRRWQWRMKKLGAPSITCVRRAF	A variety of mouse and human tumor cell lines, including breast cancer, colorectal cancer, neuroblastoma, and melanoma	Selective destruction of cytoplasmic and mitochondrial membranes in cancer cells - Antitumor angiogenesis - Inhibition of tumor cell proliferation and metastasis -Cell apoptosis induction	[139–141]
Cecropins Cecropin A Cecropin B	α-helix KWKLFKKIEKVGQNIRDGIIKAGPAVAVVGQATQIAK KWKVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKAL	A variety of human cancer cell lines, including leukemia and lymphoma, bladder cancer, liver cancer, etc	Membrane disruption - Cell cycle arrest - Cell apoptosis induction - Production of reactive oxygen species - Immunity regulation	[142,143]
Magainin Magainin 2	α-helix GIGKFLHSAKKFGKAFVGEIMNS	A variety of blood system tumors and solid tumors, including lung cancer, breast cancer, glioma, etc	Membrane disruption - Cell apoptosis induction	[144,145]
Magainin G	AIGLPLHSALLPALAPVAGIMAS	Various human tumors, such as cervical cancer, lung cancer, etc	Membrane disruption - Cell apoptosis induction	[146,147]
Melittins Melittin	α-helix GIGAVLKVLTTGLPALISWIKRKRQQ	Leukemia cells, melanoma cells, etc	Membrane disruption	[148,149]
Cathelicidins BMAP-28	α-helix GGLRSLGRKILRAWKKYGPIIPIRI	A variety of human tumor cells, including leukemia cells and lymphoma	Membrane disruption - Cell apoptosis induction	[150,151]
LL-37	LLGDFFRKSKEKIGKEFRIVQRIKDFLRNLVPRTES	Various human tumors, such as colon cancer, lung cancer, breast cancer, etc	Membrane disruption - Cell apoptosis and autophagy induction - Immunity regulation	[122,152–154]
Dermaseptins Dermaseptin-B2	α-helix GLWSKIKEVGKEAAKAAAKAAGKAALGAVSEAV	A variety of human cancers, including prostate cancer, breast cancer, etc	Membrane disruption - Inhibition of tumor cell proliferation	[155]

(Continued)

Table 3 (Continued).

Peptide Name	Secondary Structure/Sequence	Targeted Tumors	Mechanisms	Refs
Dermaseptin-PP	ALWKDMLKGIGKLAGKAALGAVKTLV	Various human cancers, such as breast cancer, lung cancer, prostate cancer, glioma cells	Membrane disruption - Inhibition of tumor cell proliferation - Cell apoptosis induction	[156]
Temporins Temporin-ICEa	α-helix FVDLKKIANIINSIFGK-NH ₂	A variety of human cancers, including melanoma, breast cancer, etc	Membrane disruption - Inhibition of tumor cell proliferation / invasion and/or metastasis - Antitumor angiogenesis - Production of reactive oxygen species	[157–159]
Tachyplesins Tachyplesin I	β-sheet KWCFRVCYRGICYRRCR	A variety of cancer cells, including liver cancer, melanoma, and prostate cancer	Interaction with hyaluronan overexpressed in tumor cells and activation of complement component (C1q) - Inhibition of tumor cell proliferation - Antitumor angiogenesis - Induction of tumor cell differentiation - Increase the expression of the tumor suppressor gene	[84,86,160,161]

Gaegurins, a family of AMPs containing 6 AMPs, were isolated from *Glandirana emeljanovi*, which can be divided into two sub-families, Gaegurin I and Gaegurin II, according to their length and sequence. Studies have found that the derivatives of the antimicrobial peptide GN6 of the Gaegurin II sub-family can induce tumor cell cycle arrest and apoptosis in multidrug-resistant cancer cells, showing the potential to treat refractory malignant tumors.¹⁶³ Besides, Temporin-1CEa, a natural AMP, was isolated and purified from the skin secretions of *Rana chensinensis*. Temporin-1CEa could selectively inhibit the cell proliferation of Adriamycin-resistant breast cancer MCF-7/Adr cells because the negative charges on the membrane surface of the drug-resistant breast cancer cell line MCF-7/Adr are higher than that of the ordinary MCF-7 tumor cells. At the same time, Temporin-1CEa can increase the sensitivity of MCF-7/Adr cells to the chemotherapy drug adriamycin. After 24 hours of combined administration with Temporin-1CEa, the IC₅₀ of adriamycin decreased by 4.52 times.¹⁶⁴ It was proved that Temporin-1CEa could co-localize with P-gP on the surface of drug-resistant cell membranes, so the anticancer mechanism of Temporin-1CEa may be acting on P-gP and affecting its efflux function, thereby reversing multidrug resistance in tumor cells.^{157,164}

Besides, LL37, an AMP of the human-derived cathelicidin AMP family, and its derivative peptides (LL17-32 and LL13-37) have been found to increase the accumulation of mitoxantrone in ABCG2 overexpression-resistant tumor cells but does not affect normal tumor cells without ABCG2 overexpression. Similar to P-gp, ABCG2 is a drug-resistant semi-transporter on the tumor cell membrane, which also acts as a “drug pump” and is closely related to the accumulation of drugs in cancer cells. Both LL17-32 and LL13-37 could directly interact with ABCG2 in a non-competitive manner and inhibit its transport activity. Also, LL17-32 and LL13-37 could indirectly down-regulate ABCG2 protein expression in drug-resistant cells through the lysosomal degradation pathway, thus reversing multidrug resistance of tumor cells and making the drug-resistant cancer cells sensitive to chemotherapies.¹⁶⁵

In another example, Wang et al¹⁰⁰ gave the cationic AMP Polybia-MPI to the multi-drug resistant leukemia cell line K562/ADM that was 201 times resistant to doxorubicin and 14.7 times resistant to actinomycin. Polybia-MPI significantly inhibited the proliferation of K562/ADM cells and showed a killing effect that is similar to its effect on ordinary cells, indicating that the drug-resistant cells are sensitive to AMPs. Moreover, Banković et al¹⁷ found that NK-lysin-derived cationic peptide NK-2 could selectively inhibit the proliferation of P-gp overexpression multidrug-resistant cells, including multidrug-resistant non-small cell lung cancer cells (NCI-H460/R) and multidrug-resistant Colorectal cancer cells (DLD1-TxR). At the same time, NK-2 could also increase the sensitivity of drug-resistant cells to adriamycin by reducing the expression of P-gp on the cell surface. Additionally, NRC-03 and NRC-07, two AMPs from the pleurocidins AMP family, exhibited equal killing effect at a concentration of 50μM on normal MCF7 cells and on paclitaxel-resistant breast cancer cell line MCF7-TX400 that possess 2.6 times of P-gp expression more than the normal MCF7 cells, achieving about 60% of cell killing rate.¹⁶²

In addition, many antimicrobial peptides have also been found to inhibit drug-resistant tumors and even reverse the drug resistance of tumor cells. For example, AMPs extracted from *Musca domestica* larva can selectively inhibit the proliferation of multi-drug resistant human hepatocellular carcinoma cell line Bel-7402/ADM. The IC₅₀ value of these AMPs against Bel-7402/ADM cells was even lower than that of AMPs against normal Bel-7402 cells. Also, these AMPs could reverse the multi-drug resistance of tumor cells by inhibiting the relative expression of multidrug resistance gene 1 (MDR1).¹⁶⁶ Another AMP named BMAP-28 was reported to inhibit the proliferation of multidrug-resistant CEM-VLB lymphocytic leukemia cells.⁸⁴ Besides, the AMP Cecropin B could also have in vitro cytotoxic activity against multi-drug resistant human breast and ovarian cancer cell lines.¹³¹

The above-mentioned studies provide experimental support for AMPs to replace chemotherapeutic drugs for tumor treatment or to help solve the problem of tumor cell chemotherapy resistance, suggesting that AMPs hold great application potential in the treatment of drug-resistant tumors.

Anti-Cancer Application of AMPs Combined with Other Drugs

Traditional anti-tumor drugs need to enter cells to function effectively, but their drug utilization is low with rapid metabolism in the body and short half-life. Therefore, high-frequency administration is needed, usually leading to serious side effects. In order to achieve clinical tumor treatment, combined medication can be considered. The AMP-mediated destruction of tumor cell membranes can help chemotherapeutics better enter tumor cells, so the combined use of

chemotherapeutics and AMPs may help reduce the effective dose of chemotherapeutics, thereby reducing dose-related side effects, and achieving the effect of increased efficiency and reduced toxicity. Thus, the combined use of AMPs and antitumor drugs has potential clinical application prospects.

AMPs Combined with Chemotherapy Drugs for Cancer Treatment

In 1992, Ohsaki et al¹⁴⁶ discovered for the first time that the antimicrobial peptide Magainin analogs combined with chemotherapy drugs such as adriamycin and cisplatin showed an additive inhibitory effect on tumor cells. Since then, more and more studies have shown that AMPs can be used in combination with anti-tumor drugs to fight tumors. For example, cecropins can significantly enhance the killing effect of S-fluorouracil and Cytarabine on acute lymphoblastic leukemia cells.¹⁴⁷ Cecropin A combined with a variety of chemotherapeutics, such as 5-fluorouracil, cytarabine, etc, showed synergistic anti-tumor activity in CCRF-SB lymphoid leukemia cells and squamous skin cancer cells.^{167,168}

In addition, Xia's group¹⁶⁹ administered a Xinjiang silkworm AMP named cecropin-XJ in combination with the chemotherapy drugs such as adriamycin (ADM), cisplatin (DDP), cyclophosphamide (CTX), and carboplatin (CBP) on esophageal cancer Eca109 cells, and results showed that the cell proliferation inhibition rate and the apoptosis rate of the combined drug group were significantly higher than those of the same concentration chemotherapeutic drug group. For another example, magainin 2, an antimicrobial peptide from *Xenopus* skin, has been proven to penetrate the cancer cell membrane and act as an ion channel, which leads to the dissolution of cancer cells, thus significantly enhancing the cytotoxicity of chemotherapy drugs in tumor cells.¹⁷⁰ Magainin 2 derivatives, magainin A (MAG A) and magainin G (MAG G), have also been found to exert synergistic antitumor activity after combined use with chemotherapeutics such as cisplatin (DDP), doxorubicin (DOX), and etoposide (VP-16).¹⁴⁶

Another example is the scorpion venom peptide APBMV extracted from *Buthus martensii*, which has also been found to show a synergistic anti-tumor effect with a variety of chemotherapeutic drugs. After the combined use of a low concentration of APBMV (20 mg/L, cell inhibition rate is only 15.5%) and chemotherapeutic drugs such as mitomycin (MMC), DDP, fluorouracil (5-FU), and VP-16, the sensitivity of colorectal cancer LoVo cells to chemotherapeutic drugs increased and the cell inhibition rate even increased to about 2 times that of those drugs used alone.^{171,172} Additionally, studies on NRC-03 and NRC-07 (two AMPs from the pleurocidin AMP family) showed that the 50% effective concentration (EC₅₀) of cisplatin on tumor cells could be significantly reduced when used in combination with NRC-03 or NRC-07, that is, the cytotoxicity of cisplatin was increased.¹⁶²

The above-mentioned studies have proven the feasibility of anti-tumor use in combination with antimicrobial peptides and chemotherapeutics and indicated that antimicrobial peptides have potential application prospects for enhancing efficacy and reducing the toxicity of chemotherapeutics.

AMPs Combined with Chemotherapy Drugs Against Drug-Resistant Tumor Cells

Importantly, the combination of AMPs and chemotherapeutic drugs has obvious advantages in the treatment of drug-resistant tumor cells. Overexpression of drug efflux protein in drug-resistant tumor cells sharply decreases the concentration of drug uptake by tumor cells, which greatly limits the effect of chemotherapeutic drugs. Unlike traditional chemotherapy drugs, the action of AMPs is not affected by the overexpression of tumor multidrug resistance genes due to their special membrane disruption mechanism. Even, certain AMPs can destroy the function of drug efflux proteins such as P-GP, allowing multidrug-resistant tumor cells to regain chemical drug sensitivity.^{84,173}

Studies have shown that the combination of chemotherapeutic drugs (such as ADM and DDP) and the antimicrobial peptide θ -defensin can improve the sensitivity of multidrug-resistant breast cancer MDA-MB-231 cells to the chemotherapeutic drugs and effectively enhance the cytotoxic effect of such chemotherapeutic drugs.¹³⁵ Besides, Johnstone et al¹⁷⁴ found that the combined use of the chemotherapeutic drug ADM and multiple mammalian and plant-derived AMPs significantly increased the sensitivity of drug-resistant cells to ADM, thus improving the anti-tumor activity of ADM. The mechanism may relate to the membrane destruction effect of the AMPs. Another research found that NRC-03 and NRC-07, the two AMPs from the pleurocidin AMP family, can also reverse the sensitivity of multidrug-resistant breast cancer MDA-MB-231 cells to some chemotherapeutic drugs. The experimental process was to pre-treat MDA-MB-231 cells for 20 min with a low concentration (10 μ M) of NRC-03 or NRC-07 20 minutes and then added a gradient

concentration of cisplatin. After 72 h or 96 hours of incubation, the EC_{50} of cisplatin in the NRC-03 pretreatment group decreased by 5.5 times at both 72 h and 96 h, while it decreased by 1.6 times after 72 h and 1.7 times after 96 h in NRC-07 pretreatment group, respectively. Meanwhile, NRC-03 could also enhance the cytotoxicity of docetaxel (DTX) on MDA-MB-231 cells.¹⁶²

The above studies indicate that AMPs can also be used as chemotherapeutic drug sensitizers, and the combination of AMPs and chemotherapeutic drugs has broad application prospects in the treatment of multidrug-resistant tumors.

AMPs-Based Cancer Gene Therapy

In addition to targeting common and drug-resistant tumors, AMPs in combination with other drugs also have many applications in gene therapy. It has been proven that good therapeutic effects can be obtained by transferring or intratumorally injecting AMPs or their precursor genes into tumor cells. For example, David Winder et al¹⁷⁵ introduced an expression vector containing cecropin A gene into EJ human bladder cancer cells, which can significantly inhibit or slow down the growth of tumors in immunodeficient mice. Besides, cancer gene therapy based on melittin has also been reported. After adenovirus-mediated transfer of melittin gene to BEL-7402 human liver cancer cells under the control of α -fetoprotein promoter could significantly inhibit the proliferation of liver cancer cells in vivo and in vitro.¹⁷⁶ For another example, Xu et al¹⁷⁷ co-transfected L1210 mouse leukemia model with murine β -defensin 2 (β -defensin 2) gene and IL-18 gene. The co-transfection group produced a stronger anti-tumor immune response than a single transfection of β -defensin 2 gene or IL-18 gene that improved the survival rate of mice. The survival rate of mice in the co-transfection group was as high as 80%, which was higher than that of the two single transfection groups, and it showed long-term protective immunity to the surviving mice. Therefore, gene therapy of AMPs alone or in combination with other drugs may be used for the treatment of human cancer.

Modified AMPs for Cancer Treatment

In addition to the combination with chemotherapeutics, AMPs could also be modified by tumor-targeting peptides or cell-penetrating peptides to enhance the tumor-targeting and anti-tumor activities of the AMPs. Tumor-targeting peptides such as NGR, RGD, and CREKA can specifically bind to specific ligands overexpressed on the surface of tumor cell membranes, so they have high tumor cell selectivity and can be used as tumor-targeting vectors.^{178–180} As an example, Tachyplesin, an AMP derived from the horseshoe crab, was modified with RGD peptide, which significantly increased the enrichment of Tachyplesin in tumor blood vessels and tumor tissues. Results proved that the anti-tumor effects of RGD-Tachyplesin both in vivo and in vitro were better than those of Tachyplesin used alone. This method could enhance the effectiveness and meanwhile reduce the toxicity of AMPs.¹⁶¹ In another research, Ma et al¹⁸¹ coupled an AMP, D(KLAKLAK)₂, with a tumor-targeting peptide, TMTP1, and found that the new fusion peptide effectively inhibited the growth and metastasis of prostate cancer and gastric cancer transplanted in nude mice, which was expected to become a new effective anti-tumor agent. For another example, when the AMP, MP, and the cell-penetrating peptide, Antp, were used alone, they only showed weak cytotoxicity with no obvious tumor cell killing effect. However, their fusion peptide, MPGA, exerted strong tumor cell killing activity by destroying the cell membranes.¹⁸²

Anti-Cancer Application of AMPs Related Nanoparticles

Although AMPs have good prospects for tumor treatment due to their broad-spectrum anti-cancer activity and low possibilities to induce drug resistance in tumor cells, some limitations in their practical applications have emerged with the increasing research. The main problem is that AMPs are easily hydrolyzed by proteases and they have certain hemolytic toxicity in vivo. Secondly, the quantity of natural AMPs is limited, but the production cost is high. Therefore, how to rationally design and optimize AMPs to maintain their tumor cell selectivity as well as their stable anti-cancer activities is of great significance.^{183,184} The development of nanotechnology has inspired researchers. In the past few decades, nanotechnology has been widely used, especially in the medical field. At present, nanoparticles in medical research are generally between 1–500nm in diameter, which has unique physical, chemical, and biological properties, such as size effect, long circulation in vivo, controlled release of drugs, and prevention of drug degradation.^{185,186} Functionalized nanoparticles have many possibilities in overcoming the shortcomings of AMPs, which provides a huge

prospect for better application of AMPs. Antimicrobial peptide nano-preparations used in anti-cancer research mainly include two aspects: (1) AMPs are used as drugs and nanomaterials are used as a drug delivery system. AMPs can be loaded onto nanocarriers for targeted delivery of the antimicrobial peptides to tumor sites. The purpose is to reduce the in vivo hemolytic toxicity and immunogenicity of exogenous AMPs, while retaining their anti-cancer activity, aiming to reduce the peptide dose required for treatment while ensuring safety;^{27,187} (2) Antimicrobial peptide modified nano-formulations are used as drug carriers. Many AMPs have similar properties to cell-penetrating peptides, for example, they are usually positively charged and the peptide sequence contains both multiple basic amino acids and hydrophobic amino acids, which help them to effectively interact with tumor cell membranes. Thus, AMPs can be used as cell-penetrating peptides to modify the surface of the nano-preparations so as to transfer anti-cancer chemicals to the tumor sites and finally achieve an enhanced anti-cancer effect.^{188–190}

AMPs Worked as Effective Component

At present, there are many reports on antimicrobial peptide nano preparations. The AMP, called CA, was isolated from *Plutella xylostella* and was found to have good anti-liver tumor activity. Liu et al¹⁹¹ prepared CA-loaded nanoparticles based on poly(lactic-co-glycolic acid) (CA-PLGA-NPs), showing good activity against liver cancer HepG2 cells. As a carrier of the peptide CA, PLGA can not only improve the stability of CA in vitro but also prolong the action time of CA in the body. As a result, these nanoparticles showed good sustained-release effect, no obvious sudden-release phenomenon, and long platform release period, which could effectively reduce the dosage of peptide administrations, thereby supplementing the pharmaceuticals basis for the delivery of the peptide CA. Another study incorporated Melittin, a typical AMP, into monolayer perfluorocarbon nanoparticles, which was verified to enhance the retention time of Melittin in vivo, prevent the peptide from being hydrolyzed by serum proteases, and significantly reduce the hemolytic toxicity of Melittin in vivo. More importantly, this nano-preparation retained the therapeutic effect of Melittin on melanoma models and pre-cancerous skin damage.¹⁹² In addition, Gamal's group loaded the black snake (*Walterinnesia aegyptia*)-derived AMP onto silica nanoparticles. This preparation could significantly enhance the immune activity of normal lymphocytes,¹⁹³ and it could induce cell cycle arrest or apoptosis in human prostate cancer cells¹⁹⁴ and in a mouse model bearing human multiple myeloma.¹⁹⁵ For another example, lycosin-I is an AMP isolated from the venom of *Lycosa singorensis*, which can interact with cell membranes and then kill tumor cells by destroying tumor cell membranes. Combining the tumor cell membrane selectivity of lycosin-I with the advantages of nano-preparation, Tan et al¹⁹⁶ modified 60 nm gold particles with lycosin-I, which can significantly improve the transfer efficiency of the gold nanoparticles in tumor cells. At the same time, the lycosin-I-gold nanoparticles had low sensitivity to non-tumor cells and could selectively accumulate at tumor sites through blood circulation in tumor-bearing mice model, but were non-toxic to normal mice. Therefore, this nano-drug delivery system can be used as a new targeted drug delivery system in tumor treatment. With the help of the photothermal effect of gold nanorods, Tan's research group also constructed lycosin-I modified gold nanorods. The modified gold nanorods maintained tumor selectivity of lycosin-I, and could generate heat under near-infrared (NIR) light irradiation to kill tumor cells. The results revealed the potential value of gold nanorods in tumor diagnosis and tumor photothermal treatment.

Liposome drug delivery system has been extensively studied among many different nano-preparations. At present, there are also some reports on the research of liposomes based on AMPs. For example, Temporin-1CEa, an AMP extracted from the skin secretions of *Rana chensinensis*, was found to have good in vitro antitumor activity.¹⁵⁸ To solve the poor stability of Temporin-1CEa in vivo, prevent it from being hydrolyzed, and increase its tumor cell uptake rate, Wu et al¹⁹⁷ constructed polyethylene glycol (PEG)-modified liposomes containing Temporin-1CEa (Temporin-1 CEa-liposomes) by using the reverse-phase evaporation method. PEG-modified Temporin-1 CEa liposomes showed better serum stability and higher cell uptake rate on the basis of retaining the anti-cancer effect of Temporin-1 CEa. In addition, L-K6, an AMP modified based on the AMP Temporin-1 CEb from *Rana chensinensis*, was proved to have good anti-cancer activity in vitro and was non-toxic to normal human cells. Besides, L-K6 was less likely to cause cancer cell resistance compared with other anticancer drugs.¹⁹⁸ Chen et al¹⁹⁹ used L-K6 as a drug to prepare L-K6 liposomes in order to maintain the activity of L-K6 while improving its biological stability. The L-K6 liposome drug delivery system better retained the selective anti-cancer activity of L-K6, and exhibited a similar inhibitory effect on the proliferation of

human breast cancer MCF-7 cells as L-K6 while showing no obvious toxicity to human immortalized epidermal HaCaT cells. To further improve the biological stability of L-K6 liposomes, the author also prepared PEG-modified L-K6 liposomes. The results showed that the particle size of PEG-modified L-K6 liposomes was reduced, the liposome appearance was more thorough, and the serum stability was improved, which was more conducive to the accumulation of the drug to the tumor site and play a more potent anti-cancer effect. In addition, Huang et al²⁰⁰ designed a hybrid cytolytic peptide, α -melittin, by linking the N-terminus of melittin to the C-terminus of an amphipathic α -helical peptide via a GSG linker. α -Melittin could interact with phospholipids and self-assemble into lipid nanoparticles, which significantly enhanced the anti-cancer activity of melittin. At the same time, this α -melittin-based lipid nanoparticle efficiently shielded the positive charge of melittin within the phospholipid monolayer, resulting in the reduced cytotoxicity and a widened safe dosage range, at the same time. In addition, Yamada et al²⁰¹ prepared transferrin modified liposomes combined with a pH-sensitive fusion peptide and encapsulated the wasp-derived peptide mastoparan. The liposomes could target mastoparan to the mitochondrial site of the human chronic myelogenous leukemia K562 cells, destroying the mitochondrial membrane and inducing the release of cytochrome c, thereby causing cell death.

The above studies indicate that the nano-drug delivery system is a good delivery system for peptide anti-cancer drugs, which can improve the tumor targeting of peptide anti-cancer drugs, effectively improve the stability of peptide anti-cancer drugs, and reduce their cytotoxicity, which is of great help to promote the clinical application of peptide drugs.

AMPs Modified as Functional Ligands

In addition to being used as anticancer drugs in nanoparticles, AMPs can also be modified on the surface of the drug-loaded nano-preparations as functional ligands. Currently, a large number of studies have been conducted to improve the cellular uptake of nanoparticles by covalently combining cationic or amphiphilic cell-penetrating peptides to the surface of nanoparticles. Tat is one of the most studied and applied cell-penetrating peptides. Tseng et al²⁰² modified the surface of Adriamycin-loaded liposomes with Tat, which increased the uptake of Adriamycin in cancer cells by nearly 12 times, thus significantly enhancing the antitumor activity of Adriamycin. Similarly, Sharma's group²⁰³ used the penetratin, a *Drosophila*-derived cell-penetrating peptide, to modify the Adriamycin-loaded liposomes, thus significantly improving the transfer efficiency of the liposomes in brain tumor cells. Similar to cell-penetrating peptides, most AMPs are also cationic or amphipathic, therefore, some researchers believe that cell-penetrating peptides and AMPs are both "membrane-active peptides". The cell penetration effect of certain AMPs has also been widely reported, and many peptides have been used both as antimicrobial peptides and cell-penetrating peptides, such as insect-derived peptide Pyrrhocoricin, bovine-derived peptide Lactoferricin, *Drosophila*-derived peptide penetratin and so on.^{190,204} Therefore, it is feasible to use AMPs as the ligands to modify the surface of drug-loaded nano preparations in order to improve the cell penetration ability, tumor-targeting ability, and the tumor cell uptake rate the nanoparticles, thus promoting drug action. In summary, the AMP-based nano preparations are also a potential delivery model of anti-cancer drugs.

Challenges and Ways Forward

Although there are many basic studies on AMPs, the potential anti-cancer mechanisms of some AMPs are still difficult to clarify. Also, there are very few in vivo and clinical experiments of AMPs, and there is a lack of effective correlation between the in vitro sensitivity tests and the animal models.¹²⁴ Therefore, strengthening the research on the pharmacokinetics and pharmacodynamics of AMPs, and establishing the analysis of the correlation between in vitro stability and bioavailability in animal models may solve this problem.

Besides, the most serious barrier to the clinical use of anti-cancer AMPs remains the enzymatic degradation and inactivation of these cytotoxic peptides through interactions with negatively charged serum proteins. The hemolytic toxicity and easy hydrolysis of AMPs are also prominent problems that restrict the clinical application of AMPs. Thus, how to maintain the stable anti-cancer activity of AMPs and at the same time reduce its hemolytic toxicity as well as rational design and optimization of AMP drugs is of great significance. To solve the above problems, two ideas can be considered. One is to optimize the structure of AMPs as the structure-activity relationship is also crucial to improve the anti-cancer effects of AMPs. The methods of peptides design and modification to improve the stability, half-life time, and

specificity of AMPs include amino acid substitution, cyclization, hybridization, fragmentation, modification of C- and N-terminal of a peptide by polymer or targeting molecules,^{205,206} etc. Amino acid substitution is the most basic modification method. Many structure–activity studies on small linear AMPs indicated that the net positive charges, hydrophobicity, amphiphilicity, and helicity were the most important factors that affect their activities.²⁰⁷ As we know, most AMPs are membrane activity peptides, and that the enhanced positive charges of AMPs usually lead to higher activity, likely because the enhanced positive charges of AMPs result in increased affinity to the negatively charged cancer cell membranes.²⁰⁸ However, higher positive charges do not always lead to better activity, and a high antimicrobial activity could be achieved within an optimal hydrophobicity window.²⁰⁹ Excessive hydrophobicity may lead to strong hemolytic toxicity, which may be caused by the self-binding of the peptides that may impede the peptides pass through prokaryotic cell membranes without hindering their passage through eukaryotic cell membranes.²¹⁰ In summary, it is better to keep a balance between the overall positive charges, the hydrophobicity, and other factors when modifying the structures of AMPs.

Another strategy is to design a new AMP drug delivery system so as to protect AMPs from being enzymatically degraded and reduce their hemolytic toxicity. Due to the special physicochemical properties of AMPs, the development of a specific delivery system to protect AMPs can increase the therapeutic index of AMPs while also improving their feasibility of intestinal absorption and oral administration. Combining AMPs with modern nanotechnology is one of the options for developing new antimicrobial peptide delivery systems, which is of great significance to promote the clinical application of new anti-cancer drugs of AMPs. Nano-drug delivery systems can well maintain the anti-cancer activity of AMPs while avoiding their enzymatic hydrolysis and reducing their hemolytic toxicity. Alternatively, a specific ligand can be used to achieve direct tumor targeting of antimicrobial peptide nanoparticles, and the targeted delivery of AMPs to the tumor sites can reduce the amount of peptide needed to achieve a therapeutic effect, thus avoiding severe side effects. Furthermore, in order to achieve the rapid release of drugs, smart stimuli-responsive nano-drug carriers have received extensive attention for anti-cancer drug delivery. Smart nano-drug carriers mainly include tumor microenvironment-respond nano-drug carriers and external stimulus-respond nano-drug carriers. Smart Stimuli-Responsive nanocarriers can be stably transported in the body, and after reaching the tumor site, by sensing the chemical environment of the tumor site or receiving external stimuli, the loaded AMPs can be released at the necessary time and a specific site. As a result, the peptide concentration in the tumor site can be increased, the anti-cancer efficiency of the AMPs can be enhanced, and the toxic side effects on normal tissues can be reduced.

Future Prospective

Due to the severe side effects of chemotherapy drugs and the emergence of tumor cell resistance, the clinical efficacy of chemotherapy drugs is greatly reduced. Better than chemotherapy drugs, most AMPs can specifically inhibit the growth of most tumor cells, but are harmless to normal cells. Many AMPs can also promote the proliferation of white blood cells and fight tumors through the regulation of the body's immunity.²¹¹ Due to the unique structural properties and unique anti-cancer mechanisms of AMPs, as well as its advantages in cell selectivity and resistance to drug-resistant tumors, AMPs bring new hope for the development of anti-cancer drugs. There are different perspectives of antimicrobial peptides in anti-cancer application. On the one hand, AMPs with obvious killing effect on tumors can be directly used as anti-cancer drugs. On the other hand, some AMPs can be used as adjuvant drugs for tumor treatment. Therefore, in the past few years, such AMPs have been extensively studied as auxiliary drugs for anti-cancer drugs.^{177,212} Additionally, some AMPs are too cytotoxic and may have a killing effect on normal cells, but when such AMPs at low-dose are combined with chemotherapy drugs, the cytotoxicity of those AMPs can be restrained and at the same time, the anti-cancer effect of chemotherapy drugs can be significantly enhanced.^{169,171}

Besides, there are many other difficulties that need to be overcome in the process of AMPs, from basic research to clinical applications. First of all, the resources of natural AMPs are limited, and the chemical synthesis of AMPs needs high cost.²¹³ Although genetic engineering is currently a relatively economical and practical method for large-scale production of AMPs, there are still many limitations.²¹⁴ For example, the antibacterial and antiviral capabilities of AMPs make it difficult to use the commonly used bacteria and viruses as expression systems. Besides, with small molecular weight, the separation and purification of AMPs are difficult, and the gene expression yield is still insufficient. Therefore,

how to reduce the production cost of AMPs is one of the problems that must be solved in the practical application of AMPs.

With the in-depth research on AMPs, the optimization of AMPs structure and the design of new nano-delivery systems for AMPs will be easier to achieve, and the application of AMPs in the clinical treatment of tumors is just around the corner.

Acknowledgments

The authors acknowledge the funding of the National Natural Science Foundation of China [No.82173989] and the support from the National Administration of Traditional Chinese Medicine [No.zyyzdxk-2023272].

Disclosure

The authors report no conflicts of interest in this work.

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