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# Cell-Penetrating Versus Antimicrobial Peptides: Comparison of Potential Use as Cancer Therapeutics

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## Abstract

The use of peptides in cancer therapies has gained recent attention in the biomedical community due to their short half-lives and selective targeting advantages. At the forefront of potential peptide candidates, the cell-penetrating peptides (CPP) and, antimicrobial peptides (AMP) await implementation and inclusion into the armamentarium of future cancer therapeutics. Although similar in some properties, the use of each of these peptide types for cancer therapies differ due to their intrinsic amino acid composition, cell membrane targeting capabilities, secondary structure manifestation, mode of cell membrane encounter and permeabilization, cytoplasmic destination, and functional capabilities. While CPPS are involved with cell pore penetration and cargo (drugs, chemicals, etc) delivery, AMPs are characterized by cell membrane disruption/destabilization of bilayer membranes, channel and/or pore formation, and immune response enhancement. The present treatise first compares and contrasts the properties, characteristics, traits, and function of each peptide type and then discusses the advantages and disadvantages of their possible adaptation for clinical use. Finally, the clinical fate of the peptides are discussed as well as the methods required to evaluate their suitability for use in future cancer therapy.

**Keywords: Cell-Penetrating; Antimicrobial; Cancer; Peptides; Therapeutics; Targeting; Immunity; Cell uptake**

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## Introduction

The use of peptides as therapeutic and/or targeting ligands has recently boomed in the fields of biotechnical and pre-clinical cancer research, but have lagged behind in clinical usage. During the last three decades, cell-penetrating (CPPs) and antimicrobial peptides (AMPs) have been attracting attention for increased biomedical applications, especially in cancer. Although, similar in certain properties, the CCPs and the AMPs mostly differ in purpose, utility, amino acid composition, and biological activities. The use of each peptide for cancer therapeutics differ primarily in that CPPs are largely involved with inducing membrane pores, causing cytolysis, and delivering cargoes (drugs, chemicals) into cells; while AMPs mostly destabilize and disrupt cell biomembranes, partition into the membrane bilayers, form transmembrane channels and/or pores, and enhance host immunity [2,3,4]. In comparison, the prime targeting objectives of CCPs are the plasma membrane (for cargo delivery) and intracytoplasmic organelle membranes that can disrupt signal pathways; while AMPs target, associate, and permealize into the cell membrane and partition into its lipid/protein bilayers which can affect various cellular activities [5,6]. Regarding amino acid (AA) composition, the CCPs display an abundance of positively-charged AAs (Arginine, lysine, histidine) together with some hydrophobic AAs, while AMPs are largely amphipathic with a notable content of hydrophobic AAs, but fewer positively-charged AAs [6,7]. Despite 30 years of research on CPPs and AMPs, their precise mechanism of cell membrane entry remains controversial. Thus, the objectives of the present commentary are to compare and contrast the prominent characteristics, traits, and properties of each peptide type and briefly summarize their potential use in clinical cancer therapeutic strategies.

## Cell-penetrating Peptides (CPPs)

Drug delivery in cancer patients are often hampered by cell/tissue and skin layers, mucus membranes, blood/brain barriers, biomembrane integrity, reduced drug bio-availability, and poor uptake of certain drugs into difficult-to-access areas. The CPPs appear to mimic the cell penetration methods of TAT peptides observed in virus transmissions that form pores in bilipid membranes [8] (Table 1). Their cargoes can either be conjugated to peptides by covalent bonds or can be complexed to peptides by natural binding affinities [9]. The mechanism of cell internalization of APPs is still

**Table 1:** The Cell-penetrating and Antimicrobial Peptides are Listed and Compared According to Their Biochemical and Biophysical Characteristics, Traits, and Properties.

| Characteristics, Traits, Properties | Cell-penetrating Peptides (CPP) | Antimicrobial Peptides (AMP) | Reference Citations |
|---|---|---|---|
| 1) Cell membrane penetration effects | Forms transient transmembrane pores, penetrates cell membrane | Forms transmembrane pores and/or channels, stabilizes the cell membrane potential | [2,3,9,16] |
| 2) Cell method of internalization | a) Energy-dependent clathrin/caveolae endocytosis<br>b) energy-independent electrostatic interaction | Transmembrane channel passage, channel receptor endocytosis | [9,15,20] |
| 3) Cell-specific targeting | Bacterial cell wall and virus coats, plasma membranes of vertebrates, Guanidinium group interaction | Microbial cell wall/membrane, plasma membranes of vertebrate (mammals), transformed cancer cells. | [4,8,10] |
| 4) Cell cargo delivery vehicles | Transports and carries conjugated and/or bound drugs, chemicals, and chemotherapeutic drugs | Mostly lacks cargo delivery capability, binds metals, dimerizes with peptides and proteins | [9,12,13] |
| 5) Cell toxicity | Cytotoxic, Cytolytic | Cytostatic, Cytolytic | [4,7,14,15] |
| 6) Number of AAs in length | Excess of polycationic AA, some polar/non-polar and hydrophobic AAs | Largely amphipathic, contains some positive and negative charged AAs and hydrophobic AAs | [10,17,18] |
| 7) Amino acid (AA) composition | 6-10 AAs | 12-50 AA | [6,7,16] |
| 8) Peptide secondary structure | Disordered in free solution, mostly lacks secondary structure | Displays, some alpha-helix, Beta sheets, and Beta Hairpin loops | [6,7,16,17] |
| 9) Effect on Host Immunity | No effects on immune response, immunity of the Host | Promotes and enhances the innate immune response of host organism, initiates Chemokine Immunomodulation | [15,16,24] |
| 10) Examples of peptides in nature and/or synthesized | a) Transactivating transcriptional activator (TAT) from HIV-1,<br>b) Heparin sulfate interacting polyarginines,<br>c) Penetration dimer,<br>d) cell penetrating Carrier peptide Pep-1<br>e) Drosophila, antennapedia Homeo-protein. | a) Amphibian-H5,<br>b) human dermcidin<br>c) human defensins,<br>d) Cecropins from insects<br>e) Magainin and bombesin from amphibians<br>f) Indolicidin from (Cows)<br>g) prophenin from pigs<br>h) horseshoe crabs | [3,4,21,28] |

controversial but is thought to occur by an energy dependent cell ingestion via clathrin/caveolae endocytosis and energy independent electrostatic interactions with negatively charged phospholipids, or by transient pore formation by interaction with charged amino acid side chains in the bilayers [10]. By such means, CPPs can smuggle cargoes into the cell's interior; such cargoes could include nucleic acids, proteins, other peptides, oligonucleotides, small molecules, and imaging contrast agents. The CPPs tend to be short cationic AA containing peptides consisting of 6-10 AAs in length.

Certain CPPs, such as Matrikines, can modulate intracellular activities such as cell growth, proliferation and differentiation, migration, adhesion, and apoptosis [11]. Other CCPs can produce knock- down of signaling pathways, can target to specific organelles, induce cytotoxicity and subsequent cell death, assume Trojan horse entry into cells, and cross difficult cell/tissue barriers [12,13]. Such obstacles could encompass the blood/brain barrier, intestinal mucosa, nasal mucosa, eye conjunctiva, skin barriers, and nerve cell membranes. CCPs can be found naturally-occurring or be synthesized in the laboratory. Two types of CCPs are known to exist; A) the linear flexible type and B) the fixed and rigid type. Both types consist largely of positively-charged, and some polar and hydrophobic AAs. The linear type is flexible, while the rigid type can be cyclic, stapled, dimeric, multivalent, and self-assembled [14]. Addition of Acetylation to the CPP chain of AAs can improve cell internalization properties.

## The Antimicrobial Peptides (AMPs)

The AMPs are host defense agents which are natural components of the innate immune response system found in a multitude of organisms. The AMPs are naturally-occurring, broad spectrum antibiotic agents that kill gram positive and gram-negative bacteria, fungi, enveloped viruses, and transformed cancer cells [15] (Table 1). Many different types of AMPs have been found in insects, sea life, fish, amphibians, and mammals including man (defensins). AMPs are known to create pores and/or transmembrane channels within cell bilayer membranes for translocation to the cell interior; such channels are often associated with cell surface receptors, especially chemokines. The AMPs function to destabilize and disrupt bilayer membranes, form pores and/or transmembrane channels, affect intracellular activities, and enhance immunity by a process of immunomodulation [16]. The AMP molecules are generally 12-50 AAs in length, contain two or more arginine, lysine, or histidines, and display several hydrophobic and polar AAs; such peptides are largely zwitterionic amphipathic in nature. Their secondary structure components can be comprised of α-helices, β-hairloops, βeta-stranded sheets, and may contain one or more disulfide linkages [17]. Some AMPs display a mostly disordered structure in free solution but can refold into tertiary structure following cell entry.

The prime target of the AMP is the cell (plasma) membrane in which they destabilize and/or disrupt the bilayer and permealize to form a lytic pore or transmembrane channel [18]. AMPs are attracted and attach to the cell membrane by means of their helical content, AA composition, and amphipathic nature . Such peptides are attracted to a net negative surface membrane charge as displayed by bacteria and transformed cancer cells (Note: normal mammalian cells display a net positive membrane charge). Thus, the AMP cell-targeting specificity depends on the electrostatic target cell surface net negative charge displayed by microbial and cancer cells versus the net positive surface charge on non-cancer normal mammalian cells [19]. The negative charge on transformed cells result from transformed cells (targeted for apoptosis ) undergoing a phospholipid bilayer flip in which negative-charged phospholipids emerge to the outer bilayer of the cell membrane; such bilayer-altered cells then display a net negative cell surface membrane charge [20]. Following cell entry, AMPs can affect biological activities such as protein folding, DNA and protein synthesis, cytoplasmic organelle membrane integrity, and angiogenesis [21]. The immuno-modulatory properties of AMPs include induction of chemokine production, mimicry of chemokine activity, modulation of antigen presenting dendritic cells, and influencing blood clearance of pathogens, microbes, and foreign bodies [22]. Furthermore, plasma membrane disturbance and disruption followed by transmembrane channel/pore formation can initiate slight membrane leakages eventually leading to eventual slow

death of the cell; this is referred to as cytostatic demise. Regarding localization, AMPs have been detected in prokaryotes, sea organisms, fish, and all vertebrates including man.

## Potential Clinical Applications of CPP and AMPs

Preclinical studies in animals and in cell culture have demonstrated that CPPs are lytic and cytotoxic to cells, while AMPs tend to be lytic but often cytostatic (Table 1). In view of previous pre-clinical studies, it would seem logical to apply the use of CPP and AMP present day technologies toward clinical cancer therapy. However, at present, use of both peptides for cancer treatment have not yet achieved routine clinical status. In contrast, the AMPs have experienced vast clinical success as therapeutic agents for infections such as pneumonia, hepatitis-C, and multiple bacterial, viral, and fungal infestations [23,24]. In comparison, CPPs have been utilized more for transport into cells of nucleic acids, siRNAs, antisense oligomers, plasmids, and contrast agents, mainly in cell culture and pre-clinical animal models [13]. Reasons for the lack of clinical utility are many, some of which are presently addressed below. For example, the CPPs have only low to moderate specificity and sensitivity for cell targeting even though they are of small size/dimensions, show ease of modification, and demonstrate ease of bio-availability. Certain areas of CPP research have yet to be more fully developed such as: 1) efficient transport of low bio-available drugs; 2) ample disruption of disease impacted G-coupled protein receptor signaling, and 3) improved delivery of poor uptake drugs. Although pre-clinical results for CPPs have prompted some clinical trials, overall implementation to the clinic have yet to be advanced. Basic issues of CPP usage have to be overcome such as; 1) more understanding of the internalization mechanisms; 2) calculation of translocation efficiency and transport kinetics; 3) clarification of metabolic degradation pathways of CPPs; 4) determination of toxicity limits; 5) elucidation of off-target side effects; and 6) further exploration of controlled delivery strategies. Disadvantages of CPP usage include the need of high molar excess ratios of CPP-to-drug cargoes, low cell/tissue homing specificity, and improved methods of implementation for fusion of CPP-to- proteins.

In comparison, the entry of AMPs into the cell interior is a result of cell membrane partition and disruption with some subsequent cell leakage, activation of the autolysin enzyme, interference with DNA, RNA, and protein synthesis, and altered enzyme activity [25]. As an advantage, AMPs target only bacteria and transformed cancer cells which display a net negative charge on their surface. Hence, improved cell target selectivity could be achieved by modulating net surface charge, helicity, hydrophobic AA content, selective determination of hydrophobic moment, reverse peptide synthesis, and use of both L-and D-amino acids in the peptide chain synthesis. The consequences of using AMPs longterm is the possible biocidal resistance as a result of AMP degradation, initiation of increased proteoglycan coating on cancer cells surfaces, reduced cell membrane fluidity, activation of ABC transporter receptors which pump AMPs to the cell exterior, and unwanted alteration of cell transmembrane protein constituents [26].

## Concluding Statements

Future improved developments of CPPS and AMPs might include procedures such as: 1) insertion of organelle signal localization AA sequences during peptide structure synthesis, and 2) enhancement of cell targeting specificity using new and novel membrane electrostatic alterations [27]. The use of additional transporter partner peptides with differing AA sequences and uptake kinetics might aid in achieving optimal low micromolar intracellular concentrations of peptides required to influence various cytoplasmic interactions. On a comparative functional basis, use of CCPs and AMPs could readily compete with rival vector transport methodologies such as electroporation, magnetofection, lipofection, viral vectors, dendrimers, and nanoparticles [24]. Greater understanding of the functional potential roles for CPP and AMP will require implementation of techniques employing solid state NMR for membrane disruption, X-ray crystallography (to identify key membrane phospholipids), neutron X-ray diffraction, fluorescent dye tracking, circular dichroism, and polarization interferometry for study of ion channel formation [28]. In summation, CPPs and AMPs have yet to achieve “prime time” usage in the rapidly advancing field of cancer therapeutics.

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