A Brief Review on Cell Penetrating Peptides for Cancer Treatment

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

School of Pharmacy

Brac University

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing my degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate reference.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The project titled "A Brief Review on Cell Penetrating Peptides for Cancer Treatment" submitted by Nazifa Mahiwyat (19346017) of Summer, 2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons) on September 2024.

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Ethics Statement

This project does not involve any clinical trial or human participants and no animals were used or harmed.

Abstract

Efficient transportation of imaging reagents and medications to tumors is vital for the detection and treatment of cancer. Peptides have several advantages, including biocompatibility, simplicity of synthesis, reduced size, ability to avoid off-target negative effects, and the ability to achieve favorable effects with lower doses. Cell penetrating peptides (CPP) possess the unique ability to traverse cell membranes and facilitate the transport of therapeutic drugs across the plasma membrane. Although there has been notable advancement in the design and use of CPP, further research is necessary to better their targeted delivery to tumors, while minimizing adverse effects and improving treatment effectiveness. CPPs have also been utilized in many pre-clinical and clinical trials for the treatment of cancer disease. Developing next-generation CPPs with higher cell penetration, stability, and selectivity can boost the drug delivery approach by creative design or chemical alterations. Recent CPP-based targeted cell penetration advancements provide emerging optimization strategies.

Keywords: Cell penetrating peptides; cancer; targeted delivery to tumors; clinical trials.

Dedication

Dedicated to my father whose appreciation and kindness encourage me even in his absence and to my Thesis Supervisor Dr. Sabrina Sharmin Ma'am whose constant support made me here today.

Acknowledgement

In the beginning, I want to express my sincere thanks to Allah for giving me the strength to keep going through this time with mercy and kindness. I'm incredibly grateful to Dr. Sabrina Sharmin, Assistant Professor in the School of Pharmacy at Brac University, for always showing me the way and helping me with my project. Her help and kindness have been very helpful throughout this whole process.

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List of Acronyms

CPP	Cell Penetrating Peptides
TAT	Transactivator of Transcription
DNA	Deoxyribonucleic acid
RNA	Ribonucleic Acids
siRNA	Small interfering RNA
PNA	Peptide Nucleic Acid
ODNs	Oligodeoxynucleotides
CTTs	CPP-conjugated treatments
DDS	Drug Delivery System
PEG	Polyethylene glycol
PEI	Polyethylenimine
EPR	Effect of Permeability and Retention

Chapter 1: Introduction

1.1 Cancer

Cancer, sometimes referred to as "a chronic disease," is characterized by the unregulated proliferation of inappropriate cells and excessive activation of the immune system. According to the World Health Organization, cancer was responsible for about 9.6 million deaths in 2018. Cancer is typically attributed to the accumulation of genetic, epigenetic, and transcriptional alterations, which confer significant characteristics upon cancer cells. These characteristics include the capacity for continuous growth, invasion, angiogenesis, metastasis, and apoptosis. These traits are widely recognized as distinctive features of cancer cells. During the development of a cancer cell, the genome of mal cells undergoes mutations in tumor suppressor genes, proto-oncogenes, and other genes that play a role in regulating cell growth. The occurrence of these mutations is crucial for the progression of cancer. Ongoing research is being conducted on molecular markers, aiming to ascertain the way the presence or absence of these markers contributes to the advancement of cancer. P53 is an instance of a tumor suppressor protein. The p53 tumor suppressor protein, a pivotal regulator of the cell cycle in both normal human cells and human cancer cells, undergoes significant alterations in nearly all instances of the latter. This phenomenon is observed in both normal human cells and human cancer cells. Under normal cellular circumstances, p53 acts as the regulator that inhibits cell development in response to physiological stress or damage. Various therapies have been created to eliminate cancer cells, including chemotherapy, surgery, radiation therapy, and the recently developed immunotherapy. Although these therapies have extended the survival of cancer patients, a significant number of individuals relapse and are unable to attain long-term survival. Hence, it is vital to possess a thorough understanding of cancer, including its inception, progression to metastasis, and subsequent recurrence.

1.2 Overview of Cell Penetrating Peptides

CPPs typically consist of 5 to 30 amino acids and possess side chains composed of basic amino acids. Additionally, CPPs frequently exhibit amphipathic properties. Basically, CPPs refer to a diverse range of short peptides that possess the ability to traverse cell membranes without causing any alterations to their structure or functionality. The initial CPPs were created using natural proteins such as TAT from HIV-TAT and piercing from the Antennapedia homeodomain . Subsequently, several novel CPPs have been synthesized, including both naturally occurring protein-derived CPPs and artificially designed ones. Extensive research has been conducted on their capacity to transport bioactive chemicals, medicines, and compounds that can eliminate tumors into cells. These features reduce the permeability of therapeutically relevant macromolecules through biological membranes, making them very desirable in this context. (Gori et al., 2023)

Numerous research studies and reports have demonstrated the effectiveness of CPPs in facilitating the intracellular delivery of various bioactive molecules, such as oligonucleotides, siRNA, anticancer drugs, bioimaging agents, DNA, nanoparticles, proteins, and other peptides. Over the past 10 years, the identification of many CPPs has demonstrated the potential of peptides for drug delivery into cells. Several CPP-conjugated treatments (CTTs) have been used to improve the extracellular and intracellular internalization of different small molecules and biomolecules, including plasmid DNA, siRNA, oligonucleotides, and peptide nucleic acid (PNA). These therapies show great potential for therapeutic success. The primary obstacle to the clinical development of CPPs continues to be their lack of cell selectivity. Comparably, inadequate antitumor efficacy and insufficient selectivity towards tumor cells are the main problems with traditional cancer treatment. (Kalafatovic & Giralt, 2017)

Chemotherapeutic medications can be coupled to targeted moieties to enhance these properties. With CPPs coupled with targeting ligands, there are several ways to target cancer cells specifically, including activatable cell-penetrating peptides, transfusible compounds, and cell targeting peptides. Activatable CPPs are made to be inactive as cell-penetrating peptides until they are activated by cancer-specific proteases. Conventional CPP sequences are often combined with tumor-targeting peptides to create cell-targeting peptides. (Gori et al., 2023)

1.3 Association of CPP with Cancer

Cell-penetrating peptides (CPPs) are transmembrane peptides, generally ranging in length from 5 to 30 amino acids, which possess the capability to traverse the outer layer of cells. They serve as vehicles for the transportation of diverse carrier molecules, including pharmaceuticals, proteins, nucleic acids, and nanoparticles, into cellular environments. The use of cell-penetrating peptides in cancer diagnostics and treatment has the potential to greatly enhance their specificity, effectiveness, and safety. Comparably, inadequate antitumor efficacy and insufficient selectivity towards tumor cells are the main problems with traditional cancer treatment. Chemotherapeutic medications can be coupled to targeted moieties to enhance these properties. With CPPs coupled with targeting ligands, there are several ways to target cancer cells specifically, including activatable cell-penetrating peptides, transfusible compounds, and cell targeting peptides. Activatable CPPs are made to be inactive as cell-penetrating peptides until they are activated by cancer-specific proteases. Conventional CPP sequences are often combined with tumor-targeting peptides to create cell-targeting peptides. The prospective uses of CPPs in targeted drug administration, imaging, and treatment are receiving major interest in the field of cancer study.

1.4: Aim of the Project

CPP has lately gained recognition as a promising therapeutic option for cancer. Cancer, which stands as the predominant factor behind mortality worldwide. In the year 2020 alone, cancer claimed the lives of over 10 million individuals, accounting for almost one-sixth of all deaths. Various therapy modalities are accessible for the management of cancer patients; nevertheless, the outcomes are suboptimal. Researchers are always endeavoring to develop novel pharmaceuticals or therapeutic approaches for various forms of cancer. Cell penetrating peptides are a kind of peptide that have the ability target tumor cells without affecting the healthy cells. The objective of the research is to create techniques necessary for discovering novel targets and analyzing clinical trials to enhance the utilization of CPP for cancer treatment.

1.5: Objectives of the Study

- To give an idea of how CPP is used to treat cancer.

- To know about application, clinical trials and effectives approaches of CPP.

- To get more information about treating cancer with CPP.

Chapter 2:

Methodology

To write this review paper, it is necessary to do a thorough and organized analysis of the existing research on the subject. This review study clearly defines its purpose and scope by providing a description of the classifications, modes of action, clinical research, and possible anticancer effects of CPP. In order to compose this review article, the first step is doing a search for relevant keywords in reliable sources to find suitable publications. Next, a thorough literature search is conducted using reliable sources, academic publications, and other scholarly databases. PubMed, ScienceDirect and SpringerLink are utilized to collect all the required information for the composition of this review paper. To precisely record the advancements in clinical research, reputable sources such as Lancet, peer-reviewed research articles, MDPI open-access publications, and Elsevier are appropriately utilized and cited. These genuine sources confirm the authenticity and accuracy of the information.

Chapter 3: Structure and Functions of CPP

3.1: Structure of CPP

Cell-penetrating peptides (CPPs) have a heterogeneous nature, without a distinct or consistent structure. Instead, they comprise a wide range of peptides with varied amino acid sequences and structural characteristics. Nevertheless, several CPPs have shared traits and themes.

CPPs are generally short peptides, typically composed of 5 to 30 amino acids. Due to their concise size, they may effortlessly infiltrate cell membranes.

Cationic residues: Numerous cell-penetrating peptides (CPPs) are rich in cationic amino acids, such as arginine (Arg) and lysine (Lys), which carry a positive charge. It is believed that these residues with a positive charge interact with negatively charged parts of cell membranes, including phospholipid head groups or glycosaminoglycans, which helps in penetrating the membrane.

Amphipathicity; It refers to the characteristic of CPPs to possess both hydrophobic and hydrophilic areas in their structure. The presence of both hydrophobic and hydrophilic regions in these molecules may enhance their capacity to bind to and move across lipid bilayers.

Flexibility: Certain CPPs include sections within their structure that are flexible or disordered. This characteristic enables them to assume various conformations when they interact with membranes or other components of cells.

Secondary Structures: Although several cell-penetrating peptides (CPPs) lack a defined structure in solution, certain CPPs can acquire secondary structures including α-helices, β-sheets, or twists when they connect to membranes or cargo molecules.

Internalization refers to the process of incorporating or internalizing external information, beliefs, or values into one's own thoughts, attitudes, or behaviors. CPPs can enter cells by many pathways, such as direct translocation across the lipid bilayer (membrane transduction), endocytosis followed by escape from endosomes, or a combination of both methods.

The structural variety of CPPs plays a crucial role in their ability to efficiently transport cargo molecules inside cells and distribute them intracellularly. Scientists are actively studying and designing CPPs with customized characteristics to improve their effectiveness, selectivity, and safety for different medicinal uses.

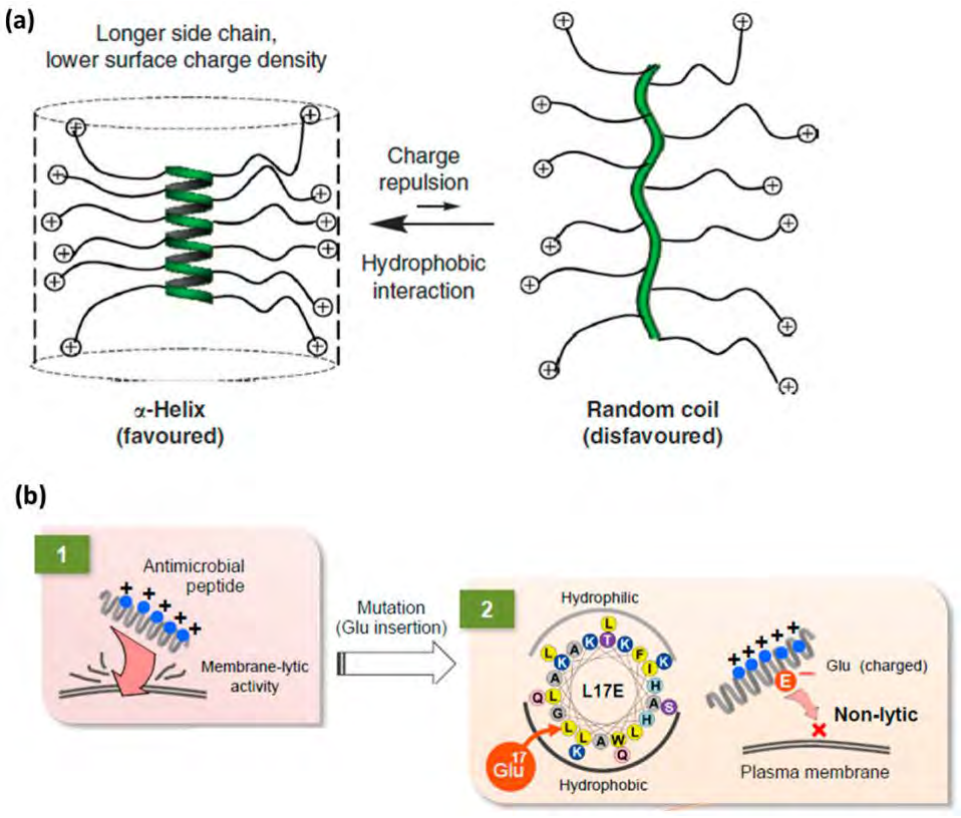

Figure 1: Structure of CPP (Kalafatovic & Giralt, 2017)

3.2 Functions of CPP

Their main purpose is to enhance the transportation of diverse substances (such as medicines, nucleic acids, peptides, or proteins) into cells. The following are the main purposes of cell-penetrating peptides:

Drug Transport: Cell-penetrating peptides can be utilized for the transportation of therapeutic compounds, including small molecule medicines and therapeutic proteins, into cells. This includes transportation to both the cytoplasm and the nucleus.

Gene Delivery: Cell-penetrating peptides can be chemically linked or combined with nucleic acids (such as DNA, RNA, or siRNA) to enhance their transportation into cells, allowing for the use of gene therapy or RNA interference.

Biomedical Screening: CPPs can be linked to imaging agents (such as fluorescent dyes or contrast agents) to aid in their transportation into cells for the purpose of imaging, enabling researchers to observe cellular activities.

Vaccine Delivery: Cell-penetrating peptides can be utilized to transport antigens or vaccine adjuvants into cells, hence augmenting the immune response and potentially boosting the effectiveness of vaccines.

Academic instruments: Cell-penetrating peptides are highly important instruments for investigating cellular processes and for advancing experimental procedures in the fields of cell biology, pharmacology, and molecular biology.

Targeted therapy involves the conjugation of cell-penetrating peptides with specific targeting ligands, such as antibodies or receptor ligands. This enables the precise delivery of therapeutic molecules to cell types or regions.

Cell Penetration: Certain Cell-Penetrating Peptides possess the capability to infiltrate many tissues, including the blood-brain barrier. This property can be advantageous for the targeted delivery of medications or therapeutic agents to the brain.

In summary, cell-penetrating peptides provide a flexible and encouraging method for delivering different types of molecules into cells, with potential uses in medication administration, gene therapy, imaging, and research. Nevertheless, the development and deployment of CPP-based technologies still face significant hurdles, including cytotoxicity, immunogenicity, and specificity of delivery. (Zhou et al., 2022)

Chapter 4: Mechanisms of CPP

4.1 Uptake Mechanisms

Much research has focused on the uptake mechanisms that transfer CPPs over the plasma membrane, the specific routes via which CPPs access cells remain unclear. Microscopy or flow cytometry were commonly used for early research on fixed cells. Although CPPs have many shared characteristics, their mechanisms of uptake differ significantly. In contrast, data show that differing experimental circumstances can change the cellular uptake and translocation mechanism of CPPs. Using varied concentrations, cell types, incubation period, and CPP physiochemical factors (e.g., hydrophobicity and net charge) might provide inconsistent findings. Therefore, it is possible that CPPs without cargoes can be absorbed by the cells through numerous paths, including direct penetration of the plasma membrane and endocytic uptake mediated by Cathrin, caveolae, and/or other molecules, depending on the nature of the peptide/cell interaction. (Copolovici et al., 2014a)

4.2 Endocytosis

Endocytosis, which encompasses phagocytosis and pinocytosis, is a meticulously controlled mechanism by which solutes and fluids are taken in by cells from the extracellular matrix. Phagocytosis is a multifaceted mechanism employed for the internalization of sizable particles, including but not limited to cells, viruses, or bacteria. Conversely, pinocytosis primarily serves the purpose of encompassing smaller particles. Endocytosis is a process that involves several routes, which may be categorized as caveolae and/or lipid-raft-mediated endocytosis, macropinocytosis, cholesterol-dependent clathrin-mediated endocytosis, or caveolae- and clathrin-independent endocytosis. The primary distinctions between energy-

independent pathways and endocytosis are mostly centered around the processes of membrane permeation and release. (Copolovici et al., 2014a)

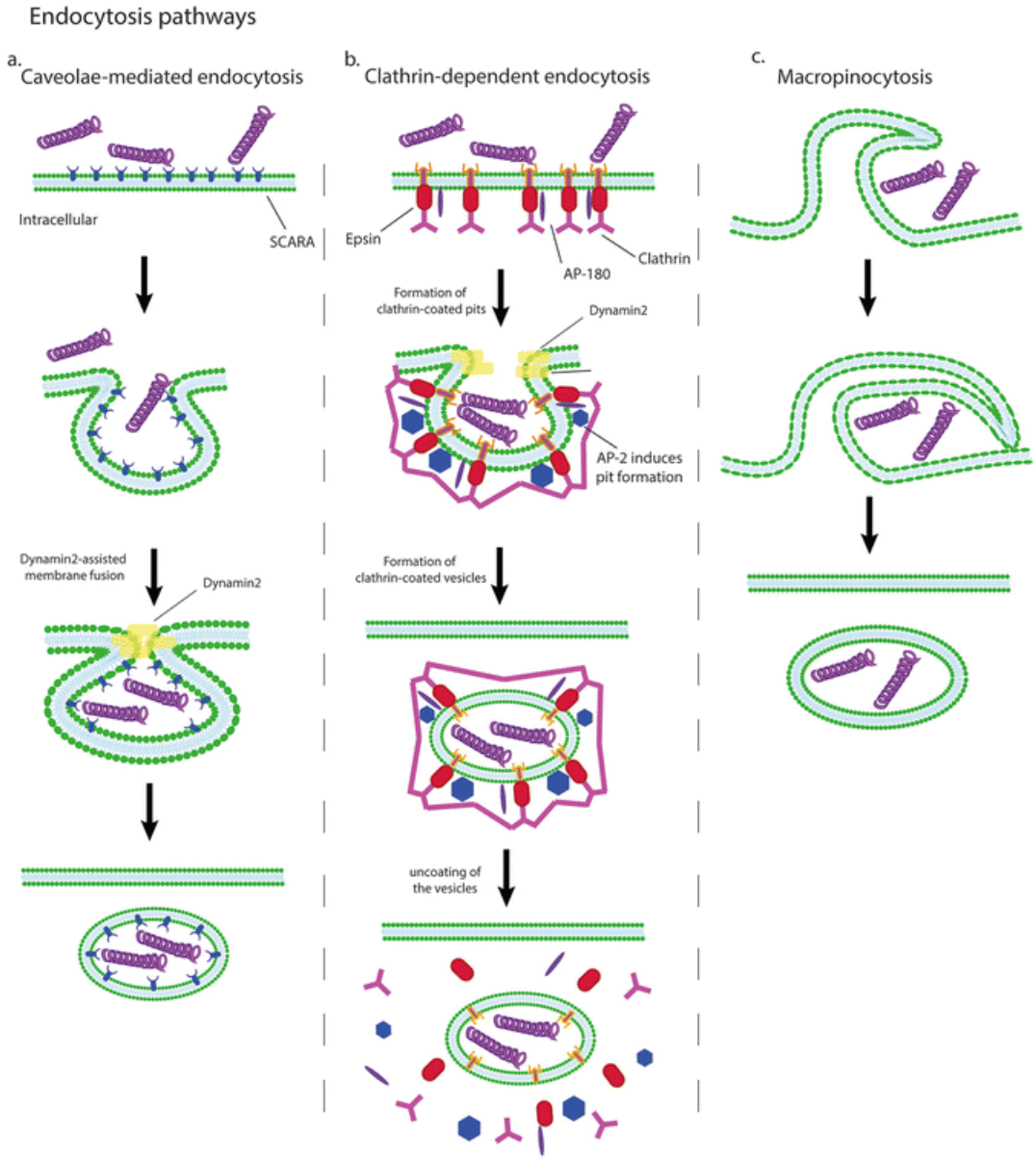

Figure 2: Endocytosis Pathways (Copolovici et al., 2014b)

Chapter 5: Applications in Cancer Treatment

5.1: Application of CPPs as Delivery Vehicles for Cancer Therapy

To treat a variety of disorders, including neurological disease, asthma, ischemia, diabetes, and cancer, CPP-based medication delivery has been investigated as a potential treatment option.

As of right now, there are over 300 research studies that have been published that are associated with CPP-based drug delivery. Cancer has been the most important application of all the investigations that have been done. It has been demonstrated that the CPP, in its capacity as an effective carrier, is capable of efficiently delivering cytotoxic medications (such as tiny anticancer agents, poisons, tumor suppressor proteins, siRNA, and so on) into tumor cells to trigger apoptosis. Although there have been reports of success in in vitro research, there have only been a few studies that have demonstrated the effectiveness of therapy in animal models. (Shin et al., 2014)

5.2: Delivery of small molecule drugs via CPP

Due to their small size and lipophilicity, tiny molecule antitumor medicines permeate into tumor cells efficiently, yet recurrent exposure to them often causes resistance to multiple drugs (MDR). Studies have examined pairing these medications with CPPs to solve this problem. Dubikovskaya et al. coupled octaarginine (R8) to taxol, an anticancer medication, via a disulfide link, which selectively released taxol inside cells.70 Both R8-taxol and taxol alone were evaluated toward cancers of the ovary models using OVCA-429 (taxol-sensitive) or OVCA-429T (taxol-resistant) cells implanted in mice's peritoneal cavities R8-taxol and taxol targeted taxol-sensitive cancers similarly, according to their findings. In a taxol-resistant tumor model, R8-taxol-treated mice outperformed taxol alone in survival. In addition to MDR

prevention, this study indicated further benefits of pairing small molecule medicines with CPP. The drug's enhanced water solubility allowed for its usage without surfactants (e.g., cremophor EL). Since CPP-drug adheres to surrounding tissues, the investigators found a significant concentration of the drug at the injection site. While this characteristic may hinder systemic delivery of the CPP-drug, it can enhance therapeutic effectiveness in intra-tumor injections. Lee et al. chemically conjugated doxorubicin and TAT to polymeric chitosan backbones to increase cytotoxicity and targeted administration of the anticancer medication. Compared to free doxorubicin or chitosan/doxorubicin without TAT, the hybrid showed enhanced cell uptake and stronger anti-tumor actions. The chitosan/doxorubicin/TAT hybrid's increased cell internalization may modify the biodistribution patterns of doxorubicin, increasing tumor localization and inhibiting tumor development in CT26 xenograft-bearing mice.71 Myrberg et al. created a chimeric peptide by combining a breast tumor-homing peptide (‘PEGA peptide’; 9-mer cyclic peptide: cCPGPEGAGC) with a CPP (pVEC) and chemically conjugating it to chlorambucil, an anticancer medication.72 In vitro, PEGA-pVEC-chlorambucil combination had better cellular absorption and cytotoxicity against breast cancer cells than chlorambucil alone. Further in vivo investigations revealed fluorescent dye-labeled PEGA-pVEC targeting breast tumor vasculature with less non-tumor tissue accumulation compared to pVEC alone. Directing CPP-coupled medication delivery is logical in this work. However, prior research indicated that CPP-mediated absorption was so effective that it might eliminate targeting specificity of conjugated antigens therefore targeting ligands should be used with caution. Shokolenko et al. found that the arrangement of targeting ligands and CPPs on chimeric peptides can impact their targeting performance. (Shin et al., 2014)

5.3: Delivery of Therapeutic Peptides or Proteins via CPP

Various cellular stressors trigger apoptosis, which is controlled by tumor suppressor proteins like p53 or p16. Mutations during carcinogenesis modify or disable tumor suppressor proteins in numerous human malignancies. (Kurrikoff & Langel, 2019)

Thus, providing full-length proteins or peptides that match these proteins' critical residues has been used to treat cancer. Snyder et al. created a retro-inverse TATp-p53 C-terminus chimeric peptide. When intraperitoneally injected into a terminal peritoneal carcinomatosis mouse model, the treatment group (mean survival time: >70 days) outlived mutant or vehicle-treated animals by 6 times. In an advanced peritoneal carcinoma model, the ones receiving the therapy group survived 50% longer than the control groups (mean survival time for vehicle and mutant peptide treated groups: 33 and 35 days with half of the mice lasting beyond 200 days. A chimeric peptide of p53's mdm-2-binding domain (residues 17–26; PNC-28) and penetration was used to treat another tumor. (Regberg et al., 2012)

5.4: Delivery of genes via CPP

Delivery of genetic material, such as antisense oligodeoxynucleotides (ODNs) or small interference RNA (siRNAs), has the potential to be a highly effective approach for treating cancer. The range is from 86 to 88. RNA interference (RNAi) has become a highly appealing therapeutic approach because of its exceptional precision in targeting genes and unparalleled effectiveness in suppressing tumors. Nevertheless, there are still several challenges to overcome to successfully administer naked siRNA in vivo. One significant obstacle in RNAi therapy is the limited capacity of siRNA medicines to enter cells, which is caused by their high charges and relatively large sizes. (Shin et al., 2014)

Several methods have been tried to overcome this barrier, including the use of cationic liposomes, cationic polymers, or cell-penetrating peptides (CPPs). (Shin et al., 2014)

CPPs have attracted significant attention among these carriers due to their proven low toxicity in both in vitro and in vivo settings. (Shin et al., 2014)

There are two primary techniques for connecting siRNA to CPP: covalent conjugation and noncovalent complex formation. While covalent conjugation is considered the most optimal method, it presents technical challenges in conjugating and purifying siRNA-CPP because of their strong charge-charge interactions. These interactions typically result in the development of firmly bound combinations and groups. The cellular uptake of these ionic siRNA-CPP aggregates is significantly lower compared to that of the 1:1 siRNA-CPP covalent conjugates. This is due to the excessive negative charges of siRNA can readily overcome the cationic amino acids of the CPP, causing both siRNA and CPP to partially lose their biological functions. Although there are drawbacks, the use of non-covalent, ionic complexation of siRNA with CPP has been the preferred method in most published investigations, mainly because of its simplicity. Although many studies on siRNA-CPP have been concentrated on establishing its viability in laboratory settings, there is a growing body of evidence showcasing its effectiveness in animal models. (Shin et al., 2014)

5.5: Co-delivery of Nanoparticles via CPP

Despite the limited understanding of the translocation mechanisms of CPPs/CPP-cargoes, these peptides have been extensively and effectively used to improve the intracellular delivery of various nanoparticles, including magnetic nanoparticles, lipid-based formulations, micelles, gold nanoparticles, quantum dots, and others. (Kurrikoff & Langel, 2019)

Josephson et al. initially documented the conjugation of cell-penetrating peptides (CPPs) to nanoparticles in 1999. The work involved the conjugation of TATp to CLIOs, which are cross-linked iron oxide particles. The TATp-CLIO combination demonstrated high efficacy in cell labeling, making it a promising candidate for applications in MRI and magnetic

separation of targeted cells in living organisms. The intracellular transport of TATp-CLIO, which had an average size of 41 nm and included TATp moieties of 6.7 per particle, was about 100 times more than that of the control group (CLIOs not conjugated with TATp) in all investigated cell lines. Subsequently, the impact of TATp density on the surface of particles and the level of sensitivity in detecting MRI were examined in mouse cells by varying the TATp/CLIO ratios. One hundred an exponential rise in the cellular absorption of TATp–CLIO was found when the TATp/CLIO ratio increased. A 15 TATps/CLIO particle was able to reach a maximum increase of about 100 times in cell uptake compared to the control. Due to the improved intracellular absorption facilitated by the TATp moieties, cells may now be monitored at concentrations that are 100 times lower by utilizing a greater ratio of the TATp/CLIO conjugates. Stroh et al. reported another effective work on cell labeling using nanoparticles treated with CPP. The team synthesized TATp-modified micelles using polyethylene glycol-phosphatidyl ethanolamine (PEG-PE) that encapsulated quantum dots. Through the process of incubating these micelles modified with TATp with primary bone marrow lineage-negative cells, the cells were effectively tagged outside of their natural environment. When the cells tagged with quantum dots were injected into the carotid artery of mice with MCaIV tumors, their attraction to the blood vessels of the tumor could be easily observed by imaging the quantum dots. The effective enhancement of intracellular transport across several cell lines (rat cardiac myocyte H9C2 cells, murine Lewis lung carcinoma cells, and human breast tumor BT20 cells) has been demonstrated by the modification of liposomes with cell-penetrating peptides (CPPs) such as TATp or penetratin. The direct connection between cell-penetrating peptides (CPPs) and the cell surface is crucial for the process of cell entrance. When CPPs are shielded, their ability to be taken up by the cell is significantly hindered. The findings indicated that an average of five CPPs per particle effectively improved the intracellular transportation of liposomes. However, the effectiveness of

liposomes in being taken up by cells was directly related to the concentration of the attached cell-penetrating peptides (CPPs).

Curiously, the rate at which the substance was absorbed varied depending on the specific cell-penetrating peptide (CPP) and cell lines used. In contrast to the TATp-liposomes, which showed a somewhat sluggish process of being taken up by cells, the penetratin-liposomes were rapidly internalized by cells and achieved their maximal uptake within one hour. (Shin et al., 2014)

Chapter 6: Efficacy and limitations of CPP

6.1: Effective Approaches for Targeted Delivery of CPP-Coupled Cargo to Cancer Sites

While CPPs alone have not been found to induce toxicity at doses below hundreds of micromolar, the nonspecific internalization of cargoes paired with CPPs may lead to unforeseen toxicity caused by the cargoes. In research conducted in living organisms, Schwarze et al. observed that the fusion protein TATp-β-galactosidase was present in several organs throughout the body, including the brain.122 Due to the widespread absence of target specificity in cell-penetrating peptides (CPPs), it is necessary to take additional precautions when delivering medicines that have limited tissue selectivity.

While the exact process by which CPP facilitates cellular internalization is still not fully understood, earlier research has demonstrated that the initial step of cell entrance involves the interaction between CPPs and negatively charged cell surface glycosaminoglycans. To achieve selective delivery of the CPP-drugs, a key approach would be to use a "smart" strategy that can inhibit the CPP activities while in circulation, until it reaches the desired location. Then, the inhibition can be reversed at the target site to expose the CPPs, allowing them to exert their cell penetrating activity locally. So far, only a limited number of drug delivery systems (DDS) utilizing the approach for precise transportation of CPP-coupled substances have been developed and examined in animal models. The inhibition of CPPs was often accomplished by the direct formation of complexes between cationic CPPs and anionic equivalents, or by physically protecting CPPs with PEG chains. Various techniques have been developed to reverse the block, such as leveraging the tumor microenvironment or employing an exogenous activating agent.

6.2: Challenges and potential risks

Even though CPPs have a great deal of benefits, it is important to point out that they also have a great deal of drawbacks, which are caused by the chemical and functional features that they possess. Low cell, tissue, and organ specificity is exhibited by a significant proportion of CPPs that belong to the first generation. Because of this, the CPPs that are intended for therapeutic application need to be delivered directly to the tissue that is being targeted. However, the development of cell-selective, organ-selective, or disease-selective peptide carriers has barely begun in the past several years, and it will take some time before they can be successfully used in clinical settings.

It is necessary to make an estimation of the transport efficiency, the cytotoxicity of each CPP cargo complex, and the necessary auxiliaries for each target. This is due to the fact that these characteristics vary from one CPP to another, from one cargo to another cargo, and from one cell type to another cell type. It may be concluded that CPPs are not the philosopher's stone. For each use, thorough optimization is required regarding the effectiveness of internalization and the cytotoxicity of the substance.

Because of the absorption into intracellular endosomes, one of the most significant deficiencies is observed. Destabilization of these cell organelles, which can be accomplished by the addition of auxiliary chemicals or charged polymers like PEIs, is necessary for release from these organelles. It is possible that each of these auxiliary chemicals is cytotoxic. Developing novel CPPs that have mechanisms that do not involve endosomal absorption is necessary to circumvent this endosomal uptake.

There are certain time-consuming drawbacks that require prolonged investigations in order to be able to select the suitable CPP for the given cargo and cell type. The benefit of simple handling that is achieved via the production and internalization of noncovalent complexes

between CPPs and cargos is accompanied by some time-consuming disadvantages. When the noncovalent approach is utilized, the risk of cytotoxic consequences is increased when there is a high molar excess of CPPs, which can range from five to twenty times the payload. The stability of conjugates or fusion proteins with CPPs is greater than that of noncovalent complexes when it comes to high ionic concentrations and by-products in the transduction media. However, in certain instances, the removal of CPP sequences after absorption is necessary.

It is possible for secreted, membrane-bound, intracellular, and serum proteases to render CPPs inactive, even though the uptake process typically takes just five to thirty minutes to complete. This is the case in sluggish processes (big cargos) and in experiments conducted on animals. To prevent proteases from rendering the CPPs inactive, it is necessary to stabilize them by either the insertion of nonproteinogenic amino acids or through cyclization. The ability to transport cargoes past the blood–brain barrier may be advantageous, but it may also become a disadvantage when the medication is administered throughout the entire body.

Chapter 7: Recent Developments and Advances

7.1: Clinical Trials

While numerous CPPs have been identified and researched, only a few of them are now being explored in (pre)clinical trials for medicinal or diagnostic uses. These investigations often focus on the originally discovered sequences Tat and penetratin, or the synthetic poly-arginine. (Prakash Tripathi et al., 2018)

Presumably, the cause of this occurrence is that researchers have acquired a more extensive understanding of the activity of these peptides, which aids in refining the pharmacological characteristics of the future CPP-based treatment. Curiously, this pattern is not strictly adhered to when examining the compounds now under investigation in clinical research for various types of cancer. It is discovered that more peptide therapies that can enter cells and have a natural ability to inhibit tumor growth. These peptides execute this by either concentrating on certain protein-protein interactions or by directly inducing cell death through other methods. CPP-based compounds are now being investigated for their potential use in treating cancers such as breast cancer, colorectal cancer, malignancy of the lungs, lymphoma and other blood cancers, as well as metastatic, refractory or advanced solid tumors. (Matijass & Neundorf, 2021)

7.2: Result of Clinical Trials

Table 1 Several anti-cancer therapies of CPP in clinical development.

CPPs	**Cargoes**	**Drugs in the trial**	**Therapeutic Application**	**Status**	**ClinicalTrial.gov ID**	**Reference**
A highly charged oligopeptide of human origin	SN38	SN38 alone	Tumor	Phase I	NA	(Zhou et al., 2022)
P28	P28	P28 alone	Solid tumors	Phase I Completed in 2014	NCT00914914	(Zhou et al., 2022)
ACPPs	Cy5 and Cy7	AVB-620	Tumor Imaging	Phase I Completed in 2017	NCT02391194	(Zhou et al., 2022)
ALRN-6924	Palbociclib	ALRN-6924 alone and in combination with palbociclib	Solid Tumor, Lymphoma, and Peripheral T cell Lymphoma	Phase 2a Completed in 2020	NCT02264613	(Zhou et al., 2022)
ALRN-6924	Cytarabine	ALRN-6924 alone and in	Acute myeloid	Phase I Completed	NCT02909972	(Zhou et al., 2022)

		combination with cytarabine	leukemia and advanced myelodysplastic syndrome	in 2019		
ALRN-6924	Paclitaxel	ALRN-692 in combination with paclitaxel	Advanced, metastatic or unresectable solid tumors	Phase I	NCT03725436	(Zhou et al., 2022)
ALRN-6924	Topotecan	Phase 1b ALRN-6924 with topotecan Phase 2 topotecan alone and in combination with ALRN-6924	Small cell lung cancer	Phase 1a completed in 2019 Phase 1b Phase II	NCT04022876	(Zhou et al., 2022)

Chapter 8: Potential Prospects:

After extensive investigation, CPPs appear to be poised to move from the laboratory to clinical applications. The cancer therapy sector is expected to be among the earliest beneficiaries of the usage of cell-penetrating peptides (CPPs) due to the absence of effective delivery mechanisms and the significant adverse effects associated with many existing medications. The initial CPP systems that will be used in clinical settings are expected to be formulations of typical cancer chemotherapeutics, which will be identical to the liposomal formulations that are now available in the market. (Regberg et al., 2012)

Subsequently, it would be possible to build delivery methods that specifically target certain areas, constructions that can be activated by cell-penetrating peptides (CPP), and therapies that utilize novel therapeutic targets. Cationic cell-penetrating peptides (CPPs) show great potential as effective carriers for delivering small interfering RNA (siRNA) therapeutics. Theoretically, a single optimized peptide sequence may be utilized to transport a wide range of siRNAs, therefore facilitating the targeting of various disease conditions and offering the potential for individualized cancer therapies. The therapeutic constructs may be formed by utilizing uncomplicated, non-covalent complexes of siRNA and CPP. These complexes can be created using a chosen siRNA or a mix of many siRNAs, tailored to each patient or specific cancer type. (Prakash Tripathi et al., 2018)

Due to the significant expenses associated with developing medicine for clinical usage, the primary focus for novel treatments is on the most prevalent kinds of cancer, such as breast and lung cancer. (Regberg et al., 2012)

However, the benefits of CPP-based therapies may also have potential applications in other forms of cancer. The minimal toxicity of peptide-based delivery systems will serve as a significant benefit of this therapy approach, in contrast to several alternative methods of drug administration such as free medicines or liposomes. Cationic peptide polymers can also have

a function in combination delivery systems that utilize different types of nanoparticles or liposomal formulations. Nanoparticles that have been modified with cell-penetrating peptides have the potential to enhance the absorption of these particles by cells. Additionally, they may enable the particles to traverse the blood-brain barrier. In the case of nanoparticles that contain both penetrating and targeting peptides, they may exhibit a higher level of specificity in delivering their cargo. Overall, the outlook for CPPs is promising and it is likely that the initial CPP-based medications will be available on the market in the coming years. (Regberg et al., 2012)

Cancer treatment requires targeted medication delivery to cancer cells while limiting harm to normal cells. Attached CPPs can permeate cancer cell membranes and deliver chemotherapy medicines directly to cancer cells. This customized administration method improves therapeutic efficacy and reduces negative effects. Targeting genes implicated in the growth of cancer, nucleic acid-based therapeutics like siRNA or antisense oligonucleotides may treat cancer. These chemicals seldom penetrate the cell membrane alone. CPPs can transport nucleic acids to cancer cells for suppressing genes or transcript regulation. CPPs can be coupled to fluorescent dyes or contrast agents to identify and visualize cancer cells. CPPs inject imaging chemicals into cancer cells to precisely localize and image tumors, helping cancer diagnosis, staging, and therapy response. Failure to cure cancer cells typically results from chemotherapy medication resistance. CPP-mediated drug delivery bypasses efflux pumps and other cell membrane resistance mechanisms by delivering therapeutic drugs directly into cancer cells. (Regberg et al., 2012)

Solid tumors have leaky blood arteries and poor lymphatic drainage, causing macromolecule buildup in the tumor microenvironment. CPPs can use the increased permeability and retention effect (EPR) to target and enter tumor tissues, increasing therapeutic drug accumulation in tumors while limiting systemic toxicity. (Matijass & Neundorf, 2021)

Chapter 9: Conclusion

In recent years, innovations in CPP-based drug delivery have proved their applicability and versatility in relation to a wide range of applications. Clearly, there is a tendency towards an increase in the number of therapeutic applications that include CPPs. On the other hand, the preclinical pipeline of research that demonstrates high translational value is just as significant as the clinical trials that are now being conducted. This is because these studies allow for the prediction of future clinical advancements. The purpose of this review is to concentrate solely on stories of this nature. It is simple to observe that clinical development has solely resulted from the "classical" CPP development, whereas the nanotechnology area has not yet shown itself. This is a summary of the latest research that has been presented above.

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