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Research Article

Therapeutic Mechanism and Clinical Applications of Cell-penetrating Peptide

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Abstract

Cell-penetrating peptides (CPPs) are short peptides composed of 30 or fewer amino acids, with the ability to penetrate cellular membranes. Their therapeutic mechanism mainly lies in their ability to conjugate with a variety of biologically active substances, such as chemotherapeutic drugs, nucleic acids, proteins etc., forming complexes that can enter cells via energy-dependent endocytosis, delivering their cargo to the cell interior to exert their effects without affecting cell viability. In terms clinical application, CPPs show broad prospects. In the treatment of tumors, they can act as "smart carriers" for chemotherapy drugs, increasing the concentration of drugs within tumor cells and damage to normal tissues; they are also "powerful assistants" for gene therapy, effectively delivering nucleic acid-like substances, such as the MPG-8 membrane-penrating peptide carrying siRNA targeting cyclin B1 inhibits the growth of mouse transplanted tumors. In addition, they can also be used as "immunostim" of tumor vaccines to enhance immune response, and as "accurate navigation devices" of molecular imaging to assist in the surgical resection of tumors. Although there are still challenges such short half-life and incomplete understanding of the mechanism of action, with the deepening of research and the iteration of technology, CPPs are expected to provide new strategies and methods for treatment of a variety of diseases.

Keywords

Cell Penetrating Peptide, Antivirus, Virus Infestation, Cervical Cancer

1. Introduction

Globally, viral infectious diseases have always been a serious threat to public health and human life safety. From the human immunodeficiency virus (HIV) that has been rampant for many years and caused AIDS, to the various cancers caused by human papillomavirus (HPV), and then to the-scale epidemics caused by influenza viruses from time to time, these viral infections not only bring great suffering to patients but also place a heavy burden on social medical resources.

Conventional antiviral therapies, such as the use of small molecule antiviral drugs and vaccines, have achieved certain effects in the prevention and of some viral infections. However, there are still many limitations for many complex and difficult-to-overcome viruses, such as the difficulty of drugs

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to effectively enter intracellular site of action and the easy generation of drug resistance.

In recent years, the emerging field of cell-penetrating peptides has gradually come into people's vision. With its unique structure and characteristics, it has shown great potential in clinical applications against viruses, bringing new hope for solving the current dilemma of antiviral therapy. However, as with any emerging making its way to the clinic, cell-penetrating peptides also face a series of severe challenges in practical application [1, 2].

2. Definition and Characteristics of Cell-penetrating Peptides and Their Classification

2.1. Definition of Cell-penetrating Peptides

Cell-penetrating peptides (CPPs), also known as cell-penrating peptides, are a class of short peptide sequences that can carry bioactive molecules (such as proteins, nucleic acids, nanoparticles, etc.) the cell membrane into the cell interior, generally consisting of 5-30 amino acid residues.

2.2. Structural Characteristics of Cell-penetrating Peptides

2.2.1. Amino acid Composition

Rich in arginine lysine and other positively charged amino acids, these cationic amino acids endow cell-penetrating peptides with the ability to interact with the negatively cell membrane. For example, the Tat peptide (from the HIV viral transcriptional activator protein) contains multiple arginine residues, and its sequence is GRKKRRQRRR.

2.2.2. Amphiphilicity

Many cell-penetrating peptides possess amphiphilicity, meaning they have both hydrophilic hydrophobic regions. This characteristic helps them interact with the phospholipid bilayer of the cell membrane. For example, penetratin, a cell-etrating peptide, has both hydrophilic and hydrophobic amino acid regions in its structure, allowing it to interact with and cross the cell membrane in an aous solution environment [3-5].

2.3. Classification of Cell-penetrating Peptides

2.3.1. Cell-penetrating Peptides from Natural Sources

Cell-penetrating peptides discovered from natural proteins, in addition to the aforementioned Tat peptide of HIV virus, include penetratin in the fruit fly antennae foot protein. Penratin can effectively enter various cell types without the assistance of any carrier.

2.3.2. Synthetic Cell-penetrating Peptides

Cell-penetrating peptides designed according to the structural characteristics of natural-penetrating peptides or based on certain theories. For example, the polyarginine peptide, composed of multiple arginine residues in series, good membrane-penetrating ability; and the PepFect series of cell-penetrating peptides designed by optimizing the amino acid sequence, which improves membrane-penetrating efficiency and the delivery capacity of bioactive molecules by adjusting the amino acid composition and arrangement [6-8].

2.3.3. Chimeric Cell-penetrating Peptides

Cell-penetrating peptides formed by combining different sources of structural domains with specific functions. For example, by connecting the structural domain cell-targeting property and the transmembrane structural domain, a chimeric peptide that can both specifically recognize the target cell and efficiently penetrate the membrane is to improve the targeted delivery ability of the cell-penetrating peptide.

3. The Mechanism and Application of Cell-penetrating Peptide Transmembrane

3.1. Direct Penetration of the Cell Membrane

Cell penetrating peptides directly insert into the cell membrane through electrostatic interaction with the cell membrane phospholipid bilayer, forming a transient channel or pore allows bioactive molecules to pass through and enter the cell. This method does not rely on energy or endocytosis. For example, the polyarginine pept may cross the cell membrane through the direct penetration mechanism [9-12].

3.2. Endocytosis

Many cell-penetrating peptides enter cells through endocyt. This includes clathrin-mediated endocytosis, caveolin-mediated endocytosis, and macropinocytosis. After to the cell membrane, the cell-penetrating peptide is wrapped by the cell membrane to form an endocytic vesicle, which then enters the cell. The endocytic vesicle undergoes a series of membrane fusion and transport processes inside the cell, and finally releases the carried bioactive molecules into the cylasm. For example, Tat peptide enters the cell mainly through clathrin-mediated endocytosis [13-16].

3.3. Application of Cell-Penetrating Peptides

Cell-penetrating peptides are a class of peptides that the ability to penetrate cell membranes, allowing them to perme-

ate into the cell interior. The applications of cell-penetrating peptides are extensive, as follows:

3.3.1. Drug Delivery

Cell-penetrating peptides can be used to enhance intracellular drug delivery. Due to their ability to penetrate cell membranes, can bind to drugs and promote their entry into the cell interior. This application can improve the intracellular concentration of drugs, increasing the efficacy of the drugs. As carrier to deliver various drug molecules into cells, it can improve the efficiency of cellular drug uptake and enhance the efficacy of the drug. For example, using cell-etrating peptides to deliver antitumor drugs allows the drugs to more effectively act on tumor cells, improving the effects of cancer treatment. TAT cell-penet peptides are widely used in the construction of drug delivery systems and can be used to deliver drugs to tumor cells or other specific cells [17-21].

3.3.2. Gene Delivery and Gene Therapy

Transmembrane peptides can be used to enhance the efficiency of gene delivery. Due to their to penetrate the cell membrane, they can bind to DNA or RNA and promote the entry of genes into the cell interior. This application can improve the efficiency of gene delivery promote the application of gene therapy. Transmembrane peptides can carry nucleic acid substances (such as plasmid DNA, siRNA, etc.) into cells achieving gene transfection or gene silencing, and providing new means for gene therapy. For example, in the treatment research of some genetic diseases, normal genes are delivered diseased cells through cell-penetrating peptides, trying to correct gene defects. HIV-1 TAT transmembrane peptide is widely used in the of gene delivery systems, which can be used to deliver genes into specific cells to achieve the purpose of gene therapy [22-25].

3.3.3. Protein Transduction

Helps proteins cross the cell membrane into the cell, studies the function of proteins in the cell, and carries protein therapy. For example, some proteins with biological activity (such as enzymes, cytokines, etc.) are fused with cell-penetrating peptides enable them to enter the cell and exert their effects.

3.3.4. Cell Imaging

Pepetides can be used to enhance cell imaging. Due to their ability to penetrate cell membranes, they can conjugated with fluorescent dyes or radioisotopes and enter the interior of cells. This application allows for real-time observation and quantitative analysis of cellular internal processes. example, Penetratin has been used in cell imaging studies to track the location and dynamic changes of specific molecules within cells [26-29].

3.3.5. Vaccine Development

Transmembrane peptides can be used to enhance the immune effects of vaccines. Due to their ability to cell membranes, they can bind to antigens and promote the entry of antigens into the cell interior, activating the immune system. This application can enhance immunogenicity of vaccines and improve the immune response to specific pathogens. For example, the Antennapedia transmembrane peptide has been used in vaccine and can be used to enhance the immune effects of vaccines.

4. The Peptide Directly Acts on the Viral Particles

4.1. Interference with Viral Adsorption

4.1.1. Mechanism of Action Against Different Viruses

The first step of a viral infection of host cells isorption, in which cell-penetrating peptides play an important “interfering” role. Some cell-penetrating peptides can bind to viral proteins, thereby blocking the recognition and binding process between the virus and the receptors on the surface of host cells. The virus relies on its specific surface proteins to precisely recognize bind to receptors on the surface of host cells, thus initiating the infection process. Taking the human immunodeficiency virus (HIV) as an, the sugar protein gp120 on the surface of HIV will specifically interact with the CD4 molecule on the surface of the host cell and the co-ceptor CCR5 or CXCR4. Some cell-penetrating peptides can cunningly simulate the partial structural domains of the host cell receptors anditively bind to the viral surface proteins. In this way, it is difficult for the virus to find the real host cell receptors, unable to complete adsorption, and block the initial step of infection. Studies have found that some cell-penetrating peptides designed based on the structure of CD4 molecules can effectively reduce the binding of HIV to host cells and significantly reduce the number of cells infected by the virus. The mechanism of viral adsorption varies with the type of virus, and cellpenetrating peptides also show diverse interfering ways accordingly. In addition to HIV, such as hepatitis C virus (HCV), it binds to multiple on host cells, such as sulfated acetyl heparin protein glycan, CD81, and so on, through a variety of proteins on the surface It was found that some cell-penetrating peptides can competitively bind to these receptors' key binding sites and compete with HCV surface proteins. When these-penetrating peptides are present, HCV virus particles are difficult to normally dock with host cell receptors, greatly reducing the efficiency of viral adsorption and thereby the infection rate. During the Ebola virus infection process, the viral surface glycoprotein GP interacts with receptors such as NP1 on the host cell, and cellpenetrating peptides designed based on the NPC1 receptor structure can effectively block this binding, signifi-

cantly inhibiting the adsorption of Ebola virus to cells, and provide research ideas for the prevention and control of the Ebola epidemic [30-32].

4.1.2. Correlation Between Transmembrane Peptide Structure and Interference Effects

The amino acid sequence and structural characteristics of cell-penrating peptides significantly affect their ability to interfere with viral adsorption. Transmembrane peptides rich in arginine residues often have stronger electrostatic interaction capabilities, them to bind more tightly to viral surface proteins. For example, polyarginine transmembrane peptides can strongly attract to the negatively charged regions of viral surface proteins to their high density of positive charges, thus effectively hindering the binding of viruses to host cell receptors. In addition, the secondary structures of transmembrane peptides such as α-helices and β-sheets, also affect their interactions with viruses and receptors. Transmembrane peptides with specific secondary structures may be more likely mimic the structural domains of receptors, competitively binding to viral surface proteins in a more accurate manner, enhancing the interference effects on viral adsorption.

4.2. Disruption of Viral Structure

4.2.1. The Special Physicochemical Properties of Cell-penetrating Peptides

Cell penetrating peptides with special physicochemical properties can directly "attack" the viral structure. Many viruses have envelopes, which are composed of a phospholipidayer and membrane proteins and are essential for maintaining the integrity and infectivity of the viral particles. Some cell-penetrating peptides with amphipathic orationic properties can interact with the viral envelope. They may insert into the phospholipid bilayer of the viral envelope, causing perturbations to the envelope structure, rupting the integrity of the envelope, and causing perturbations, fusion, or the formation of holes in the membrane, resulting in the leakage of the viral genetic material inside, of infectivity, and disintegration of the viral particles. For example, the envelope of the influenza virus will show abnormal fusion or form holes after being treated by specific-penetrating peptides. Once the integrity of the envelope is destroyed, the genetic material inside the virus, such as nucleic acids, will be leaked out and the virus will lose its infectivity. Experiments have shown that after treating influenza viruses with specific cell-penetrating peptides, obvious damage to the viral can be observed under an electron microscope, the morphology of the viral particles becomes irregular, and the ability to infect cells is greatly reduced.

4.2.2. Differential Effects on Different Viral Envelopes

The composition and characteristics of the envelopes of different viruses are different, and the of cell-penetrating peptides on them also vary. For herpesviruses, their envelopes are more complex, containing a variety of glycoproteins lipid components. Cell-penetrating peptides with amphipathic properties can use their hydrophobic regions to insert into the phospholipid bil of the herpes virus envelope, and their hydrophilic regions interact with water molecules on the surface of the envelope. This action disrupts the lipid arrangement of envelope, leading to local structural disorder of the envelope and the formation of small holes, which causes the leakage of genetic material inside the virus. For rabies virus, envelope structure is relatively more compact, but certain cationic cell-penetrating peptides can interact electrostatically with negatively charged membrane proteins on the rabies envelope, changing the conformation of membrane proteins, causing instability of the envelope, and ultimately leading to the rupture of the envelope and the inactivation of the particles [33-35].

4.2.3. The Influence of Concentration and Action Time of Cell-penetrating Peptides

The effect of cell-penetratingides on the destruction of viral structures is closely related to their concentration and action time. Within a certain range, increasing the concentration of cell-penetrating peptides increase the chance of interaction with the viral envelope, thus more effectively destroying the viral structure. For example, in the study of influenza virus, it was found that as concentration of a specific cell-penetrating peptide increased, the degree of damage to the viral envelope became more obvious, and more viral particles with envelope rupt and morphological deformation could be observed under the electron microscope. Action time is also important. A shorter time of action may only cause slight disturbances to the viral envelope while with prolonged action time, cell-penetrating peptides can continue to destroy the envelope structure, eventually leading to the complete loss of infectivity of the virus Studies have shown that treating hepatitis B virus with cell-penetrating peptides for 2 hours compared to 6 hours, the latter has a higher degree damage to viral particles and almost completely loses the ability to infect cells [36-39].

5. Cell-penetrating Peptides Affect the Viral Replication Cycle in Host Cells

5.1. Inhibition of Viral Gene Expression

5.1.1. Delivery and Mechanism of Action of Nucleic Acid-like Substances

Cell-penetrating peptides can serve as efficient carriers to deliver antisense oligonucleotides, siRNA, and other nucleic acid-like substances into host cells. Once inside the, these nucleic acid-like substances play a crucial role in the com-

plex intracellular environment. These nucleic acid molecules can specifically bind to viral mRNA within the through complementary base pairing, and through the RNA interference (RNAi) mechanism, they can block the transcription or translation processes of viral genes. Taking RNA interference (i) mechanism as an example, after successful delivery of siRNA into the cell by cell-penetrating peptides, siRNA will form RNA-induced silencing (RISC) with relevant proteins in the cell. The siRNA in RISC will specifically recognize and bind to viral mRNA through its complementary base sequence. Once bound the endonuclease in RISC will cleave and degrade the viral mRNA, thus blocking the translation process of viral genes and preventing the virus from synthesizing necessary for its replication. For example, in studies targeting human papillomavirus (HPV), by delivering siRNA specific to certain genes of HPV through cellpenetrating peptides, it is possible to significantly reduce the expression level of viral-related proteins and effectively inhibit the replication of HPV in host cells. Initis B virus-infected cells, the use of cell-penetrating peptides to deliver siRNA targeted against hepatitis B virus mRNA can effectively reduce the of viral-related proteins and inhibit viral replication.

5.1.2. Regulate Transcription Factors in Host Cells

Cell-penetrating peptides can also indirectly inhibit viral gene expression by affecting factors in host cells. After infecting host cells, viruses use the transcription mechanism of host cells to initiate the transcription of their own genes. Some cell-penet peptides can enter the nucleus and interact with transcription factors involved in the regulation of viral gene transcription in host cells. For example, during herpesvirus infection, cell-penetrating peptides can bind to specific transcription factors activated by the virus in host cells, changing their conformation or inhibiting their activity, so that transcription factors cannot normally bind to the promoter region of viral genes, thus blocking the initiation of viral gene transcription, reducing the production of viral mRNA, inhibiting the expression viral genes at the source, and then hindering the replication process of viruses.

5.1.3. Blocking Viral Assembly and Release

(i). Blocking Viral Assembly Pathway

Interference with the Viral Assembly Process After completing replication and protein synthesis in host cells, viruses need to undergo assembly and be released from the cells. Cell-penetrating peptides can interfere with this process, example, by interacting with key proteins involved in viral assembly, disrupting the assembly structure of the viral particles; or by affecting the host cell's vesicle pathways related to viral release, preventing the newly formed viral particles from being released from the cells and reducing the spread of the virus. Viruses, after completing genome replication protein synthesis in host cells, need to undergo precise assembly to form infectious viral particles. Cell-penetrating peptides can play a role in interfering with this process The assembly of viruses relies on the interaction between various viral proteins and the binding to the viral genome. Certain cell-penetrating peptides can specifically bind to key involved in viral assembly, changing the spatial conformation of these proteins and disrupting their normal interaction networks. For example, in the assembly process of influenza viruses, the1 protein is essential for the morphology and structural stability of the viral particles. It has been found that specific cell-penetrating peptides can bind to the M protein, interfering with the interaction of the M1 protein with other viral proteins and viral RNA, resulting in the failure of influenza virus particles to assemble correctly, and the viral particles often have abnormal structures and do not have infectious activity.

(ii). Blocking the Viral Release Pathway

The release of viruses from host cells is an important link in their spread and diffusion.-penetrating peptides can affect the vesicular transport pathway related to viral release in host cells. Many viruses are transported from the endoplasmic retic or the Golgi apparatus and other organelles of host cells to the cell membrane step by step through vesicular transport, and finally released outside the cell bying and other means. Cell-penetrating peptides can interfere with this transport process, such as interacting with key proteins or regulatory factors involved in vesicular transport. In the process of HIV virus release, cell-penetrating peptides can bind to proteins in host cells involved in the budding and release of HIV, the normal assembly and function of the ESCRT complex, thereby inhibiting the budding and release of HIV from the surface of host cells and reducing the spread of in the host body.

6. Examples of Research on Antiviral Mechanisms of Cell-Penetrating Peptides

6.1. Research Examples on HIV Virus

The Tat peptide is a segment of cell-penetrating peptide in the HIV viral transcriptional activator protein. Studies have shown when the Tat peptide is fused with certain functional structural domains that can inhibit the replication of HIV, such as a small molecule peptide segment that can inhibit the activity HIV integrase, the resulting fusion peptide can enter cells with the help of the Tat peptide's membrane-penetrating ability, effectively inhibiting the integrase from integrating the viral genome into the host genome, thereby significantly reducing the level of HIV replication within the cell. In the study of HIV virus, researchers have out many explorations using the cell-penetrating peptide Tat (from the HIV viral transcriptional activator

protein). The Tat peptide itself has a efficient membrane-penetrating ability. Researchers fuse a small molecule peptide segment that can inhibit the activity of HIV integrase with the Tat peptide. When this peptide enters HIV-infected cells, it can reach the site of action in the cell smoothly with the help of the Tat peptide's membrane-penrating properties. Inside the cell, the inhibitory small molecule peptide segment in the fusion peptide can specifically bind to HIV integrase, blocking the key step ofase that integrates the HIV viral genome into the host genome. The experimental results show that after treating infected HIV cells with this fusion peptide, the efficiency of integrating the viral genome in the cell is significantly reduced, which in turn significantly reduces the level of HIV replication in the cell, effectively inhibiting the life cycle process of HIV in host cell.

6.2. Research Examples on Human Papillomavirus (HPV)

Human papillomavirus (HPV) is closely related to the of various cancers, such as cervical cancer. Based on a deep understanding of the mechanism by which HPV enters cells, a research team at Yale University has designed a to inhibit HPV infection using cell-penetrating peptides. They found that short synthetic peptides derived from HPV capsid proteins have the ability to cross membranes. Once these synthetic peptides enter the cells, they can interfere with protein interactions crucial for HPV transport within the cell. Specifically, the cell-penet peptide can block HPV from entering its normal intracellular transport pathway. In cell culture experiments, treating HPV-infected cells with this cell-penrating peptide significantly reduced the infection rate of HPV. In mouse experimental models, it was also observed that this cell-penetrating peptide could effectively inhibitV infection in vivo, providing new ideas and potential means for the prevention and treatment of HPV-related diseases.

6.3. Research Examples on Influenza Viruses

In the study of influenza viruses, research focuses on the impact of cell-penrating peptides on the assembly process of influenza viruses. The M1 protein of the influenza virus plays a central role in the morphological and structural stability of the viral. Scientists have designed and synthesized specific cell-penetrating peptides that can bind specifically to the M1 protein. When the cell-penetrating peptideinds to the M1 protein, it changes the spatial conformation of the M1 protein, disrupting the normal interaction network between the M1 protein and other viral and viral RNA. Observations under an electron microscope show that after treatment with cell-penetrating peptides, influenza virus particles cannot be correctly assembled, forming virus with abnormal structures, and these abnormal virus particles almost completely lose their ability to infect cells. This research result indicates that interfering with the assembly process of influenza viruses through cellpenetrating peptides may become a new strategy for anti-influenza virus therapy.

PepFect series in anti-influenza virus: PepFect is a class of artificially synthesized cell-penetrating peptides. Studies have shown that PepFect can efficiently deliver siRNA targeted influenza virus genes into infected cells. In animal experiments, when siRNA delivered by PepFect is used to treat mice infected with influenza virus, a significant decrease viral load in the lungs of mice, alleviation of lung inflammation, and improved survival status can be observed, showing good anti-influenza virus effects.

6.4. Research Examples on Herpes Viruses

The research group of Professor Zheng Chunfu from Soochow University has made progress in the field of herpes virus research. Herpes viruses, such as human herpes simplex virus type I (HSV-1), have a genome that can encode a variety of viral proteins and can establish lifelong latent infection in human neuronal cells. Studies have found that certain cell-penetrating peptides enter the nucleus, bind to specific transcription factors activated by HSV-1 in host cells. This binding changes the conformation of the transcription factors, inhibits their, and prevents the transcription factors from normally binding to the promoter regions of HSV-1 genes, thus blocking the transcription initiation of HSV-1 genes. Experimental show that after treating cells infected with HSV-1 with this cell-penetrating peptide, the production of viral mRNA is significantly reduced, suppressing the of viral genes at the source, and then hindering the replication process of HSV-1 in host cells, providing new targets and potential treatment methods for the treatment herpes virus infection.

7. Application Prospects and Challenges of Cell-Penetrating Peptides in Antiviral Therapy

7.1. Prospects 1. Development of New Antiviral Drugs

7.1.1. Efficient Delivery Vector

Cell-penetrating peptides, as a new type delivery vector, provide new strategies for the development of drugs against difficult-to-treat viral infections, such as AIDS and hepatitis C. By connecting various molecules antiviral activity (such as small molecule inhibitors, nucleic acid-based drugs) with cell-penetrating peptides, it is expected to improve intracellular delivery efficiency of drugs, enhance the efficacy of drugs, and develop more effective antiviral drugs. Traditional antiviral drugs are often limited in efficacy they cannot effectively enter the intracellular site of action.

Cell-penetrating peptides can carry various antiviral active molecules (such as small molecule inhib, nucleic acid-based drugs) across the cell membrane, precisely delivering the drug to the interior of the cell, increasing the concentration of the drug in the cell and thus enhancing the antiviral effect of the drug. For example, by connecting small molecule inhibitors targeting key viral proteins with cell-penetrating peptides the inhibitors can be more efficiently delivered to the site of viral replication, directly blocking the replication process of the virus [40-43].

7.1.2. Development of New Mechanism of Action Drugs

Cell-penetrating peptides inherently possess mechanisms of action such as interfering with viral ads, destroying viral structures, and affecting the replication cycle of viruses in host cells. Based on these properties, it is possible to design antiviral drugs with completely new of action. For example, by using cell-penetrating peptides to mimic the structural domain of host cell receptors to block viral adsorption, this new drug a different mode of action from traditional drugs is expected to provide new solutions for dealing with drug-resistant viral infections.

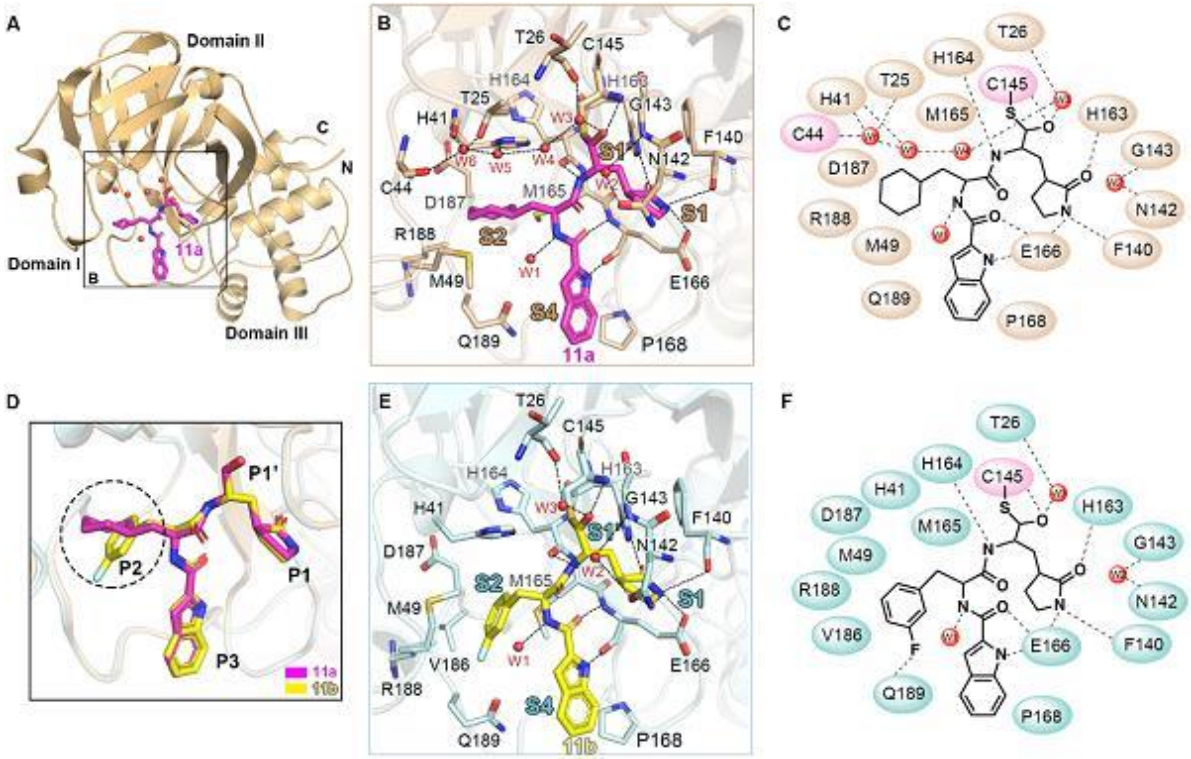

Figure 1. *Structural diagram of various peptide antiviral drugs.*

7.2. Combined Treatment Strategy

7.2.1. Synergistic Effect

It can be used in combination with traditional antiviral drugs exert synergistic effects. For example, antiviral nucleic acid drugs delivered by cell-penetrating peptides can inhibit viral replication at the genetic, and when combined with traditional drugs that act on other links of viral replication, they can block the process of viral infection at multiple targets and improve the therapeutic effect. the same time, it may reduce the dosage of traditional drugs and reduce the side effects of drugs. The nucleic acid drugs delivered by cell-penetrating pept inhibit viral replication at the genetic level, while traditional antiviral drugs act on other links of viral replication. The synergistic effect of the two can block process of viral infection at multiple targets and significantly improve the therapeutic effect. In the treatment of AIDS, the use of cell-penetrating peptide-deliveredRNA against HIV genes in combination with traditional antiretroviral drugs can more effectively inhibit the replication of HIV in patients [44-47].

7.2.2. Reduce the Side Effects of Drugs

The combined use of cell-penetrating peptide drug delivery and traditional drugs could potentially the dosage of traditional drugs. Because cell-penetrating peptides can improve the efficiency of intracellular drug delivery, it allows the drug to work better at lower dose, thereby reducing the

side effects of traditional drugs caused by high-dose use. For example, in the treatment of influenza, combined drug use can reduce dosage of neuraminidase inhibitors and reduce their potential adverse reactions.

7.3. Challenges 1. Safety Issues

The long-term safety of cell-penetrating peptides in vivo is still not understood. Their cationic properties may lead to non-specific binding with various negatively charged biomolecules (such as nucleic acids, membrane phospholipids etc.) within cells, causing issues like cytotoxicity and immunogenicity. For example, certain cell-penetrating peptides may induce changes in structure and function of the cell membrane, affecting normal physiological activities of the cell, which requires further in-depth research and optimization to improve their safety.

7.3.1. Cytotoxicity

The cationic nature of cell-penetrating peptides makes them prone to non- binding with various negatively charged biomolecules inside cells, such as nucleic acids, membrane phospholipids, etc. This non-specific binding may alter the and function of the cell membrane, affecting the normal physiological activities of the cell and leading to cytotoxicity. For example, certain cell-penetrating pept can cause an increase in the permeability of the cell membrane, leakage of intracellular substances, and even cell death at high concentrations [48-50].

7.3.2. Immogenicity

As exogenous substances entering the body, cell-penetrating peptides may trigger immune reactions. The immune system recognizes them as foreign bodies and generates responses, which may not only reduce the antiviral effects of cell-penetrating peptides but also lead to immune-related adverse reactions in the organism. example, certain synthetic cell-penetrating peptides have induced antibody production in animal experiments, affecting their action time and efficacy in vivo.

7.3.3. Lack of Target Specificity

Most of the current cell-penetrating peptides lack specific targeting for particular cell types or tissues and may be taken up by non-target cells during in vivo delivery, reducing the effective concentration of the drug in target cells and affecting the therapeutic effect, while increasing risk of off-target effects. How to endow cell-penetrating peptides with good target specificity, so that they can precisely deliver antiviral to virus-infected cells, is an urgent problem to be solved [51-54].

Uptake by non-target cells: Most cell-penetrating peptides lack specific targeting to particular cell types tissues, and they can be easily taken up by non-target cells during in vivo delivery. This leads to the waste of antiviral drugs in non-target, reducing the effective concentration of the drug within target cells and affecting the therapeutic effect, while also increasing the risk of off-target effects. For example, in the of hepatitis B, drugs delivered by cell-penetrating peptides may be taken up by cells in tissues other than the liver, resulting in poor antiv effects of the drug in the liver.

Difficulties in improving targetability: Although the targetability of cell-penetrating peptides can enhanced by linking them with structure domains with targetability, it is still a technical challenge to precisely design and construct such targetable molecules that can both maintain the membrane-etrating ability of cell-penetrating peptides and achieve efficient and specific targeting. Current research in targetability is still in the exploratory stage, and is a certain gap from clinical application.

8. Conclusion

The clinical application of cell-penetrating peptides in the field of antiviral is currently in the exploratory stage, showing while facing great challenges.

From a positive perspective, in the research and development of new antiviral drugs, cell-penetrating peptides, as efficient delivery, can carry small molecule inhibitors, nucleic acid drugs, etc., into cells, enhancing the antiviral effects of the drugs, and providing new approaches the development of drugs against difficult-to-treat viral infections (such as HIV, HPV, etc.). Moreover, based on its unique mechanism of, which interferes with viral adsorption, destroys viral structure, and affects the viral replication cycle, it is expected that new drugs with completely new mechanisms of action be designed to deal with drug-resistant viral infections. In terms of combined treatment strategy, when used in combination with traditional antiviral drugs, the drugs delivered by-penetrating peptides can synergize and enhance the effects, block multiple targets of viral infection, and reduce the dosage and side effects of traditional drugs the same time, such as in the research of AIDS and influenza treatment, which has initially achieved results.

However, it is important to note that cell-penetrating peptides face severe challenges in clinical application. Safety issues pose an obstacle, as their cationic properties can lead to cytotoxicity, which changes the structure and function of the cell membrane, affecting normal physiological activities and even cell death. Additionally, as foreign substances, cell-penetrating peptides can trigger immune reactions, generating immunogenicity, reducing their antiviral effects, causing immune-related adverse reactions. Inadequate targeting is also a pressing issue that needs to be addressed. Most cell-penetrating peptides lack specificity certain cell types or tissues, and they are prone to be taken up by non-target cells in vivo, leading to drug wastage, decreased drug concentration in target, and increased risk of off-target effects. Although attempts can be made to improve this by

connecting them with targeting domains, the precise design and construction of such targeted are technically challenging, and there is still a long way to go before they can be applied clinically.

In summary, cell-penetrating peptides show promising in antiviral clinical applications. However, to truly achieve widespread clinical use, it is imperative that researchers make significant breakthroughs in addressing safety and targeting issues. in-depth research and technological innovation, it is possible to advance cell-penetrating peptides from the laboratory to clinical practice, bringing new and effective treatment options antiviral therapy.

Abbreviations

CPP	Cell-Penetrating Peptidex
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
siRNA	Small Interfering RNA
GFP	Green Fluorescent Protein
asONs	Antisense Oligonucleotides
PMO	Phosphodiamide Morpholine Oligomer
dsDNA	Double-stranded DNA

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Not applicable.

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Conceptualization, J.M. and C.W.; data curation, J.M.; writing—original draft preparation, J.M.; writing—review and editing, C.W. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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