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A Review of Peptide Applications in Influenza Virus Research

Influenza viruses are major respiratory pathogens responsible for seasonal epidemics and potential pandemics. Despite preventive measures such as vaccines and neuraminidase inhibitors, the virus's high mutation rate often leads to fluctuating vaccine efficacy and the emergence of drug-resistant strains. Therefore, developing novel, broad-spectrum anti-influenza strategies is crucial. Peptides, as biologically active molecules composed of amino acids, have emerged as a frontier in influenza virus research and antiviral drug development due to their design flexibility, precise targeting, and low propensity to induce resistance. This review summarizes the application of peptides in influenza virus research, covering strategies, advances, and challenges in their use as viral entry inhibitors, immunomodulators, and vaccine design components.

I. Peptides as Inhibitors of Viral Entry and Fusion

Influenza virus infection begins with the binding of the viral surface protein hemagglutinin (HA) to sialic acid receptors on host cell surfaces. Subsequently, genetic material is injected into the cell via endocytosis and

membrane fusion triggered by low pH. Peptides have been engineered as effective intervention tools targeting these two critical steps.

1. Receptor-binding competitive peptides

These peptides mimic key structures of HA's receptor-binding domain or host cell receptors, thereby blocking viral adsorption.

Strategy: Utilize structural biology to determine the precise conformation of HA's head-end receptor-binding pocket, designing peptides that bind with high affinity or synthesizing segments mimicking the structure of sialic acid receptors.

Case and Challenge: Some studies have synthesized constrained peptides mimicking the ring structure of HA's receptor-binding site, demonstrating inhibitory activity in vitro. However, as the HA head is the most frequently mutated region of the virus, peptide inhibitors targeting this area are prone to inactivation due to viral antigenic drift, limiting their broad-spectrum application potential.

2. Membrane Fusion Inhibitors (Targeting the HA2 Stem Region)

This strategy holds greater promise for broad-spectrum efficacy. The HA2 subunit mediates membrane fusion, with its N-terminal fusion peptide and subsequent curled-helix structure being highly conserved.

Core Mechanism: Design peptides mimicking the core region of the HA2 coiled-coil (e.g., derived from C-terminal or N-terminal HA2 sequences). These peptides bind to the intermediate conformations of viral

HA2 exposed during fusion, preventing the formation of the correct hexameric bundle and thereby inhibiting pore formation.

Representative Advances: Peptide fusion inhibitors designed based on HA2 sequences demonstrate broad-spectrum inhibitory activity against multiple influenza A subtypes (e.g., H1N1, H5N1, H7N9) in vitro and in animal models. In vivo stability and half-life of such peptides can be significantly enhanced through chemical modifications (e.g., polyethylene glycolation, fatty acid chain modifications).

3. Peptides Targeting Other Viral Proteins

Neuraminidase Inhibitors: Peptides designed to mimic transition state analogues of NA substrates (sialic acid) or competitive inhibitors directly targeting the NA active site. However, compared to existing small-molecule drugs (oseltamivir), peptides currently show no significant advantage in activity or oral bioavailability.

M2 Ion Channel Blockers: The proton channel activity of the M2 protein is critical for viral uncoating. Short peptides designed to insert into the M2 tetramer channel and block proton flow could theoretically mimic amantadine's mechanism. However, due to widespread resistance issues, research in this area has cooled.

II. Peptides as Host-Targeted Antiviral Agents and Immunomodulators

Rather than directly targeting variable viral proteins, another approach to achieving broad-spectrum antiviral activity involves targeting

relatively conserved host factors essential for viral replication within host cells, while also modulating excessive immune responses.

1. Peptides Targeting Host Proteases

The HA precursor of influenza viruses requires cleavage by host proteases to gain infectivity. Designing peptides that inhibit the activity of these key host proteases (e.g., transmembrane serine protease 2, cathepsins) can block viral maturation. This strategy holds promise for effectiveness against multiple viral subtypes that rely on the same protease cleavage, while being less likely to induce viral resistance.

2. Host cell membrane-penetrating peptides and their derivatives

Certain host defense peptides or their derivatives with membrane-disrupting activity can nonspecifically inhibit viral entry by damaging viral envelopes or cell membranes. Furthermore, coupling antiviral active sequences with cell membrane-penetrating peptides can enhance the entry of antiviral peptides into cells, targeting intracellular viral replication stages.

3. Immunomodulatory Peptides

Severe influenza is often accompanied by excessive inflammatory responses. Synthetic peptides with immunomodulatory functions can be engineered to suppress excessive pro-inflammatory cytokine release, mitigate immunopathological damage, and improve host survival rates without directly affecting viral load.

III. Application of Peptides in Influenza Vaccine Research

1. Antigen Candidates for Universal Vaccines

Traditional influenza vaccines primarily induce antibodies targeting the variable head region of HA. To develop “universal” vaccines against multiple subtypes, researchers are focusing on highly conserved “Achilles' heel” sites within viral proteins, such as the HA2 stem region, the extracellular domain of M2 protein, or T-cell epitopes on NP or M1 proteins.

Strategy: Chemically synthesize peptides from these conserved regions or design them as tandem polyantigenic peptides, combined with potent adjuvants.

Objective: Aim to induce broad-spectrum neutralizing antibodies against the HA stem or stimulate cytotoxic T lymphocyte responses capable of cross-recognizing different subtypes, thereby providing broader protection.

2. As Vaccine Delivery Vectors and Adjuvants

Peptides themselves can serve as precise carriers for antigenic epitopes. Through self-assembly into nanoparticles that mimic virus-like particle structures, they enhance antigen immunogenicity and delivery efficiency. Certain peptides with immunostimulatory activity can also function directly as adjuvants, boosting the overall vaccine response.

IV. Optimization Strategies and Challenges for Peptide Drugs

To transform promising anti-influenza peptides from the laboratory

into clinical candidates, several challenges must be overcome:

1. Enhancing protease stability and in vivo half-life

Strategy: Employ chemical approaches such as backbone cyclization, introduction of D-amino acids, terminal modifications, and polyethylene glycolation to counteract degradation by abundant peptidases in serum and tissues.

2. Enhancing antiviral activity and broad-spectrum efficacy

Strategy: Identify key residues through alanine scanning and perform rational design based on structure; construct multivalent peptides (linking multiple active units) to enhance affinity for viral targets; combine peptides targeting different conserved epitopes to achieve synergistic effects and reduce resistance risks.

3. Improving Delivery and Administration Routes

Strategies: Develop inhalation formulations (e.g., dry powder inhalers) suitable for pulmonary local administration, enabling direct peptide action at respiratory infection sites to increase local concentrations and reduce systemic side effects.

4. Key Challenges

In vivo efficacy validation: Many peptides with strong in vitro activity may exhibit diminished protective effects in animal models due to poor pharmacokinetics.

Cost: The high chemical synthesis costs of long peptides may limit

their accessibility as preventive drugs (e.g., vaccine alternatives).

Immunogenicity: Repeated administration may induce unwanted antibody responses.

V. Summary and Outlook

Peptides play multiple roles in influenza virus research: from serving as “molecular shields” that directly inhibit viral entry and fusion, to acting as “immune modulators” that regulate host responses, to functioning as “core components” in novel vaccine design. Their greatest appeal lies in the ability to precisely target highly conserved “weaknesses” within the viral lifecycle, offering hope for developing broad-spectrum antiviral drugs and universal vaccines free from the constraints of seasonal updates.

Future advancements in this field will increasingly rely on multidisciplinary collaboration:

Computational and Structural Biology-Driven Design: Leveraging artificial intelligence to predict viral-host protein interaction interfaces and engineer ideal peptide sequences with ultra-high affinity and broad-spectrum activity.

Advanced Delivery Technologies: Integrating nanotechnology and novel biomaterials to develop smart delivery systems enabling sustained-release and targeted pulmonary delivery.

Combination therapy strategies: Exploring synergistic use of peptides with traditional small-molecule drugs, antibodies, or peptides with

different mechanisms to enhance efficacy and delay resistance.

Although translating investigational peptides into clinical drugs still faces challenges such as stability, delivery, and cost, with the continuous accumulation of knowledge in peptide chemistry, formulation science, and virology, peptides hold promise as a powerful weapon against future influenza threats, particularly against novel cross-species transmitted viral strains.