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REVIEW

Overview of host-directed antiviral targets for future research and drug development

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Abstract Viruses constitute a significant group of pathogens that have caused numerous fatalities and substantial economic losses in recent years, particularly with the emergence of coronaviruses. While the impact of SARS-CoV-2 appears to be diminishing in daily life, only a limited number of drugs have received approval or emergency use authorization for its treatment. Given the high mutation rate of viral genomes, host-directed agents (HDAs) have emerged as a preferred choice due to their broad applicability and lasting effectiveness. In contrast to direct-acting antivirals (DAAs), HDAs offer several advantages, including broad-spectrum antiviral activities, potential efficacy against future emerging viruses, and a lower likelihood of inducing drug resistance. In our review article, we have synthesized known host-directed antiviral targets that span diverse cellular pathways and mechanisms, shedding light on the intricate interplay between host cells and viruses. Additionally, we have provided a brief overview of the development of HDAs based on these targets. We aim for this comprehensive analysis to offer valuable perspectives and insights that can guide future antiviral research and drug development efforts.

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1. Introduction

Viruses, estimated to encompass approximately 10^{31} diverse species, have evolved in tandem with their hosts, notably humans, engaging in an enduring and subtle conflict[1]. Although most viruses do not lead to lethal outcomes in humans, several pandemics caused by viruses have occurred in the past 20 years, including severe acute respiratory syndrome coronavirus (SARS-CoV) (2002), "swine" influenza (2009), Middle East respiratory syndrome coronavirus (MERS-CoV) (2012), H1N1 chikungunya (2014), Ebola (2014), Zika (ZIKV) (2015), and most recently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (2019)[2,3]. The impact of SARS-CoV-2 has been profound, leading to a significant number of infections and fatalities, prompting a three-year disruption in both society and economics. Despite substantial progress in understanding SARS-CoV-2 and mitigating its virulence and morbidity, the emergence of viruses as high-risk pathogens in future outbreaks remains a foreseeable concern.

The conventional method for controlling virus-induced pandemics is vaccination, a convenient and cost−effective long-term measure[4-6]. However, challenges such as the expenses associated with low-temperature preservation and transportation, inadequate or absent immune responses in certain populations, and the limited effectiveness for individuals already infected render vaccination an incomplete solution for specific viruses[7]. An alternative option involves utilizing chemicals or natural substrate mimics inspired by virus−host interactions. Viruses possess a constrained genome size, encoding structural and non-structural proteins essential for their replication and propagation. Targeting virus-encoded proteins or other viral factors, such as the genome, represents an optimal approach, as the absence of human homologs would likely result in minimal cytotoxicity. Nevertheless, the heightened mutational frequency of viruses can render pathogen-targeted therapeutic interventions ineffective, particularly under selective pressures.

As a result, the scientific community is increasingly shifting towards an alternative strategy known as HDAs, which offer supplementary and compensatory options for combating viruses. In comparison to DAAs, HDAs present several advantages. Viruses are obligate parasitic pathogens that depend on host resources and cellular machinery for their survival. Targeting host-directed factors provides a broad-spectrum antiviral strategy, given that multiple viruses may utilize many of the same host factors during replication[8]. Additionally, compounds that interfere with host targets create a higher barrier to antimicrobial resistance due to the relatively stable host genome. However, the development of HDAs relies heavily on a comprehensive understanding of viral pathogenesis, which can be particularly challenging in unexpected pandemic scenarios. Understanding virus−host interactions and associated cellular pathways aids in the development of effective HDA strategies and facilitates the management of public health emergencies. This approach allows for the repurposing of existing drugs and offers insights into viral evolution and adaptation mechanisms within hosts.

In this review, we have compiled potential cellular targets involved in virus-host interactions, including microRNAs, transcription and nuclear factors, heat shock proteins, kinases and their associated signaling pathways, metabolism-related targets, the ubiquitin−proteasome system and ubiquitylation, and other host-directed targets. Furthermore, we have analyzed the progress in developing inhibitors and drugs based on these host-directed antiviral targets. While certain targets are known to be associated with specific viral infections, they may offer valuable insights applicable to other viral pathogenesis scenarios. Host-directed antiviral targets related to host immune and inflammation responses, which have been extensively covered elsewhere[9-11], are not discussed in this review.

2. MicroRNAs (miRNAs)

miRNAs are short, non-coding RNA sequences typically consisting of around 20 oligoribonucleotides. They are derived from larger RNA polymerase II (RNAP II) transcripts known as primary miRNAs (pri-miRNAs). A pri-miRNA can undergo processing to generate multiple miRNAs or a single miRNA. miRNAs combine with various components to form the RNA-induced silencing complex (RISC), with argonaute proteins playing a crucial role, particularly argonaute 2 (AGO2)[12-14]. miRNAs guide RISC to target mRNAs, primarily by recognizing the 3′UTRs of mRNAs, although they may also bind to the 5′UTR or the coding region[15-18]. The degree of complementarity between miRNAs and their target mRNAs dictates the fate of the mRNAs: direct degradation by AGO2 in cases of high complementarity, or translational repression in cases of lower complementarity (Fig. 1)[14,15].

Compared to other regulatory factors, miRNAs have a relatively recent history. The first discovery of miRNAs dates back to 1993 in *Caenorhabditis elegans*, with the identification of the miRNA *lin-4*, followed by *let-7*[19,20]. Through extensive research efforts, miRNAs were eventually acknowledged as a conserved group of endogenous regulatory factors present in various species, spanning animals and plants, and were officially termed miRNAs[21-24]. Over the course of approximately two decades, there has been a substantial expansion in the understanding of miRNAs, ranging from the identification of novel miRNAs to the elucidation of their roles in physiology and disease. The microRNA database miRbase now contains 38,589 entries, including 1917 miRNA entries from Homo sapiens (as of November 23, 2024)[25]. The discovery of miRNAs has significantly transformed the scientific comprehension of gene regulation, leading to Victor Ambros and Gary Ruvkun jointly receiving the Nobel Prize in Physiology or Medicine "for the discovery of microRNA and its role in post-transcriptional gene regulation"[26]. miRNAs introduce a novel mechanism of post-transcriptional gene regulation and play crucial roles in various cellular processes such as development, differentiation, proliferation, apoptosis, homeostasis, stress responses, and immune responses, particularly in IFN-mediated immune activation during viral infections[14,15]. Through interactions with their target mRNAs, miRNAs either degrade or transcriptionally repress these mRNAs *via* RISC, thereby shaping the cellular expression profile and influencing cellular evolution. Notably, based on reported and predicted miRNA targets, miRNAs are known to regulate over half of the protein-coding genes in humans[16].

In the context of viral infections, miRNAs play a dual role. On one hand, they can initiate an antiviral response by directly interacting with the viral genome or altering host mRNA expression patterns. Conversely, viruses may exploit host miRNAs to establish a more conducive cellular environment for their replication.

2.1. *Antiviral miRNAs*

Building upon prior research, McCaskill et al. expanded on the antiviral properties of miRNA mimics against influenza A virus (IAV) and respiratory syncytial virus (RSV). They pinpointed miR-124, miR-24, and miR-744 as targeting the p38 mitogen-activated

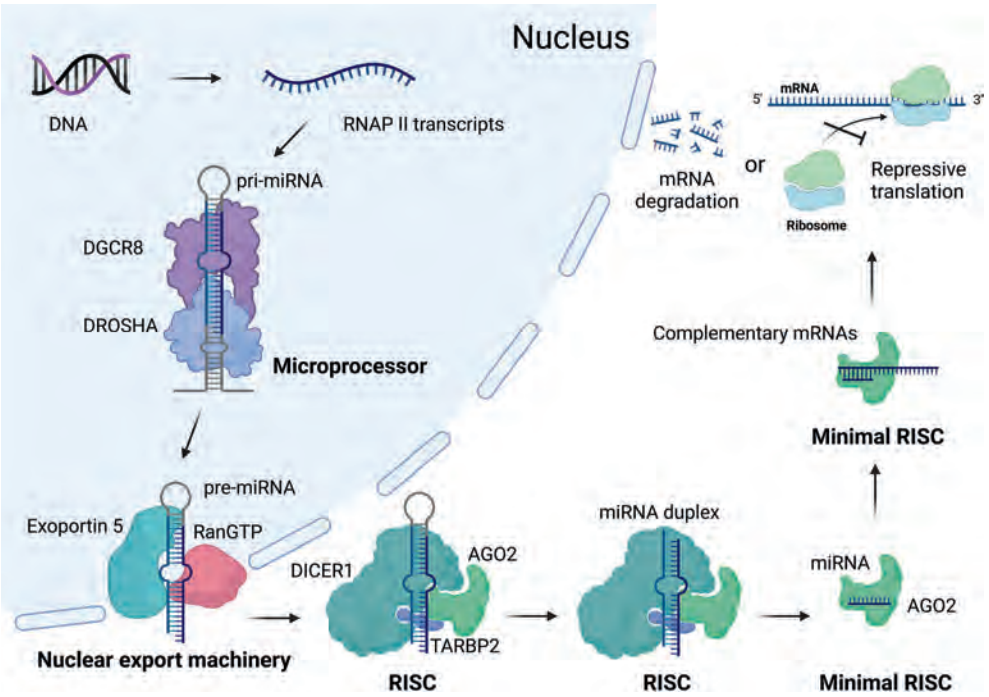

Figure 1 The biogenesis of miRNA. RNAP II transcripts (pri-miRNAs) undergo processing into miRNAs by the microprocessor and RISC, and meanwhile are transported out of the nucleus *via* nuclear export machinery. miRNAs associate with AGO2 to form the minimal RISC, guiding it to target mRNAs primarily by recognizing the 3′UTRs of mRNAs. The level of complementarity between miRNAs and their target mRNAs determines the fate of the mRNAs: direct degradation (high complementarity) or translational repression (lower complementarity). (Created in BioRender. Gu, X. (2025) https://BioRender.com/z08y439).

protein kinase (MAPK) signaling pathway, specifically honing in on the MAPK-activated protein kinase 2 (MK2) (Fig. 2). Meanwhile, MK2 was identified as a versatile antiviral target with potential for the development of therapies against both IAV and RSV[27].

A recent study has unveiled a novel pathway of IFN-mediated antiviral response that governs cholesterol biosynthesis through miR-342-5p. This miRNA employs a multi-hit strategy by repressively modulating key regulators (*SREBF2* and miR-33) and crucial enzymes (*IDI1* and *SC4MOL*) within the sterol pathway (Fig. 3). Leveraging the significance of this pathway, miR-342-5p achieves broad-spectrum antiviral activity against human cytomegalovirus (HCMV), herpes simplex virus 1 (HSV1), and IAV (H1N1)[28].

Several other antiviral miRNAs demonstrate diverse mechanisms in combating viral infections. For instance, miR-223 targets the Forkhead Box Protein O3 (FOXO3) to combat vesicular stomatitis virus (VSV), miR-let-7c targets the HO-1 transcriptional repressor Bach1 to fight against hepatitis C virus (HCV), and hsa-miR-1-3p directly inhibits the supportive host factor ATP6V1A to counteract influenza virus H1N1[29-31]. These examples showcase the varied ways in which miRNAs can be utilized in antiviral strategies, whether by modulating the host's immune response or by disrupting the virus's ability to exploit host factors for replication.

2.2. *Proviral miRNAs*

Host miRNAs can modulate proviral activity by interfering with host antiviral immune responses, with their effects varying depending on the specific virus. For instance, miR-124, known for its antiviral activity, has been found to exhibit proviral activity against enterovirus 71 (EV71) by targeting *IL-6R* and *STAT3* mRNAs (Fig. 4)[32]. This highlights the need for further exploration into the diverse roles miRNAs play across various viral pathogens.

Another miRNA, miR-1225-3p, is down-regulated by type I interferon through the IFN/JAK/STAT signaling pathway. Its inhibition leads to increased expression of growth factor receptor-bound protein 2-associated binding protein 3 (GAB3) (Fig. 4), which enhances antiviral responses against multiple IFN-susceptible viruses, including HCV, Sendai virus (SeV), and Newcastle disease virus[33]. The study suggests that the down-regulation of miR-1225-3p may serve as a mechanism by which host cells defend against viral infection by boosting the antiviral response. This discovery not only elucidates the biological function of miR-1225-3p but also proposes a novel antiviral regulatory pathway involving miRNA and GAB3.

Two other miRNAs, miRNA-548 and miRNA-23a, are also engaged in the IFN response by targeting IFN-λ1 and IRF1 (Fig. 4), respectively, thereby promoting the replication of EV71 and VSV, or human HSV1 separately[34,35].

Toll-like Receptors (TLRs) are pivotal pattern recognition receptors for pathogens that mediate innate immune responses against viruses. For instance, miR-135a targets myeloid differentiation primary response 88 (MyD88) in the TLR signaling pathway, along with related serine/threonine kinase 2 (RIPK2) and the antiviral chemokine CXCL12 (Fig. 4), thereby preferentially regulating HCV propagation[36]. Similarly, miR-125a, upregulated in response to HCV infection, suppresses the expression of mitochondrial antiviral signaling (MAVS) and TNF receptor-associated factor 6 (TRAF6) (Fig. 4), both crucial components of the antiviral IFN response and TLR signaling pathway[37].

Furthermore, proviral miRNAs like miR-214 facilitate viral infections by enhancing host supportive factor thrombin while

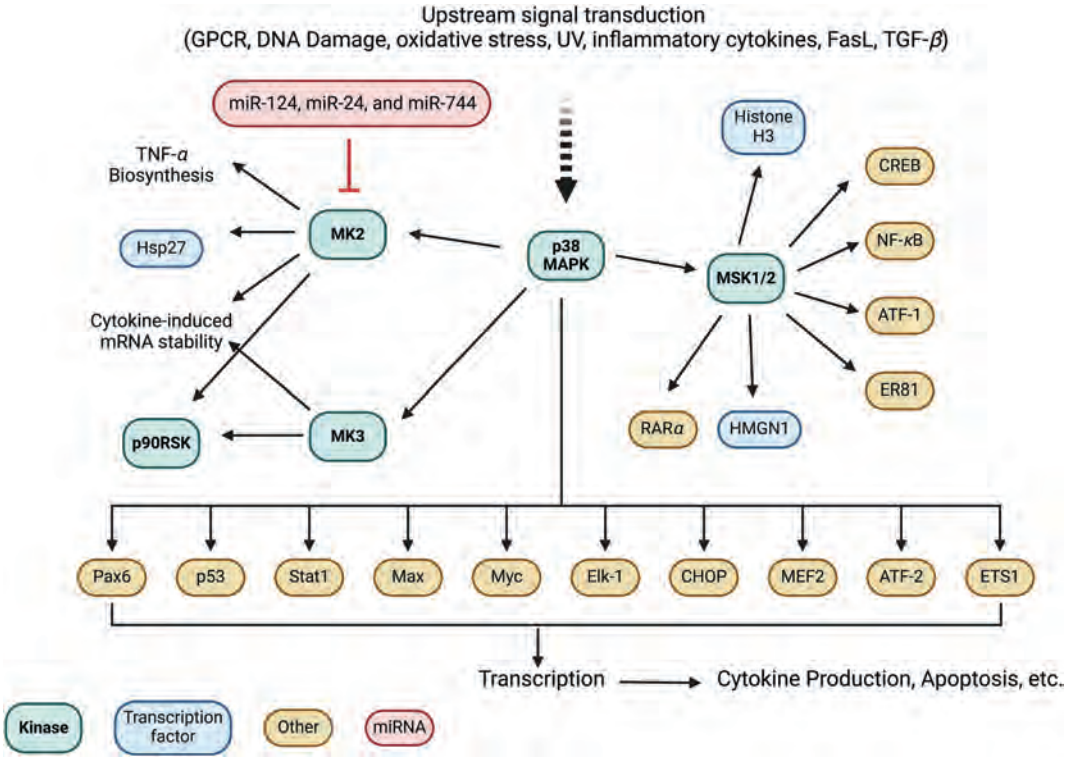

Figure 2 The antiviral mechanism of miR-124, miR-24, and miR-744. The p38 MAPK pathway receives upstream signal transduction, including signals from GPCR, DNA damage, oxidative stress, UV radiation, inflammatory cytokines, FasL, and TGF-β, and regulates downstream effectors, activating transcription and leading to cytokine production, apoptosis, and other cellular responses. miR-124, miR-24, and miR-744 have been identified as targeting the p38 MAPK signaling pathway, specifically focusing on the MK2 kinase. (Created in BioRender. Gu, X. (2025) https://BioRender.com/z08y439).

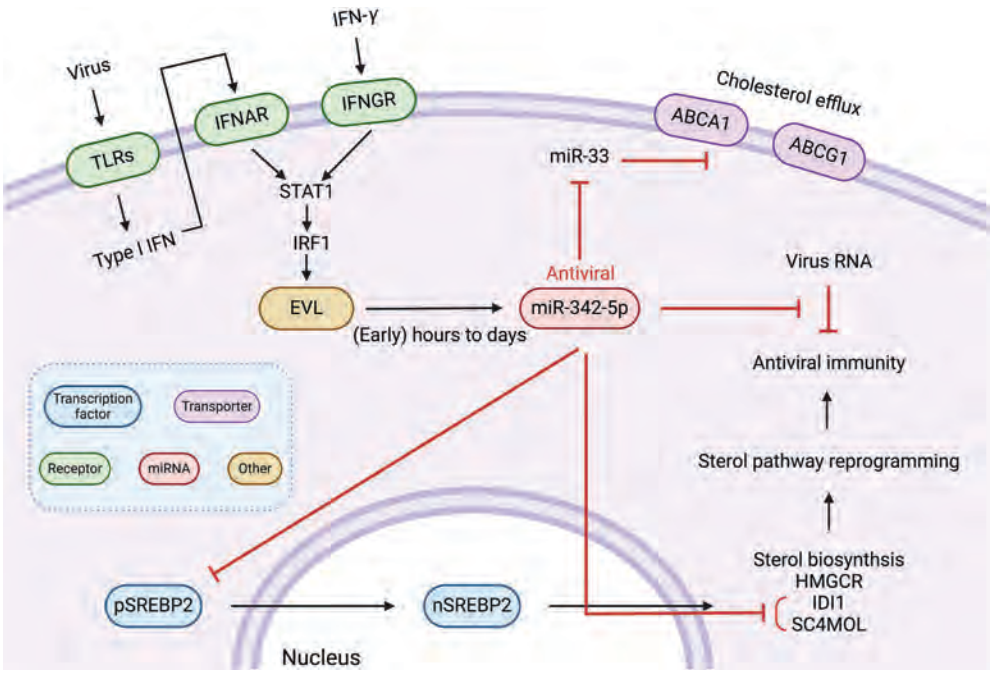

Figure 3 The mechanism of miR-342-5p regulating sterol metabolism. miR-342-5p targets the mevalonate-sterol biosynthesis pathway through a multi-hit mechanism, suppressing the pathway at various functional levels: transcriptionally *via SREBF2*, post-transcriptionally *via* miR-33, and enzymatically *via IDI1* and *SC4MOL*. This leads to a reduction in intermediate sterol pathway metabolites and total cholesterol levels. (Created in BioRender. Gu, X. (2025) https://BioRender.com/z08y439).

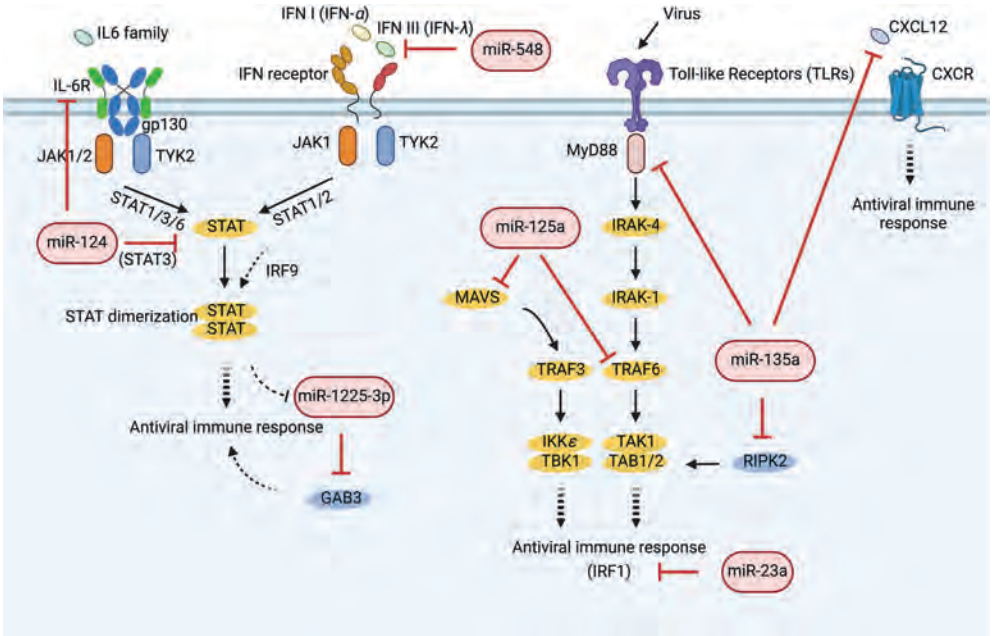

Figure 4 The proviral mechanisms of miRNAs. The IL-6 family/IL-6R, IFN I/IFN receptors, TLRs, and CXCL12/CXCR pathways mediate diverse antiviral immune responses. In contrast, miRNAs such as miR-124, miR-1225-3p, miR-548, miR-23a, miR-135a, and miR-125a disrupt these antiviral responses at various levels. (Created in BioRender. Gu, X. (2025) https://BioRender.com/z08y439).

simultaneously targeting the antiviral factor 2′,5′-oligoadenylate synthetase[38]. This regulatory effect of miR-214 may create a conducive environment for viral replication while evading the host immune response.

The modulation of gene expression by miRNAs can occur through various mechanisms. Some miRNAs directly target mRNAs[32], while others influence positive or negative regulatory factors[28,31,35,38]. This diversity in miRNA-mediated regulation enables precise control of gene expression in response to viral infections.

It is crucial to recognize that the antiviral or proviral factors and signaling pathways regulated by miRNAs are not mutually exclusive. For example, FOXO3, MAVS, TRAF6, and GAB3 are all linked to the IFN-mediated antiviral response[30,33,37], emphasizing the multi-hit regulatory nature of miRNAs. Furthermore, miRNAs and the IFN pathway can directly or indirectly regulate each other, forming a regulatory loop or network against viral infections[30,35]. This intricate interplay between miRNAs and the IFN pathway adds an additional layer of control to the host's antiviral defenses.

This multi-layer regulatory mechanism may assist host cells in effectively combating viral infections while preventing excessive immune responses that could lead to damage. Simultaneously, this complex regulatory network may offer viruses multiple avenues to evade the host's immune defenses, contributing to the ongoing evolutionary battle between host and virus. Exploring the interaction between these miRNAs and their targets, whether from hosts or viruses, is crucial for developing novel antiviral therapeutic strategies. Modulating miRNAs or their targets in immune responses could enhance the host's antiviral capabilities or mitigate pathological damage caused by viral infections.

As a relatively new and efficient post-transcriptional genetic regulatory mechanism, it was soon discovered that miRNAs are encoded not only in living organisms but also in viruses[39]. Compared to viral protein-mediated gene regulation, viral miRNAs offer several advantages in intervening with cellular activities and promoting viral replication, such as occupying much less space in the limited genome and being less likely to induce immune responses due to their non-immunogenic nature[14,40]. Overall, viral miRNA-mediated gene regulation creates a conducive cellular environment for virus replication and propagation by aiding immune evasion during early viral entry and infection, counteracting apoptosis, and maintaining viral latency in later infected cells[41-43]. Both cellular and viral miRNAs have the potential to serve as targets for antiviral interventions due to their extensive role in post-transcriptional regulation[44].

2.3. *Strategies for targeting miRNAs*

In the realm of drug development, the viable strategy for targeting miRNAs in antiviral therapy encompasses several key elements. Firstly, the utilization of miRNA mimics and anti-miRNA therapy plays a pivotal role. miRNA mimics are employed to restore or enhance the function of miRNAs that are diminished or underexpressed in diseases, while anti-miRNAs function by binding to overexpressed endogenous miRNAs, thereby silencing their activity. Secondly, miRNAs are harnessed for viral inhibition, exemplified by Miravirsen, an antisense RNA strand employing locked nucleic acid (LNA) technology to target the 5′ end of miR-122 for treating HCV infection. miR-122 is a supportive host factor for HCV infection and related hepatocellular carcinoma[45]. Miravirsen has exhibited efficient liver delivery, reduced cholesterol accumulation, and diminished HCV titers. Even though the clinal trial of Miravirsen was ceased, there are still more successors on the way, such as RG-101, the next-generation GalNAc-conjugated antagomiR against miR-122 under Phase II clinical trial[46]. Furthermore, disease-specific miRNA drug development is exemplified by Phase II clinical trials for MRG-201 and MRG-106, miR-29 analogs for scleroderma, and antimiR-155 nucleotides for fungoid cutaneous T-cell lymphoma.

Nevertheless, targeting miRNAs for antiviral therapy presents several challenges. Firstly, there is difficulty in target selection and validation due to the intricate nature of miRNAs targeting multiple genes simultaneously, making it exceedingly complex to accurately discern their mechanisms of action. Despite the availability of advanced algorithms, extensive sequence data, and tools like the MiRBase database to aid in predicting miRNA−mRNA binding sites, the functions of most miRNAs remain ambiguous and cannot be validated across all biological contexts. Secondly, concerns regarding off-target effects and safety arise from the multi-target nature of miRNAs, potentially leading to unintended consequences during treatment, such as the inhibition or activation of non-target genes, resulting in adverse reactions. For instance, anti-miRNA therapy may inadvertently impact certain tumor suppressor genes or genes crucial for normal cell homeostasis, disrupting fundamental cellular functions. Lastly, delivery and toxicity issues pose significant challenges as the delivery system for miRNA therapy must ensure effective targeting of the drug to the intended tissue with minimal toxicity. Currently, the development of delivery systems remains a hurdle, necessitating the reduction of side effects while ensuring efficacy. In conclusion, while antiviral therapy targeting miRNAs holds substantial promise, numerous challenges including target selection, off-target effects, and delivery system development must be addressed before clinical application can be realized.

In addition to their antiviral potential, miRNAs can serve as clinical biomarkers for chronic and persistent viral infections, including human immunodeficiency virus, human papillomavirus, and HCV. In some instances, miRNAs can also function as predictive biomarkers for prognosis, particularly for carcinogenic viruses[47-53]. As modern medical practices increasingly focus on precise and personalized healthcare, miRNA profiles could offer a robust diagnostic tool for identifying pathogen-resistant or susceptible populations. However, current research in this area predominantly concentrates on plants and livestock[54-62]. Furthermore, specific miRNAs combined with oncolytic viruses can be formulated as novel targeted anti-cancer agents[63-65], holding promise for the development of more effective and targeted cancer therapies.

The methodologies employed to identify cellular or viral miRNAs and investigate their functional roles can inspire other genetic research endeavors. These methods encompass next-generation sequencing technology, genome-wide miRNA functional screening, in silico prediction or bioinformatics-based integrative analysis, transcriptomics analysis, gene reporter assays, and various other techniques[66-70]. These approaches provide valuable tools for unraveling the intricate roles of miRNAs in viral infections and other biological processes, contributing to the advancement of new antiviral strategies and drug targets.

3. Transcription and nuclear factors

Transcription factors play a pivotal role in governing gene expression, exerting a profound impact on cellular adaptation in reaction to internal and external cues. Acting as key regulatory proteins, they act as mediators that bridge various cellular antiviral responses, rendering them significant focal points for antiviral investigations. Despite being historically deemed challenging to target pharmacologically, recent research and clinical trials are progressively endorsing a reassessment of transcription factors as promising targets within the antiviral domain[71,72]. Nevertheless, caution is warranted when targeting transcription factors due to their broad-reaching effects on gene expression and diverse cellular functions.

3.1. Interferon regulatory factors (IRFs)

Upon viral infection, host cells typically initiate the innate immune response by engaging pattern recognition receptors and subsequent adaptor proteins like STING and MAVS, along with effector kinases. These signaling cascades culminate in interferon-mediated antiviral signaling, a process tightly regulated by IRFs, notably IRF3 and IRF7[73]. Given their pivotal role in antiviral defenses, IRFs are frequently targeted by viral proteins (Fig. 5). For instance, rotavirus (RV) NSP1, pestivirus Npro, pseudorabies virus (PRV) US3, and H1N1 IAV PA have all been identified as antagonists of IRF3[74-77]. Similarly, duck hepatitis A virus (DHAV)-1 3C, PRV UL24, and H1N1 IAV NS2 have been reported to interfere with IRF7[78-80]. The direct interaction between these viral proteins and IRFs disrupts the activation and downstream signaling of IRFs, sometimes leading to their degradation. This manipulation empowers the virus to effectively subvert the host's innate antiviral response, thereby facilitating its replication and spread.

IRFs and their associated antiviral responses are under the regulation of host restriction factors to uphold physiological homeostasis. However, this regulatory framework also presents opportunities for viruses to manipulate the network and undermine host immune defenses (Fig. 5). For instance, NBR1 acts as a cargo receptor, sequestering IRF3 into autophagosomes for degradation, a process that is exploited by SeV to enhance viral replication[81]. Similarly, A20, induced by the IAV NS1 protein, suppresses the IRF3 signaling pathway[82], enabling viruses to evade the host's antiviral response.

AGO2, another restriction factor, impedes the assembly of the transcriptional complex involving IRF3 and CBP/p300 in the nucleus. H5N1 infection disrupts the nuclear distribution of AGO2, leading to the activation of the IFN signaling pathway[83].

Additional restriction factors like human noncoding RNA nc886, Rubicon, and Fas-associated factor 1 (FAF1) have been identified to negatively modulate the IRF3-mediated antiviral pathway[84-86]. However, their specific interactions with viral infections remain unclear and necessitate further exploration.

In addition to exploiting cellular factors, viruses can encode their own IRF to subvert the host immune response and promote pathogenesis[87]. These viral IRF homologs mimic host IRF functions, enabling the virus to manipulate antiviral signaling pathways and create a conducive environment for viral replication. Investigating viral IRF homologs offers valuable insights into host−virus interactions and viral strategies to evade immune responses, potentially guiding the development of innovative antiviral approaches.

A comprehensive understanding of the interplay among host restriction factors, IRFs, and viral proteins is essential for delineating host−virus dynamics. This knowledge may inspire the design of therapies aimed at restoring IRF function and bolstering the host's antiviral response[88,89]. Exploring the repercussions of IRF inhibition by viral proteins can shed light on the broad impact of viruses on the host immune system and response to viral infections. Furthermore, unraveling how host restriction factors regulate IRF-mediated antiviral pathways can offer insights into maintaining homeostasis and preventing excessive immune responses that could lead to tissue damage.

Moreover, IRFs could potentially serve as biomarkers for antiviral therapy[90]. Monitoring IRF expression levels or activation

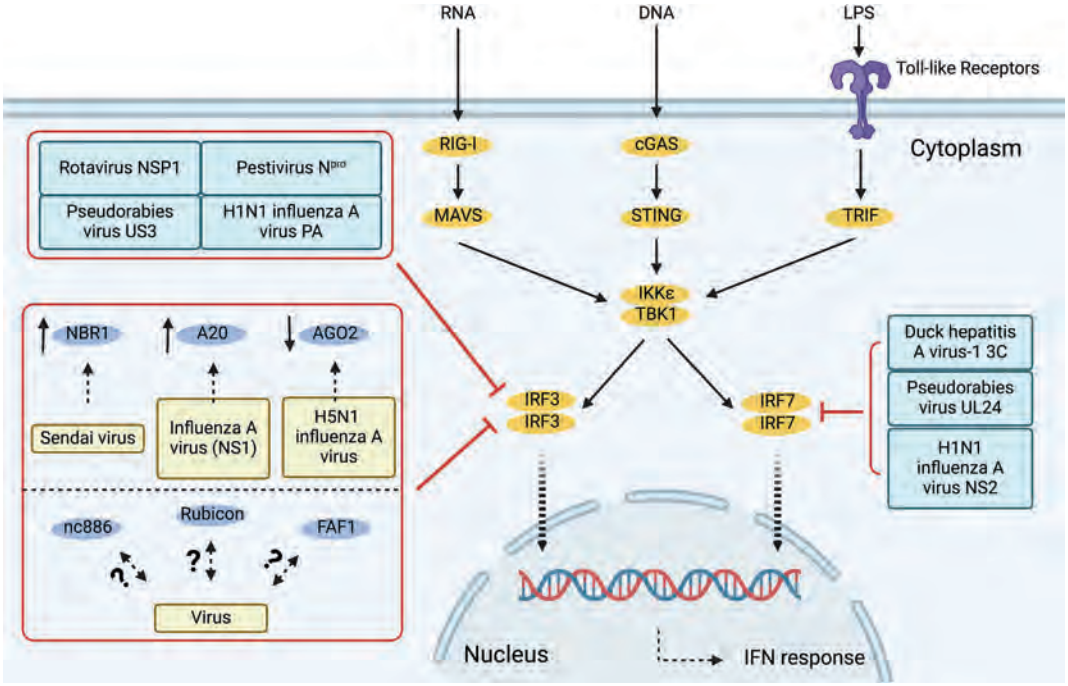

Figure 5 The IRF-mediated antiviral response and their regulation by viruses. IRF3 and IRF7 are activated by upstream signal transduction triggered by stimuli like DNA, RNA, and LPS from intracellular or extracellular sources, leading to the activation of the interferon response. Given their critical functions in antiviral responses, various viruses have developed specific proteins that target IRF3 and IRF7. (Created in BioRender. Gu, X. (2025) https://BioRender.com/z08y439).

status during viral infections may enable clinicians to evaluate the efficacy of antiviral treatments and make informed decisions regarding patient care.

3.2. Zinc finger antiviral protein (ZAP)

Metal ions play crucial roles in biological systems, with Zn(II) ions being particularly essential for approximately 10% of encoded proteins[91,92]. This section delves into a specific group of Zn(II)-binding proteins that possess one or multiple domains coordinating with Zn(II), known as zinc finger domains. Zinc finger domains represent common DNA-binding domains in transcription factors, playing a pivotal role in gene regulation. These domains are prevalent in cellular proteins and contribute to the resilience of animals and plants against external stress[93-99].

One notable example is ZAP, also referred to as zinc finger CCCH-type antiviral protein 1 or inactive poly (ADP-ribose) polymerase 13 (PARP13). ZAP comprises three integrated domains: the N-terminal domain, the central domain, and a third PARP-like domain, with various signal peptides and cofactor binding sites intersecting these domains. The N-terminal domain contains four C3H1-type zinc finger domains that bind to RNA. In conjunction with diverse cofactors like poly(A)-specific ribonuclease PARN and the decapping complex DCP1−DCP2, this domain facilitates the degradation of viral mRNAs from both ends[100]. The central domain of ZAP includes a fifth zinc finger domain and two WWE domains that bind to ADP-ribose. Although the PARP-like domain of ZAP reportedly lacks ADP-ribosyltransferase activity, the binding of ADP-ribose enhances its antiviral function. Conversely, a single Q668R mutation disrupts poly(ADP-ribose) (PAR) binding and reduces ZAP's antiviral efficacy[101]. The absence of ADP-ribosyltransferase activity is attributed to the closure of the NAD^+ binding cleft, facilitated by a newly formed short α-helix (Asp803−His807), the hydrogen bond between His810 and Tyr826, and the absence of PARP consensus residues crucial for nicotinamide anchoring and catalysis (Fig. 6)[102].

ZAP typically identifies viral genomes through ZAP-responsive elements (ZREs) that consist of high GC content. Consequently, frequent CpG nucleotides, which invariably result in high GC content, are considered targets of ZAP. Viruses, adapting over time to host antiviral immune pressures, have shown a gradual decrease in CpG dinucleotides, a trend observed in viruses like SARS-CoV-2 and related species[103]. Analyzing and comparing CpG dinucleotides in viral genomes can aid in vaccine development[104]. To effectively exert its antiviral function, ZAP must discern between host and viral genomes based on CpG dinucleotide content. Moreover, ZAP's role in IFN-mediated antiviral responses involves recognizing CpG dinucleotides, influencing the gene expression of related interferon-stimulated genes (ISGs) and interferon-repressed genes (IRGs)[105]. However, CpG dinucleotides alone do not solely determine ZAP recognition, as evidenced by the lack of a positive correlation between lentiviral vector production and CpG abundance[106]. The precise targeting mechanism of ZAP remains to be fully elucidated.

Furthermore, a multitude of nuclear factors partake in host antiviral defenses and host−virus interactions. Some of these factors are integral to IFN signaling or other antiviral immune responses, including various ISGs and modulators[107,108]. Others play crucial roles in normal cellular functions, even during viral infections, such as importin proteins, mRNA splicing factors, or complexes[84,109-113]. Effector molecules implicated in other diseases, like p53 linked to cancer, also exhibit activity in viral infections[114-116]. As scientific understanding advances, more instances of such crossovers are anticipated to emerge.

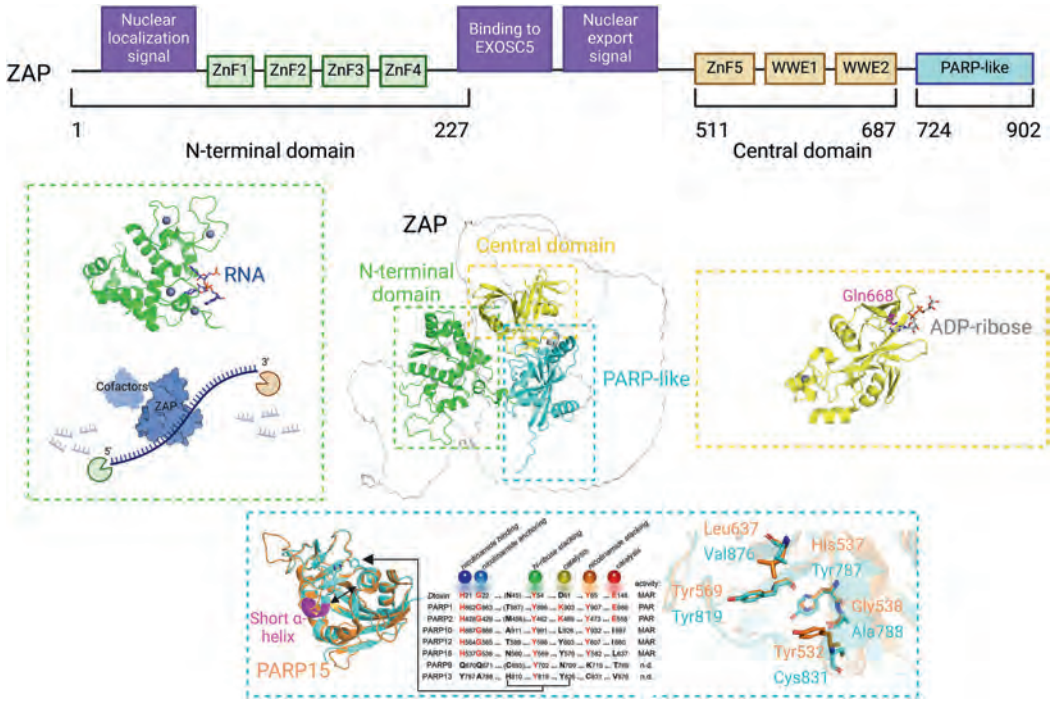

Figure 6 The domain arrangement of ZAP and schematic of each domain's function. The comparison of PARP consensus residues is sourced from a recent report[102]. The full-length structure of ZAP is revealed through the AlphaFold-predicted model, while the complex structures of the N-terminal domain with RNA, the central domain with ADP-ribose, and the alignment of the PARP-like domain with PARP15 are elucidated by PDB entries 6UEJ, 7TJQ, 2X5Y (and 3BLJ for PARP15), respectively. (Created in BioRender. Gu, X. (2025) https://BioRender.com/z08y439).

Despite the significant involvement of transcription and nuclear factors in host−virus interactions, targeting these factors poses challenges due to their structural diversity, limited druggable sites, nuclear localization, and multifaceted cellular functions. Nonetheless, with technological progress, certain factors previously deemed "undruggable" have shown promise for drug development. These advancements offer valuable insights and avenues for crafting innovative antiviral therapeutic strategies and identifying potential drug targets.

4. Heat shock proteins (Hsps)

Hsps have long been acknowledged for their significance in viral infections since the previous century[117-122]. The interplay between Hsps and viral proteins, along with the roles of Hsps in viral replication and transcription, has been progressively explored. Hsps, a class of molecular chaperones, play pivotal roles in various cellular processes beyond the well-studied heat shock response. Operating at the core of the protein quality control system, Hsps, in conjunction with their adaptable co-chaperones or complexes, facilitate the proper folding, refolding, and degradation of specific client proteins. These diverse functions of Hsps are intricately tied to their ATPase activity, with the affinity for client proteins being modulated by their ATP-bound state. Additionally, a variety of co-chaperones or complexes regulate the interactions of Hsps with client proteins based on their specificity, collectively overseeing the diverse cellular functions of Hsps[123-127].

Given the central involvement of Hsps in cellular processes and the reliance of viruses on host translation machinery, the association of Hsps with viral infections is expected. Among the array of Hsps, Hsp70 and Hsp90 have been extensively studied. Multiple investigations have demonstrated that Hsp70 plays a role in various stages of the viral infection cycle, encompassing entry, replication, assembly, and release from host cells (Fig. 7). Hsp70 also influences viral protease activity and stabilizes viral proteins through its chaperone function[128-134]. Hsp90 collaborates in these processes, particularly during the assembly phase[131]. The proviral impact of Hsp70 is evident across a spectrum of viruses, including several flaviviruses (such as ZIKV, dengue, yellow fever, West Nile, and Japanese encephalitis viruses), primate lentiviruses (such as HIV-1, HIV-2, and simian immunodeficiency viruses SIV_{MAC} and SIV_{AGM}), avian virus CELO, porcine epidemic diarrhea virus (PEDV), Hepatitis B virus (HBV), and IAV. Different viruses utilize distinct viral proteins to engage with Hsp70, enhancing or relocating its expression to facilitate specific stages in their replication cycles. Furthermore, the induced expression and proviral impact of Hsp70 have been observed in virus-infected plants[135].

While Hsps have been associated with proviral effects in numerous viral infections, several studies have indicated that Hsps can also exhibit antiviral effects, potentially through the degradation of viral proteins[136-139]. Nonetheless, chemical inhibitors, antibodies, or competitive recombinant Hsp proteins have been demonstrated to reverse the proviral function of Hsps in viral replication, suggesting their therapeutic potential against viruses[130,140-146].

4.1. Representative Hsp70 inhibitors

It is anticipated that Hsp70 inhibitors will exhibit broad-spectrum antiviral activity. The high affinity of Hsp70 for ADP and the conformational state of Hsp70 make the ATP-binding site challenging to access, posing difficulties in designing inhibitors that target these sites. Several representative Hsp70 inhibitors are detailed in Supporting Information Table S1.

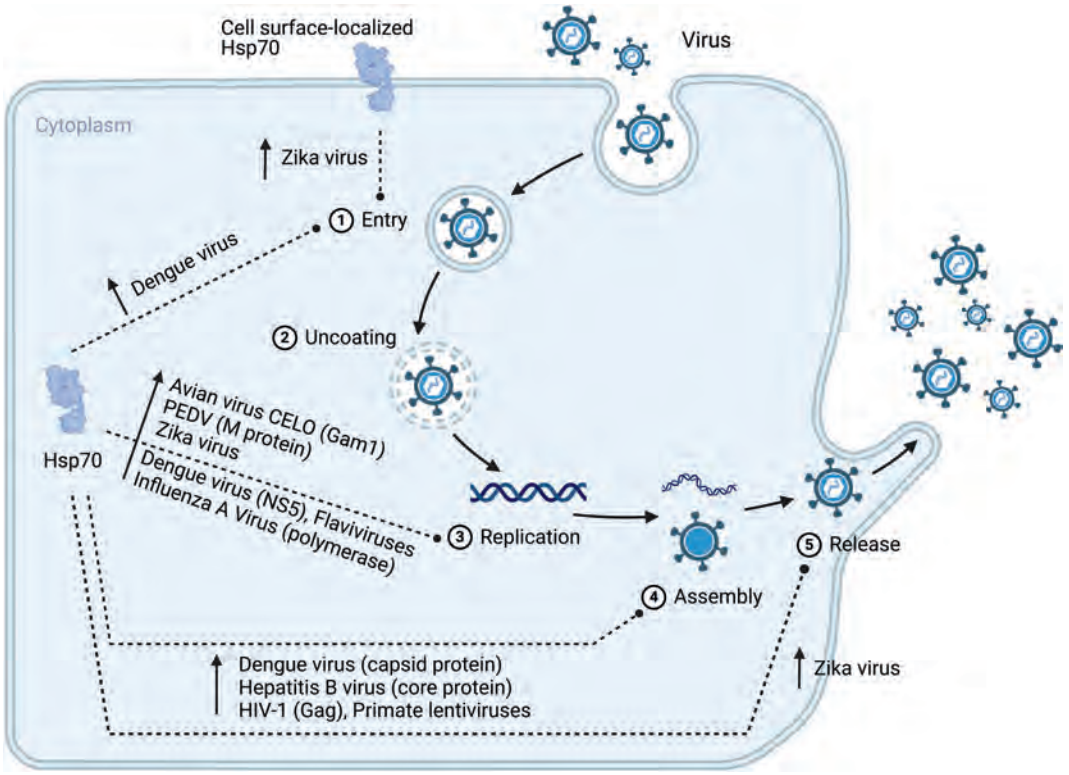

Figure 7 The proviral effects of Hsp70 impact multiple stages of the viral infection cycle, such as entry, replication, assembly, and release, and involve various viruses, with their interacting proteins specified in the brackets. (Created in BioRender. Gu, X. (2025) https://BioRender.com/z08y439).

MKT-077 functions by inhibiting Hsp70 through disrupting its interaction with nucleotide exchange factors, leading to the release of Hsp70 binding substrates[147]. MKT-077 and its analogues JG-18 and JG-40 have been shown to inhibit the transmission of DENV, with no observed toxicity to host cells at concentrations that effectively inhibit viral replication[129]. Another inhibitor, HS-72, selectively targets Hsp70i (the inducible isoform of Hsp70) by focusing on an allosteric site. HS-72 has been demonstrated to inhibit DENV entry primarily by disrupting the binding of Hsp70i to the DENV receptor complex[148]. On the other hand, IMB-DM122, a derivative of the natural compound oxymatrine, acts as a downregulator of Hsc70 (the constitutive isoform of Hsp70). IMB-DM122 effectively reduces the encapsidation of Hsc70 into HCV virion particles by targeting the Hsc70 mRNA 3′ untranslated region sequence and destabilizing the mRNA. This action limits HCV assembly and restricts its ability to infect cells[149].

4.2. Representative Hsp90 inhibitors

Several representative Hsp90 inhibitors are listed in Table S1, with the most renowned being geldanamycin[150]. Geldanamycin was the first Hsp90 inhibitor identified to bind to the N-terminal ATP-binding pockets. It has been reported to exhibit antiviral activity against a range of viral infections, including HSV2, HSV1, HCMV, EBOV, HIV1, and influenza viruses. However, due to its poor water solubility and severe liver toxicity, geldanamycin has undergone structural modifications. Modified 17-AAG has been shown to inhibit RSV replication at concentrations as low as 1.9 nmol/L[151].

Radicicol, an Hsp90 inhibitor derived from *Monosporium bonorden*[152], destabilizes the newly synthesized L protein (the large subunit of VSV polymerase) by inhibiting Hsp90. This action has been demonstrated to inhibit the replication of SV5, HPIV-2, HPIV-3, SV41, and La Crosse Bunyavirus[153]. Gedunin, a noncompetitive Hsp90 inhibitor *versus* ATP, has also been reported to inhibit DENV *in vitro* with an EC_{50} value of 10 μmol/L[154].

Many Hsp90 inhibitors in clinical trials are linked to serious side effects such as cardiotoxicity and gastrointestinal toxicity. Subtype-selective Hsp90 inhibitors may present a novel strategy for developing broad-spectrum antivirals.

In a study, interfering with the upstream regulator of Hsps, heat shock transcription factor 1 (HSF1), using its dominant-negative mutant (mHSF1), reversed Hsp70's anti-apoptotic function and induced cancer cell death[155]. This approach could be a valuable consideration for intervening in the proviral influence of Hsps, alongside the use of chemical inhibitors or antibodies. Moreover, leveraging the interaction of Hsps with viral proteins could enable the use of viral proteins or even entire virions for delivering Hsps, allowing them to exert their cytoprotective effects[156].

5. Kinases and their related signaling pathways

Kinases, a group of post-translational regulators, play a crucial role in connecting various intracellular or intercellular signaling pathways and are involved in numerous physiological events. The study of cellular kinases dates back to the 1950s, and their wide-ranging and critical involvement in life sciences led to researchers Edmond H. Fischer and Edwin G. Krebs being awarded the Nobel Prize in Physiology or Medicine in 1992 for their discoveries related to "reversible protein phosphorylation as a biological regulatory mechanism"[157]. The term "kinome" was later

introduced as a counterpart to the "genome", underscoring the significance of kinases and advocating for their systematic study[158]. Cellular kinases and their associated signaling pathways serve as important regulatory factors, functioning not only in physiological conditions but also in diseases such as viral infections and cancer[159,160]. While most kinase inhibitors are approved for use as anti-cancer agents, recent research has increasingly recognized their potential antiviral effects, particularly through drug repurposing during various virus-caused pandemics[161-165].

It is worth noting that viruses also encode their own kinases, although this discovery occurred much later than the identification of host kinases[166-168]. Viral kinases play a vital role in pathogenesis, although they are not the primary focus of this review, and only a brief introduction will be provided regarding their role in viral infections.

5.1. IKKε and TBK1

During viral infections, cellular kinases can play a crucial role in mediating antiviral responses by regulating related signaling pathways. For example, kinases such as IKKε and TBK1 are involved in RIG-I/MAVS and TLR signaling pathways.

In the context of the prototypic arenavirus lymphocytic choriomeningitis virus (LCMV), the nucleoprotein (NP) binds to the kinase domain (KD) of IKKε. This interaction blocks the autocatalytic activity of IKKε, thereby preventing the IRF3-mediated antiviral response to SeV infection. This mechanism is conserved among various arenaviruses, including the Old World arenavirus Lassa virus (LASV) and New World arenaviruses from clades A (White Water Arroyo virus (WWAV)), B (Junin virus (JUNV)), and C (Latino virus (LATV))[169].

Similarly, in the case of HSV1, the tegument protein UL46 interacts with TBK1. This interaction leads to a reduction in TBK1 activation and its downstream signaling. UL46 specifically inhibits the dimerization of TBK1 and interferes with its interaction with IRF3, ultimately inhibiting IRF3 activation and the subsequent production of type I interferons (IFN-I)[170].

5.2. p38 MAPK

The p38 MAPK signaling pathway is involved in a variety of cellular processes in response to environmental stresses and inflammatory cytokines, encompassing proliferation, development, differentiation, transformation, and apoptosis. While p38 MAPK can facilitate antiviral immune responses by stimulating transcription and cytokine production, promoting immune cell proliferation and differentiation, it can also support viral replication by modulating translation and the cell cycle. Early apoptosis during viral infection often impedes productive virus proliferation. Viral proteins like HIV-1 Nef interact with kinases (such as the CAMKIIδ−ASK-1 complex) within the pathway, disrupting p38 MAPK-mediated apoptosis. Competitive peptide inhibitors targeting Nef binding domains to CAMKIIδ have reversed this effect, restoring p38 MAPK phosphorylation and apoptosis[171]. Effective antiviral treatments have been demonstrated by targeting other kinases in the p38 MAPK pathway, including p38 itself (SB203580 and SB202190), MSK1/2 (H89 and SB747651A), and MNK1 (CGP57380) (Table S1)[172,173]. Given its influence on cytokine production, inhibiting this pathway could prevent virus-induced cytokine storms and potentially enhance prognosis.

5.3. RIPK3 and MLKL

Necroptosis, akin to apoptosis, serves as an early defense mechanism against viral infections and is recognized as a component of innate immunity. This process relies on two key effectors: receptor-interacting protein kinase 3 (RIPK3) and the pseudokinase mixed-lineage kinase-domain-like (MLKL). Certain poxviruses carry MLKL homologs in their genomes, which imitate the function of MLKL and bind RIPK3, thereby obstructing cellular MLKL activation and preventing necroptotic cell death[174]. However, in the later stages of viral infections, viruses tend to promote apoptosis and necroptosis to hasten replication and dissemination. Following 6 h of Rhinovirus infection, the phosphorylation of MLKL (indicative of necroptosis) coincides with caspase cleavage (an apoptotic marker), leading to compromised plasma membrane integrity and the release of alarmin molecules into the culture media. The application of inhibitors targeting both necroptosis and apoptosis reversed the cellular damage induced by the release of viral progeny[175].

5.4. AMPK

The AMPK signaling pathway, functioning as a cellular energy sensor, plays a pivotal role in various cellular processes such as cell growth, proliferation, autophagy, stress response, and metabolic reprogramming (Fig. 8). These processes are crucial for viral propagation and can be exploited by viruses. Manipulating this pathway offers a means to regulate viral infections. For instance, metformin, a biguanide derivative prescribed for type 2 diabetes mellitus (T2DM), was repurposed for COVID-19 treatment during the pandemic. Subsequent research elucidated its antiviral mechanism, which involves AMPK activation and associated metabolic reprogramming[176]. The intricate nature of the AMPK pathway in viral infections underscores the necessity for caution when devising AMPK-targeted antiviral strategies. While AMPK activators may impede viral replication in certain scenarios, they could potentially facilitate viral replication or exacerbate inflammatory responses in others[176,177]. Therefore, comprehending the specific mechanisms of AMPK in distinct viral infections is paramount for the development of efficacious antiviral therapies.

5.5. Pyruvate kinase (PK)

Another instance pertinent to energy metabolism and viral infections involves PK. Like AMPK, PK plays a pivotal role in cellular processes, exhibiting versatility in its interactions with viruses. Pyruvate kinase muscle type 2 (PKM2) has been identified as an antiviral factor by incorporating into virions and hindering their ability to assimilate cellular tRNAs such as $tRNA^{Lys3}$, $tRNA^{Lys1,2}$, and $tRNA^{Asn}$, thereby diminishing HIV-1 virion infectivity[178]. In the case of tomato bushy stunt virus (TBSV), the virus exploits PK to boost ATP levels, aiding in the function of recruited cellular DEAD-box helicases and consequently enhancing the production of viral (+)RNA progeny[179]. Furthermore, IAV NP and matrix protein (M1) both interact with pyruvate kinase along with another host glycolytic enzyme, alpha-enolase[180,181]. Nevertheless, the study did not elaborate on the implications of these interactions in viral infections.

5.6. Others

Focal adhesion kinase (FAK), a non-receptor tyrosine kinase, and casein kinase 2 (CK2), a ubiquitous serine/threonine kinase, are

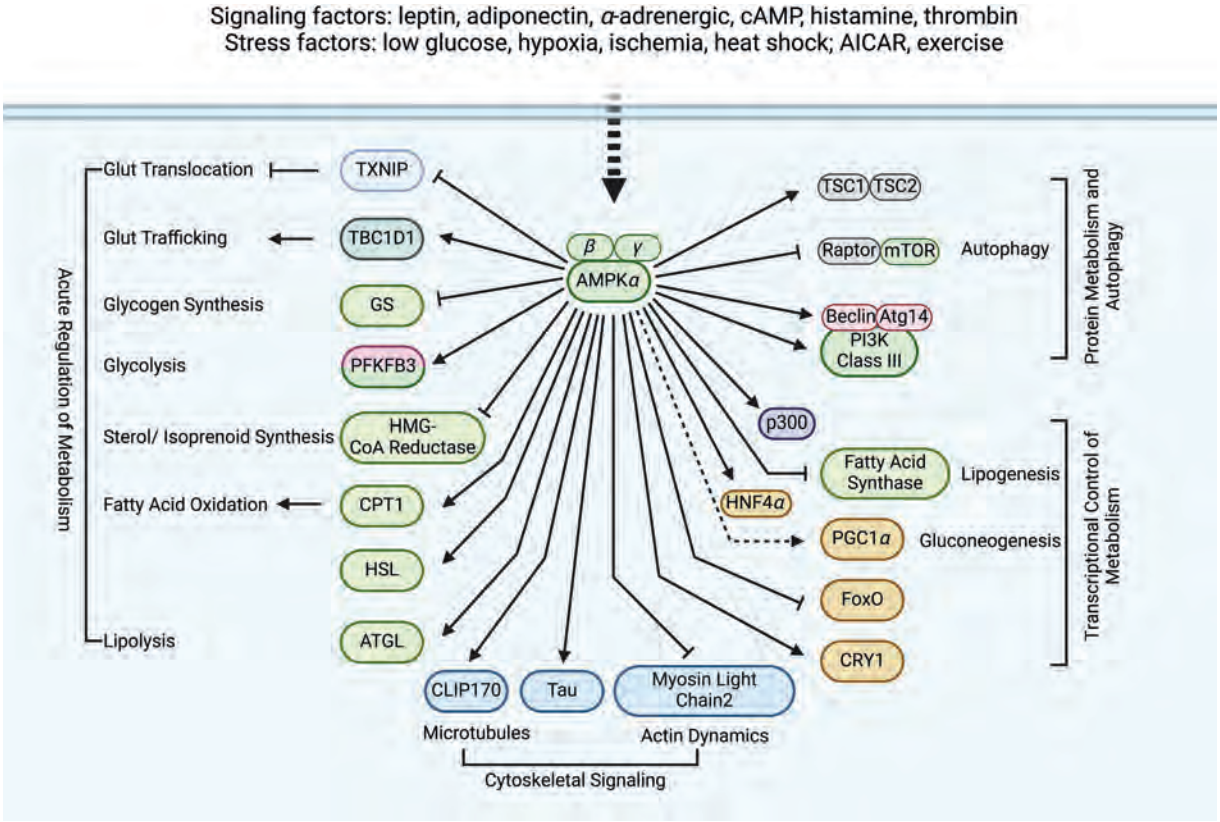

Figure 8 The vital role of the AMPK signaling pathway in cellular progress. This pathway integrates various signaling factors to govern diverse and essential cellular processes, encompassing cytoskeletal signaling, acute metabolic regulation, transcriptional control of metabolism, as well as protein metabolism and autophagy. (Created in BioRender. Gu, X. (2025) https://BioRender.com/z08y439).

additional kinases that hold pivotal roles in both cellular processes and viral infections. These kinases have been observed to interact with IAV proteins, specifically NP and NS1, respectively, enhancing their polymerase activity[182,183].

The identification of more kinases involved in virus-host interactions enhances our understanding of viral infections. Delving into these interactions offers valuable insights for crafting targeted antiviral therapies that disrupt viral pathogenesis while preserving essential cellular functions. Various viruses or subtypes manipulate different kinases and associated signaling pathways, potentially influencing their cellular or tissue tropism and host specificity, thereby elucidating why certain populations exhibit more susceptibility or resistance phenotypes[184]. This knowledge can aid in tailoring antiviral agents to specific virus types or subtypes and even in devising personalized antiviral treatments when necessary. Conversely, some viruses utilize redundant cellular kinases, enabling them to develop resistance by switching to alternative kinases upon treatment with a single kinase inhibitor[185,186]. In such scenarios, broad-spectrum kinase inhibitors or combination therapies may be more effective.

5.7. The problems and development challenges of kinase inhibitors in antiviral therapy

The repurposing of kinase inhibitors as antivirals, despite their broad antiviral potential, presents significant concerns and limitations. Kinases, integral for cellular function regulation, raise toxicity worries when targeted by inhibitors. The narrow therapeutic window of current antiviral drugs, with a small margin between efficacy and toxicity, restricts their clinical application. Operating within a well-defined therapeutic window is crucial to minimize potential toxicity. Most kinase inhibitors, designed to target conserved ATP-binding sites shared across kinase families, exhibit cross-inhibitory activity against multiple kinases, posing specificity challenges. Host kinases adaptor protein 2 (AP2)-associated protein kinase 1 (AAK1) inhibitors, for instance, affect various kinases beyond AAK1, complicating the assessment of their antiviral effects. Future research aims to identify highly selective AAK1 inhibitors to elucidate the antiviral mechanism. Given AAK1's structural resemblance to numerous kinases, developing selective and specific AAK1 inhibitors presents a challenging task for pharmaceutical chemists. Balancing the adverse effects of AAK1 inhibitors on normal cellular transport, crucial for cell function, is another obstacle. When formulating antiviral AAK1 inhibitors, maintaining a safe and effective compound window is paramount. Developing allosteric kinase inhibitors encounters hurdles in hits discovery, optimization, and activity evaluation. While drug repurposing appears promising during emergencies, challenges encompass considerations of prior knowledge of pharmacokinetics/pharmacodynamics, safety profiles, delivery routes, and formulation of repurposed candidates.

In essence, drug resistance and the narrow therapeutic window of kinase inhibitors in antiviral therapy underscore the hurdles in developing kinase inhibitors tailored for antiviral use. Overcoming these challenges demands multidisciplinary collaboration and innovative strategies to enhance the effectiveness of antiviral treatments.

6. Metabolism-related targets

In the realm of antiviral strategies, while many miRNAs and kinases primarily function as upstream regulatory factors, downstream effectors also hold promise as antiviral targets due to their pivotal roles in cellular antiviral responses. Targeting these effectors, however, may yield a less significant impact compared to disrupting an upstream regulator. In addition to effectors, proteases, complexes, or pathways involved in the host's fundamental metabolism are sometimes viewed as potential targets in viral infections. These elements play critical roles in providing energy and essential materials for host cells, making them attractive targets for antiviral interventions.

6.1. Angiotensin-converting enzyme 2 (ACE2)

Proteases play a significant role in viral infections, with ACE2 serving as a prominent example in the context of SARS-CoV and SARS-CoV-2 during viral entry and fusion processes. In normal physiological conditions, ACE2 functions by catalyzing the conversion of angiotensin I to angiotensin 1−9 and angiotensin II to angiotensin 1−7, acting as a negative regulator within the renin-angiotensin system. In 2003, ACE2 was identified as a receptor for the SARS coronavirus[187], and subsequently, the complex structure of ACE2 with the SARS-CoV spike (S) receptor-binding domain (RBD) was elucidated (Fig. 9)[188]. Its structure was later resolved with NL63-CoV S RBD and SARS-CoV-2 S RBD as well[189,190]. ACE2 binds to the "up" conformation of one or multiple RBDs, triggering conformational changes in the S2 subunits. This process leads to the formation of a six-helix bundle (6-HB), creating a conducive environment for viral fusion and entry[191,192]. Virus-binding motifs (VBMs) (Fig. 9) within ACE2 have been proposed to explain how NL63-CoV S and SARS-CoV S can bind to the same receptor despite lacking structural homology in their RBD cores or receptor-binding motifs (RBMs)[189]. The binding mode between ACE2 and the RBD from SARS-CoV-2 S closely resembles that of SARS-CoV S, with minor differences at the distal end (Fig. 9)[190]. By examining the contacting residues from ACE2, many of them can be categorized as VBMs, offering a potential strategy for developing broad-spectrum vaccines or blocking agents that target these critical interaction points. This understanding of the interaction between ACE2 and viral spike

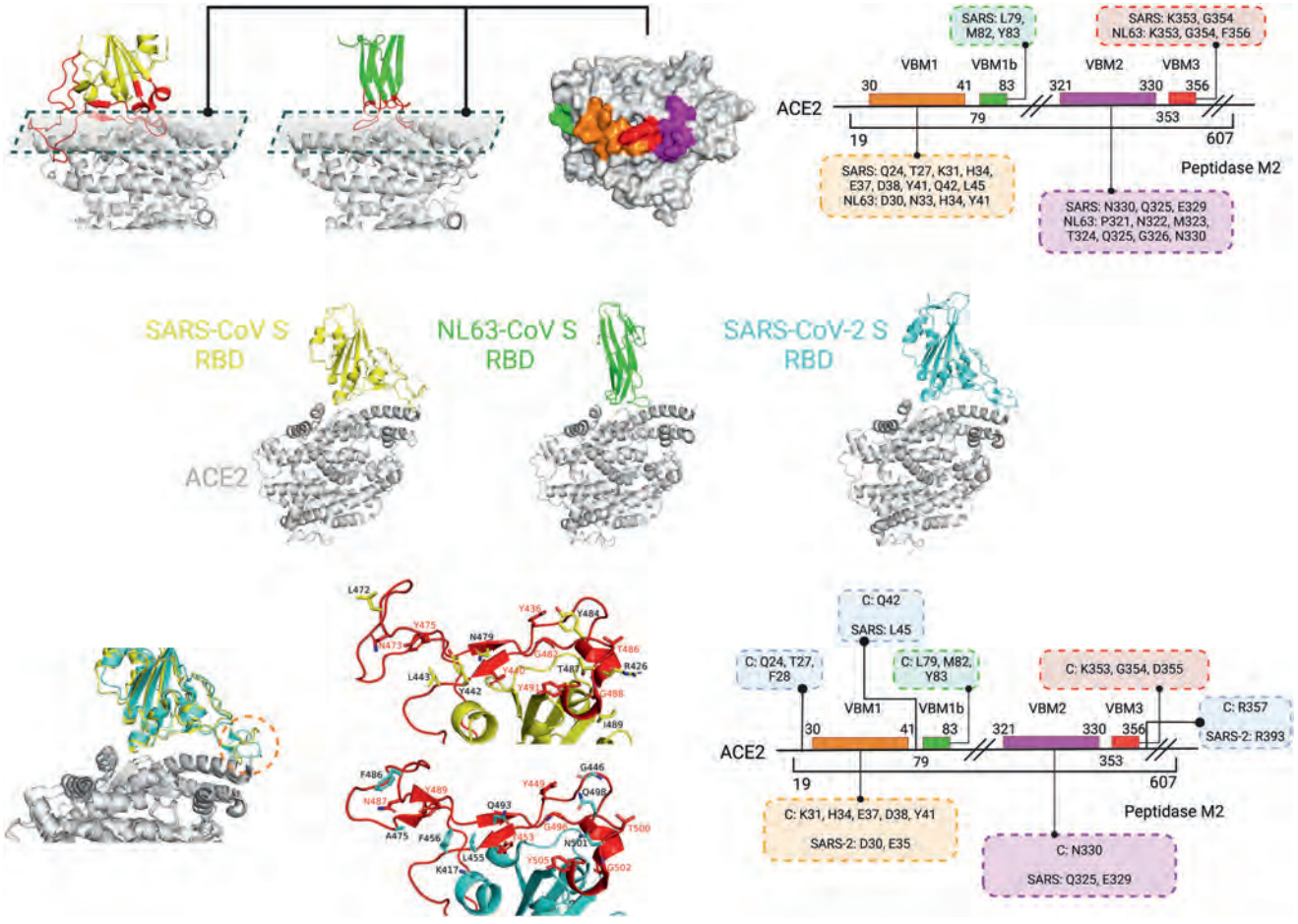

Figure 9 The binding modes between ACE2 and the RBDs from the S proteins of SARS-CoV, NL63-CoV, and SARS-CoV-2. The upper left panel illustrates the binding mode between ACE2 and the S RBD from SARS-CoV or NL63-CoV, with VBMs distinguished by different colors. The upper right section displays the location of residues in ACE2's VBMs, color-coded to match the left side, and highlights contacting residues within a labeled dotted box (mirrored in the lower right diagram). The lower left section depicts the interaction between ACE2 and the S RBD from SARS-CoV or SARS-CoV-2, featuring RBMs in red, contacting residues represented as sticks, and equivalent residues marked in red. The letter 'C' signifies the shared contacting residues between SARS-CoV and SARS-CoV-2 during their ACE2 interaction. (Created in BioRender. Gu, X. (2025) https://BioRender.com/z08y439).

proteins provides insights that could aid in the development of therapeutics with broader efficacy against related coronaviruses.

6.2. *Furin, TMPRSS2, and cathepsin W*

In addition to ACE2, two ubiquitous endoproteases, furin and TMPRSS2, play crucial roles in the activation of SARS-CoV-2 S and viral entry. Furin recognizes the multibasic motif (R-X-R/K-R↓), cleaving the S protein into non-covalently bound subunits (S1 and S2), while TMPRSS2 targets the monobasic cleavage site (R/K↓) within S2, exposing the fusion peptide and facilitating the formation of the 6-HB structure (Fig. 10)[193,194]. Notably, the furin-mediated cleavage process is present in SARS-CoV-2 S but absent in the related SARS-CoV, potentially imparting greater flexibility to SARS-CoV-2 S and enhancing its infectivity upon ACE2 binding[195]. Conversely, the recognition site of TMPRSS2 is more conserved across coronaviruses, underscoring its critical role in viral fusion and entry. Furthermore, TMPRSS2 enhances SARS-CoV entry by processing ACE2 at residues 697 to 716 (Fig. 10), independently of S protein activation[196].

Beyond SARS-CoV-2, furin and TMPRSS2 are implicated in the pathogenesis of various viruses. Furin facilitates the entry and infectivity of viruses such as HIV-1, ZIKV, measles, and IAV[197], while TMPRSS2 is involved in the entry of human parainfluenza viruses (HPIVs) and SeV[198]. Additionally, cathepsin W, a lysosomal cysteine protease, is involved in the release of IAV from late endosomes, although the specific substrate and mechanism of action remain to be fully elucidated[199,200].

6.2.1. *Representative TMPRSS2 inhibitors*

Camostat mesylate (Table S1) demonstrates promising antiviral activity as a TMPRSS2 inhibitor. *In vitro* studies have revealed its potent antiviral effects against both influenza virus type A and type B. Notably, treatment with camostat mesylate significantly reduced SARS-CoV entry into Calu-3 cells tenfold[201], indicating its potential as an antiviral agent for combating SARS-CoV-2 infection. Several clinical trials have been approved to assess its efficacy and safety in human subjects (ClinicalTrials.gov Identifier: NCT04730206, NCT04657497, NCT04355052, NCT04608266, and NCT04455815, among others).

Nafamostat mesylate (Table S1), structurally akin to camostat mesylate, has shown inhibitory effects on the entry of SARS-CoV, MERS-CoV, and SARS-CoV-2 S proteins into host cells[202]. Moreover, it has demonstrated continued efficacy against SARS-CoV-2 variants, including lineages B.1.1.7 and B.1.351[203]. Multiple clinical trials have been registered to evaluate the efficacy of nafamostat mesylate in treating COVID-19 (ClinicalTrials.gov Identifier: NCT04390594, NCT04418128, NCT04352400, NCT04628143, and NCT04623021).

Bromhexine (Table S1), an inhibitor of TMPRSS2, exhibits favorable effects in treating cytopathies induced by SARS-CoV-2 infection[204]. Furthermore, numerous clinical trials have been sanctioned to assess its efficacy and safety in COVID-19 patients (ClinicalTrials.gov Identifier: NCT04355026, NCT04273763, NCT04405999, NCT04424134, and NCT04340349).

Collectively, these results underscore the promising potential of targeting TMPRSS2 for drug development aimed at combating COVID-19.

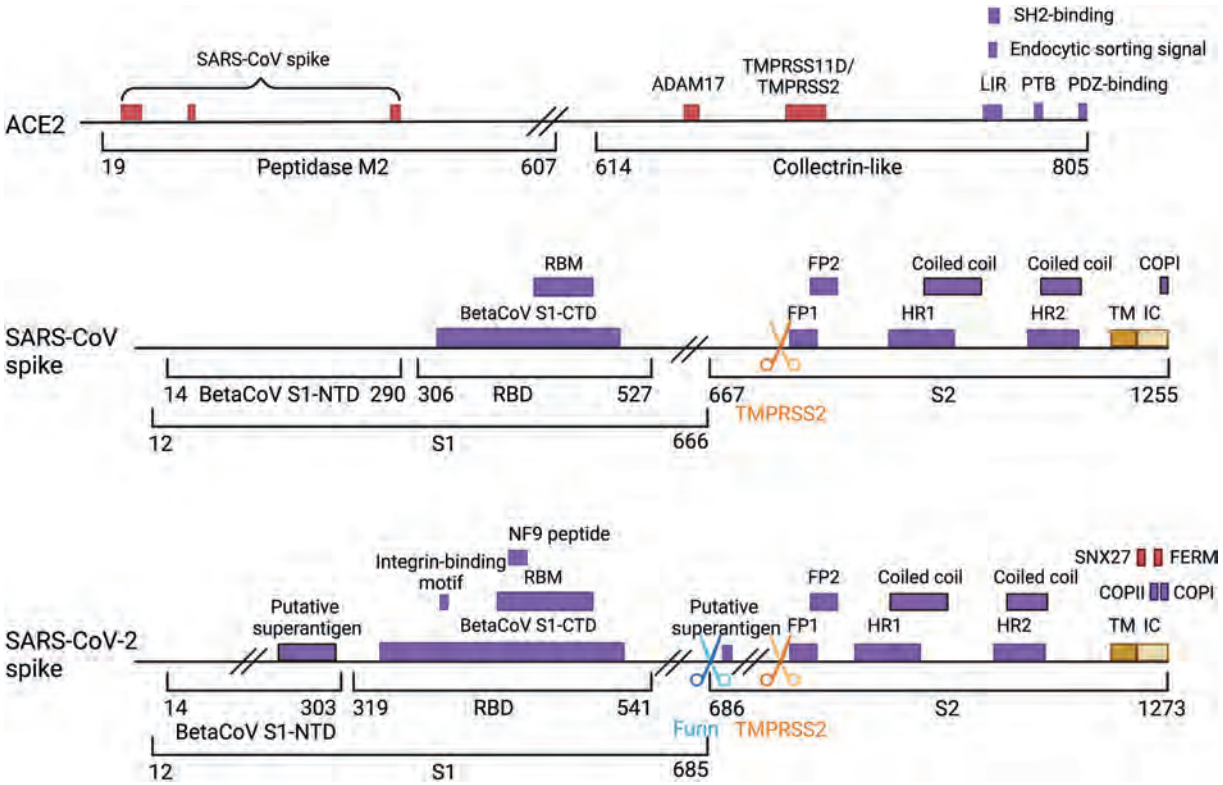

Figure 10 The domain arrangement of ACE2 and the S glycoprotein from SARS-CoV and SARS-CoV-2. Domains are depicted as purple boxes, while the transmembrane (TM) and intracellular (IC) regions are shown as dark and light yellow boxes, respectively. Interacting domains are illustrated as red boxes, with corresponding interacting proteins labeled above. The recognition sites of the two proteases, furin and TMPRSS2, are denoted by blue and orange arrow symbols, respectively. (Created in BioRender. Gu, X. (2025) https://BioRender.com/z08y439).

6.3. Lipid metabolism

Lipids, a vital group of biomolecules, play diverse essential roles within cells, serving as integral membrane components, energy reservoirs, and mediators of signal transduction. It has been elucidated that lipids and lipid metabolism are intricately involved in nearly every stage of viral infection. During early infection, lipids facilitate the endocytosis and fusion processes of viruses, sometimes acting as cellular receptors for viral entry. After virus entry and the initiation of replication, viruses may induce alterations in membrane structure to compartmentalize processes, aiding in the establishment of viral replication complexes and conferring resistance against cellular proteolysis. At this stage, viruses may also manipulate cellular lipid metabolism, potentially for energy provision, storage of viral components, and intracellular transport. In the later stages of infection, newly synthesized lipids can serve as structural frameworks for viral assembly, directly incorporating into virions, facilitating virus release from host cells, and influencing the pathogenicity of viral progeny. Detailed reviews on this subject can be found in references[205-207].

Numerous factors associated with lipid metabolism have emerged as potential targets for antiviral interventions, including acyl-coenzyme A: cholesterol acyltransferase (ACAT), fatty acid synthase (FASN), oxysterol-binding protein (OSBP), the cholesterol uptake receptor NPC1L1, and diacylglycerol *O*-acyltransferase 1 (DGAT1)[208-212].

DGAT1 plays a pivotal role in the production of infectious particles of HCV, facilitating the interaction between two viral proteins, NS5A and capsid protein core[213,214]. Additionally, DGAT1 contributes to the development of steatosis as a complication of HCV infection[215]. Inhibition or depletion of DGAT1 has been shown to impede HCV infection and safeguard mice from core-induced steatosis. Notably, pradigastat (Table S1), a specific DGAT1 inhibitor with potential anti-HCV properties *in vitro*, exhibited no significant effect in patients during a randomized clinical trial[216]. This underscores the need for further investigation into DGAT1 inhibitors as potential anti-HCV agents.

Identifying a critical intersection that is essential for viral replication but dispensable and redundant for host lipid metabolism is a fundamental step in developing host-directed broad-spectrum antiviral targets. This strategy necessitates a comprehensive understanding of the intricate interplay between viral infection and host lipid metabolism, alongside careful consideration of potential side effects and toxicity concerns.

6.4. Purine and pyrimidine biosynthesis pathways

The purine and pyrimidine biosynthesis pathways represent potential antiviral targets due to their role in providing essential materials for the extensive DNA or RNA synthesis necessary for viral replication.

Dihydrofolate reductase (DHFR) is a crucial enzyme in folate metabolism and DNA precursor synthesis[217,218]. Various reports have highlighted DHFR inhibitors with antiviral properties against a range of viruses, including HIV, ZIKV, influenza virus, and RSV[219-222].

Inosine-5′-monophosphate dehydrogenase (IMPDH) is a key enzyme in the *de novo* biosynthesis of guanine nucleotides. Inhibiting IMPDH disrupts gene synthesis for DNA and RNA viruses, thereby impeding viral replication. IMPDH inhibitors, such as mycophenolic acid, its prodrug mycophenolate mofetil, and mizoribine (Table S1), exhibit broad-spectrum antiviral activity against both DNA and RNA viruses *in vitro*[223]. For instance, mycophenolic acid, a non-competitive IMPDH inhibitor, demonstrates antiviral efficacy closely linked to intracellular GTP levels, suggesting that IMPDH inhibition plays a crucial role in its antiviral mechanism[224,225].

Alternatively, the pyrimidine biosynthesis pathway has emerged as an antiviral target in various high-throughput phenotype screens, focusing on three proteases: the multifunctional protein CAD, dihydroorotate dehydrogenase (DHODH), and the bifunctional uridine monophosphate synthetase (UMPS) (Fig. 11). Corresponding inhibitors have shown effectiveness against diverse viruses, including RSV, hepatitis D virus (HDV), and Ebola virus (EBOV)[226-228]. While these proteases and pathways are traditionally considered targets for anti-tumor therapies, they have regained significance in the development of host-directed antivirals[229].

Due to its pivotal role in the pyrimidine biosynthesis pathway, DHODH is currently a favored host target for antiviral drug development[230]. GSK983 (Table S1) hinders viral replication and impedes the proliferation of rapidly dividing cells by targeting DHODH. Through phenotypic screening, a potent new small molecule (RYL-634, Table S1) has been identified as an effective DHODH inhibitor, displaying remarkable potency against HIV, DENV, EV71, and ZIKV. An analog of RYL-634, RYL-687 (Table S1), has also demonstrated efficacy against Ebola virus infection in *vitro*[231]. A recent study by Cordsmeier et al.[232] assessed DHODH inhibitors (Table S1) against monkeypox virus (MPXV) (sourced from diagnostic samples), vaccinia virus (VACV), and cowpox virus (CPXV). The inhibitors demonstrated activity against all three viruses, leading the authors to speculate that they might exhibit inhibitory effects against orthopoxviruses in general. DHODH inhibitors offer significant advantages when used in conjunction with DAAs. The combined use of DHODH inhibitors and DAAs results in cumulative inhibition over the disease course compared to using a single drug alone. Combining DHODH inhibitors with nucleoside analogue DAAs enhances the incorporation efficiency of nucleoside analogues.

Beyond the purine and pyrimidine biosynthesis pathways, various nucleotide metabolism-related proteins or complexes have been implicated in viral replication and transport. These include Mx GTPases, RNA helicase A, ATPase/RNA helicase X-linked DEAD-box polypeptide 3 (DDX3), guanylate-binding protein 1 (GBP1), Ras-related protein Rab-5A, and the SKI complex[233-238]. These proteins or complexes modulate diverse cellular processes, exhibiting either proviral or antiviral activities by interacting with the viral genome or associating with viral structural or non-structural proteins. They influence the stability of the replication complex, disrupt virus fusion, trafficking, and egress from cells, or regulate innate immunity.

DDX3 is a cytokine crucial for DNA and RNA viral replication, making it a valuable host target for broad-spectrum antiviral drug development. Several DDX3 inhibitors targeting ATP-binding sites have been developed, showing promising anti-HIV activity *in vitro*. However, inhibitors that target ATP-binding sites may also inhibit other kinases with similar sites. To circumvent this issue, inhibitors have been designed to target the RNA binding sites of DDX3. The first DDX3 inhibitors targeting the RNA binding site, such as DDX3 inhibitors **1** and **2** (Table S1), can impede the helicase and ATPase functions of DDX3. DDX3 stands out as a promising target for broad-spectrum antiviral drug development, with DDX3 inhibitor **3** (Table S1) serving as a promising starting point for further detailed research[239-242].

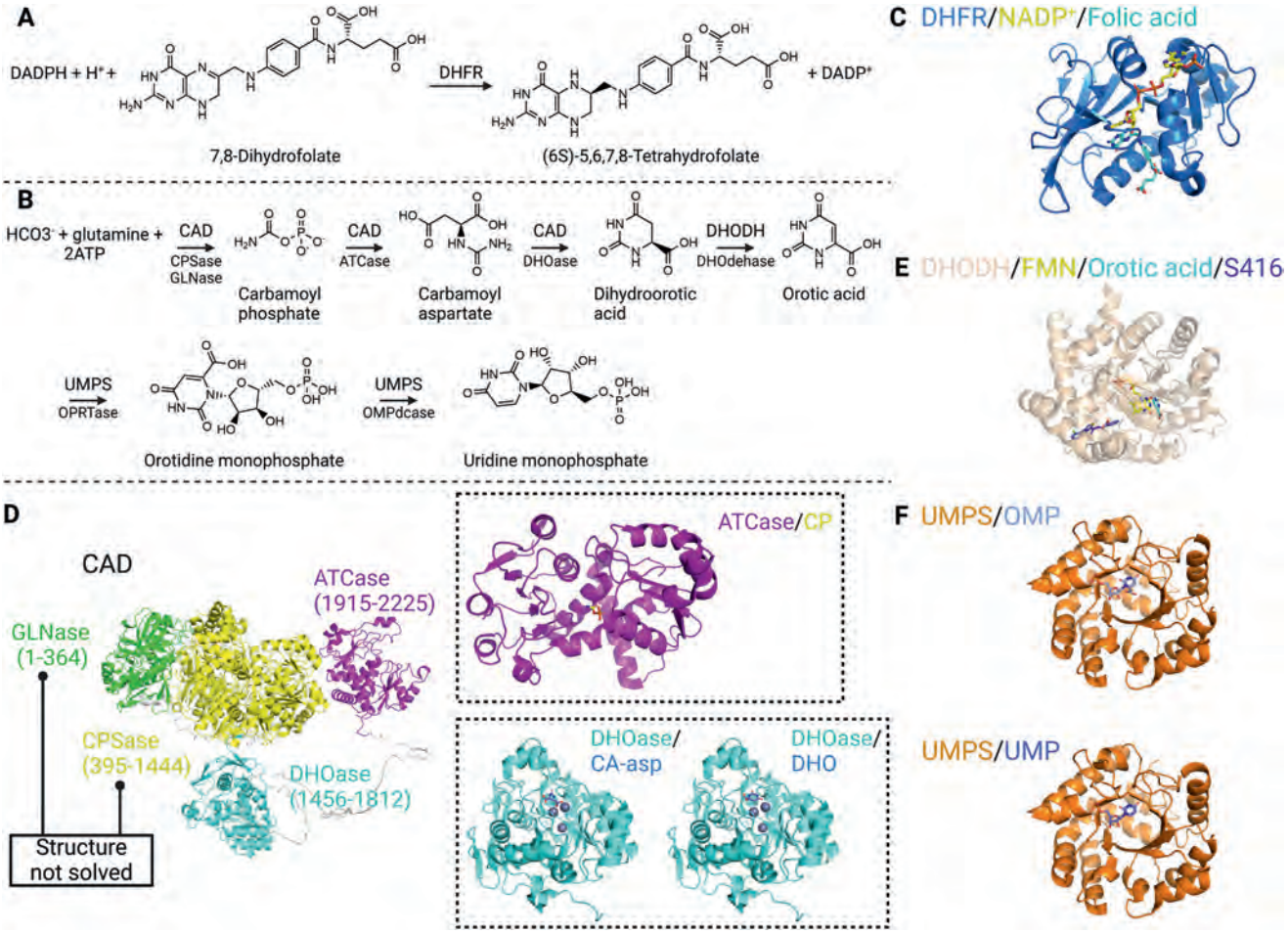

Figure 11 Host-directed targets in the purine and pyrimidine biosynthesis pathways. Panels (A) and (B) display the catalytic activities of four proteases (DHFR, CAD, DHODH, UMPS), with specific activities of multifunctional proteases indicated below the arrows. Panels (C−F) showcase complex structures of these proteases with their substrates, products, or inhibitors. Specifically, the structure of DHFR is detailed by PDB 4M6K with $NADP^+$ and folic acid. The full-length CAD structure is depicted by the AlphaFold-predicted model, colored by domain, with specific domains detailed using PDB models. Notably, the GLNase domain and CPSase domain are yet to be resolved. The ATCase domain is illustrated by PDB 5G1P (CP denotes the substrate carbamoyl phosphate), while the DHOase domain is depicted by PDB 4C6I (CA-asp and DHO represent the substrate carbamoyl aspartate and the product dihydroorotic acid, respectively). The structure of DHODH is elaborated by PDB 6M2B, featuring the product orotic acid, the cofactor flavin mononucleotide (FMN), and the inhibitor S416. Lastly, the structure of UMPS is detailed by PDB 7AM9 with its substrate OMP and by PDB 7ASQ with the product UMP. (Created in BioRender. Gu, X. (2025) https://BioRender.com/z08y439).

6.5. Others

Additional metabolism-related targets, such as GAPDH in glycolysis, sirtuins involved in epigenetic modifications regulation, and cyclophilin A (CypA) in cellular responses to oxidative stress, have emerged as potential antiviral targets following phenotypic screening and subsequent inhibition assays[243-245]. Their implication in antiviral mechanisms is unsurprising, given their conserved roles in host biological processes.

For example, CypA plays a crucial role in HCV replication. Cyclosporin A (CsA, Table S1) exhibits a strong binding affinity with CypA and is an approved immunosuppressive drug. The antiviral response observed in the combination group of CsA and IFN-α2b proved significantly more effective. CypA and NIM811 (Table S1), an analogue with a methyl-isoleucine at position 4 of CsA, both demonstrated anti-HCV activity *in vitro*[246,247].

Overall, targeting downstream effectors and cellular metabolic pathways presents a robust antiviral strategy with potentially broad applicability. This strategy should be implemented with careful consideration of potential side effects.

7. The ubiquitin−proteasome system and ubiquitylation

Ubiquitination, an indispensable posttranslational modification for cellular proteins, involves attaching the 76-amino acid protein ubiquitin (Ub) to specific proteins. This process is orchestrated by three crucial enzymes: ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3), and antagonized by ubiquitin hydrolases/deubiquitinating enzymes (DUBs). Ubiquitin can be conjugated to targeted proteins at seven internal lysine residues (K6, K11, K27, K29, K33, K48, K63) or the amino-terminal methionine, resulting in diverse ubiquitination modifications. These modifications can lead to the degradation of modified targets by the proteasome or facilitate various cellular processes (Fig. 12)[248]. The ubiquitin−proteasome system plays a pivotal role in upholding protein homeostasis,

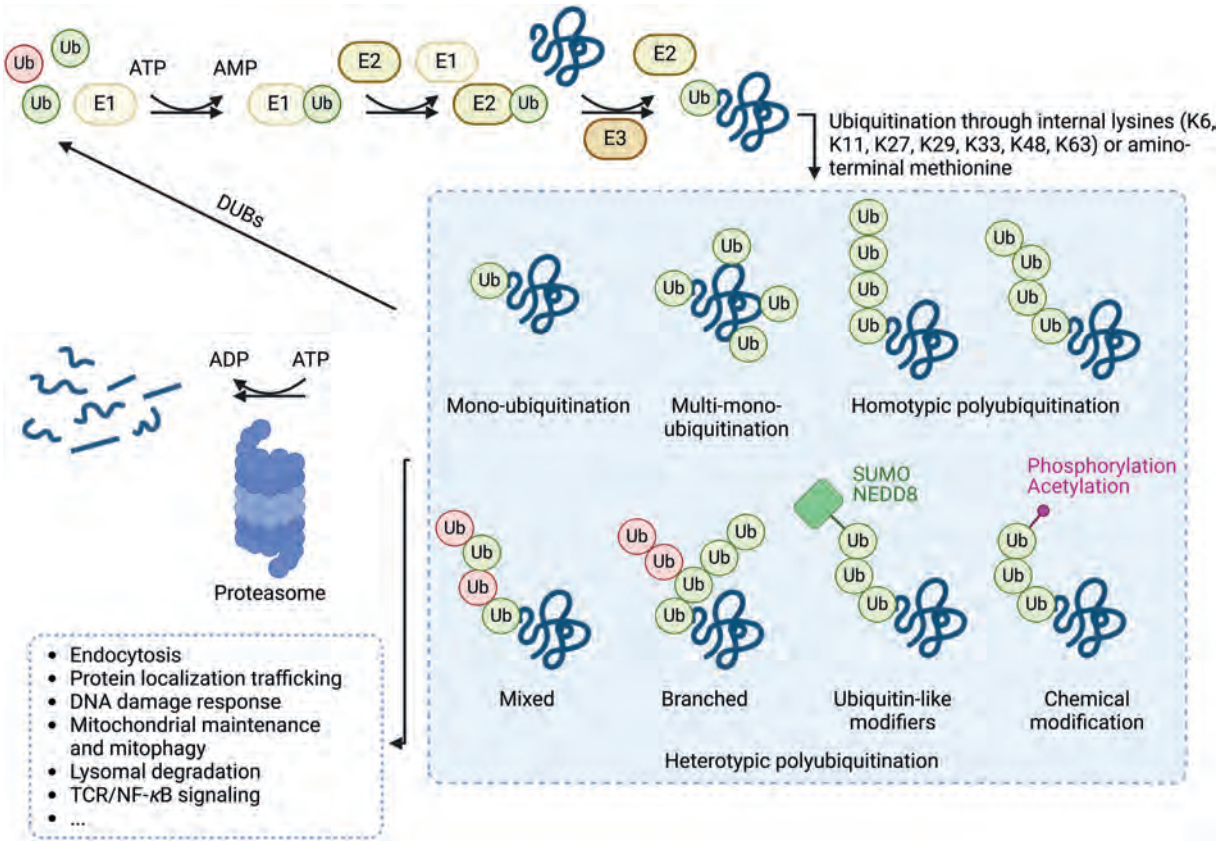

Figure 12 The scheme of ubiquitin−proteasome system. Ubiquitination relies on three pivotal enzymes: ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). Ubiquitin (Ub) can attach to targeted proteins *via* internal lysines (K6, K11, K27, K29, K33, K48, K63) or the amino-terminal methionine, resulting in a spectrum of ubiquitination modifications. This process can be counteracted by ubiquitin hydrolases/deubiquitinating enzymes (DUBs). These modifications may trigger the degradation of modified targets by the proteasome or facilitate diverse cellular processes. (Created in BioRender. Gu, X. (2025) https://BioRender.com/z08y439).

serving as a cellular defense mechanism against pathogens and diseases like neurodegenerative disorders, cancer, and viral infections, where the accumulation of misfolded and aggregated proteins is a common hallmark of their pathogenesis[249]. Similar to other conserved regulatory or metabolic pathways, the ubiquitin−proteasome pathway can function as a cellular antiviral mechanism by breaking down viral components. Nevertheless, viruses have developed strategies to evade and exploit this pathway, diminishing antiviral responses as a tactic in the enduring co-evolutionary struggle between viruses and their hosts[250,251].

7.1. Tripartite motif (TRIM) family

Within the extensive array of over 600 E3 ubiquitin ligases, the TRIM family stands out for its significant involvement in viral infections (Fig. 13)[251]. TRIM proteins can exert antiviral effects by directly marking viral proteins for degradation. For instance, TRIM69 targets the DENV NS2B-NS3 protease complex, TRIM41 targets the IAV NP, and TRIM7 targets the enterovirus 2BC protein[252-254]. Moreover, TRIM proteins can influence the interaction of other restriction factors with viral components. For example, TRIM25 facilitates K63-linked polyubiquitination of ZAP, enhancing its binding to target mRNA[255]. On the other hand, TRIM proteins may also exhibit proviral effects by dampening immune responses. For instance, TRIM29 mediates K48 ubiquitination of STING, thereby compromising the innate immune response triggered by DNA viruses and cytosolic DNA, particularly affecting the STING−TBK1−IRF3 signaling pathway[256]. A continuous co-evolutionary interplay is evident between TRIM proteins and viruses. TRIM5 has developed broad-spectrum antiviral activity with a flexible active site, prone to conformational changes when targeting the HIV capsid[257]. In response, virus-encoded proteases have evolved to counteract the TRIM-mediated degradation of crucial proteins. For example, while TRIM7 targets the enterovirus 2BC protein for degradation, it is concurrently cleaved by the enterovirus 3C protease, with the cleavage site being conserved across mammals (except in marsupials)[254]. The involvement of TRIM proteins in antiviral responses underscores their potential as targets for antiviral interventions.

7.2. APOBEC3G-Vif-E3 ubiquitin ligase complex

The APOBEC3G-Vif-E3 ubiquitin ligase complex represents a significant antiviral target. APOBEC3G, an innate host antiviral protein initially known as CEM15, exhibits cytidine deaminase activity, leading to detrimental hypermutations in retroviral DNA (Fig. 14A). Notably, APOBEC3G can manifest its antiviral function independently of its enzymatic activity[258]. Moreover, APOBEC3G disrupts the interaction between the Moloney leukemia virus 10 (MOV10) protein and AGO2, thereby impeding the normal assembly of the miRNA-inducing silencing complex (miRISC) and inhibiting miRNA-mediated translation repression[259]. However,

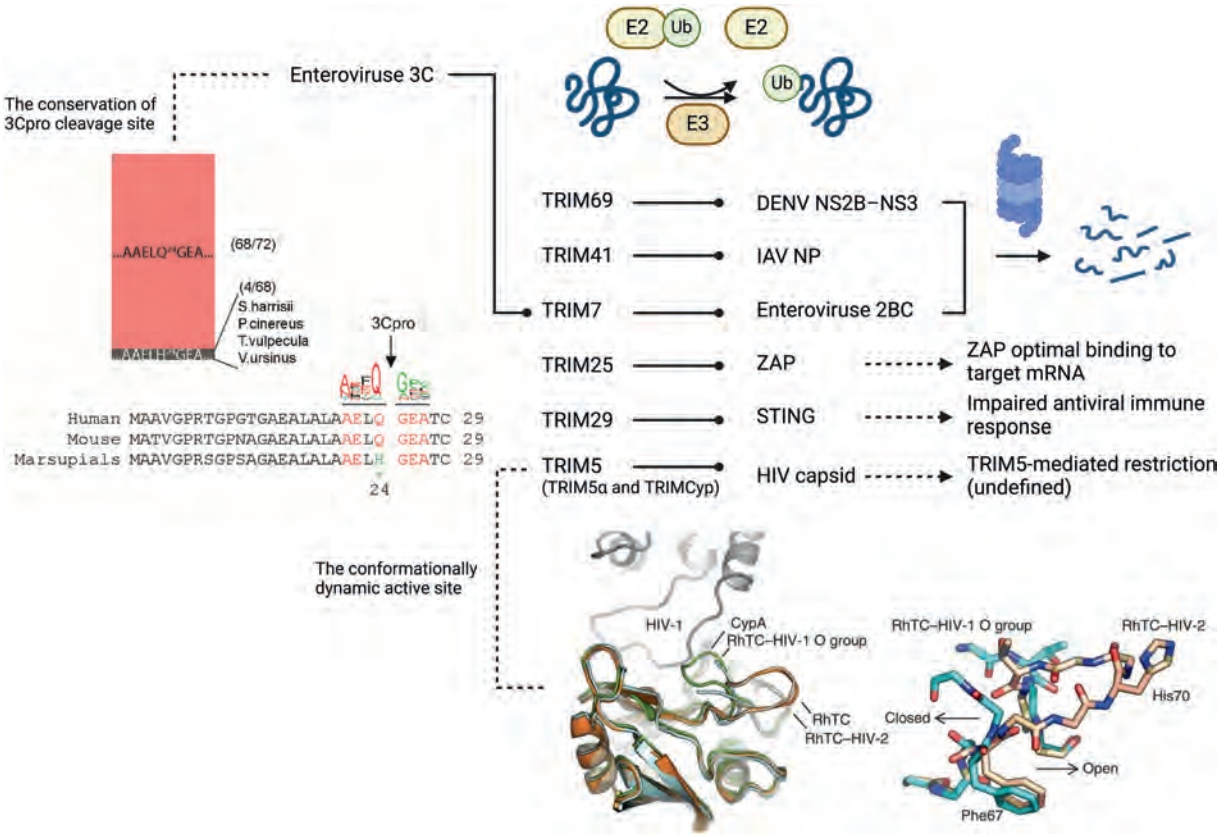

Figure 13 The intensive involvement of TRIMs in viral infection and the co-evolution between TRIMs and viruses. TRIM proteins play a role in targeting viral proteins for proteasomal degradation, coordinating with restriction factors, and modulating the antiviral immune response through ubiquitination. Specifically, Enterovirus 3C acts to counteract the restriction imposed by TRIM7, with its cleavage site being conserved among mammals (except in marsupials)[254]. Additionally, Rhesus macaque TRIMCyp (RhTC) has developed a conformationally dynamic binding site (loop 66−74) for the HIV capsid, leading to the acquisition of broad-spectrum antiviral activity[257]. (Created in BioRender. Gu, X. (2025) https://BioRender.com/z08y439).

HIV and related retroviruses encode the Vif protein, which thwarts the incorporation of APOBEC3G into progeny virions. Vif recruits the ElonginB/C-Cullin5 E3 ubiquitin ligase to target APOBEC3G for proteasomal degradation (Fig. 14A and B)[260-265]. Beyond ubiquitin-dependent inhibition, Vif may employ various strategies to target APOBEC3G/APOBEC3, including interference with its synthesis and transcriptional regulation[266-269], although the precise mechanisms remain unclear.

Subsequent research has broadened the antiviral spectrum of APOBEC3G to encompass HBV, foamy viruses (FVs), xenotropic murine leukemia virus-related virus (XMRV), and more[270-272]. Investigations into the antiviral activities of APOBEC3G isoforms (such as APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3DE, APOBEC3F, APOBEC3H) (Fig. 14C) and their specific contributions have been thorough[273-277]. The structure and mode of action of APOBEC3G have been continuously explored, with recent advancements including the elucidation of the cryo-EM structure of the human APOBEC3G/HIV-1 Vif/CBF-β/ELOB/ELOC complex (Fig. 14B)[278].

Given the critical reliance of viral Vif functions on the APOBEC3G/Vif/CBF-β/CRL complex formation, compounds disrupting the interaction between Vif and the E3 ubiquitin ligase or between Vif and APOBEC3G have demonstrated efficacy as antiviral agents[279-283]. Additionally, the transcription cofactor core-binding factor beta (CBF-β) plays a pivotal role in Vif-mediated APOBEC3G inhibition, making the CBF-β-Vif interface a promising target for antiviral agent development.

Two compounds, IMB-26 and IMB-35 (Table S1), have been discovered to directly bind to APOBEC3G (A3G) and disrupt its interaction with Vif, thereby rescuing A3G from Vif-mediated degradation[284]. Both compounds exhibited A3G-dependent anti-HIV-1 activity. IMB-26 also demonstrated potent antiviral effects against HCV *in vitro* by stabilizing intracellular A3G.

Furthermore, two Vif inhibitors, RN-18 and RN-19 (Table S1), have been identified. These compounds inhibited HIV-1 replication specifically in A3G-positive cells, with IC_{50} values exceeding 100 μmol/L in A3G-negative cells[285]. Treatment with compound RN-18 not only elevated the levels of A3G but also resulted in the degradation of Vif.

7.3. Others

Various E3 ubiquitin ligases can be co-opted by viral proteins to enhance virus replication. For instance, the PRV protein UL13 recruits the E3 ligase RNF5 to target STING, evading STING-mediated interferon production[286]. The non-structural protein 5 (NS5) of ZIKV utilizes the host CRL3−ZSWIM8 complex to degrade STAT2, inhibiting the host's antiviral immune response[287]. Similarly, the E3 ubiquitin ligase TRAF6 exerts a proviral effect on tick-borne flaviviruses (TBFVs) through its

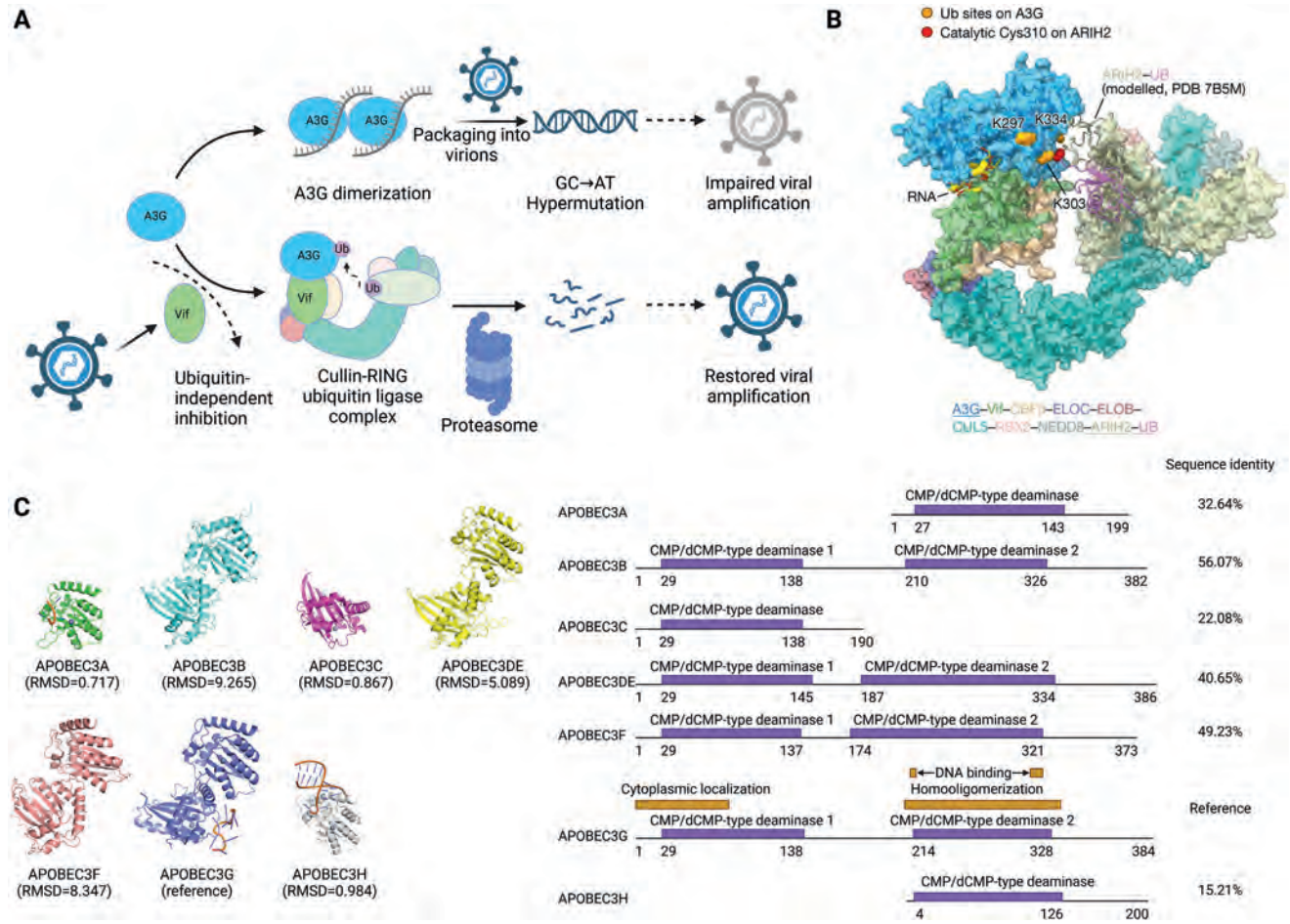

Figure 14 The APOBEC3G (A3G)-Vif-E3 ubiquitin ligase complex. (A) The mechanisms of action of APOBEC3G and Vif. (B) The structure of the APOBEC3G/RNA complex in association with HIV-1 Vif, CBF-β, and the host Cullin−RING ubiquitin ligase (CRL) complex. The model depicting the APOBEC3G/RNA/Vif/CBF-β/CRL complex is derived from a recent publication[278]. (C) A comparative analysis of human APOBEC3 isoforms. The structures of human APOBEC3 isoforms are represented using PDB 5KEG, AlphaFold model (UniProt Q9UH17), PDB 3VOW, AlphaFold model (UniProt Q96AK3), AlphaFold model (UniProt Q8IUX4), PDB 8CX0, and PDB 8FVI, respectively. The Root Mean Square Deviation (RMSD) and sequence identity are computed with APOBEC3G as the reference. (Created in BioRender. Gu, X. (2025) https://BioRender.com/z08y439).

interaction with non-structural protein 3 (NS3), although the exact mechanism remains unclear[288].

Apart from mediating protein degradation, E3 ligases can display antiviral activity by disrupting virus assembly. For example, the E3 ligase MARCH8 hinders the integration of VSV and HIV glycoproteins into virions, reducing their infectivity[289].

Beyond the proteasome pathway, ubiquitination regulates various cellular processes like autophagy and signal transduction. The aforementioned E3 ligase MARCH8 participates in PABPC4-mediated ubiquitin modification of viral N proteins, leading to autolysosome degradation in eight coronaviruses[290]. Ubiquitination of NF-κB essential modulator (NEMO) by the linear ubiquitin chain assembly complex (LUBAC) activates downstream NF-κB signaling and innate immune responses against viruses. However, viral proteins can disrupt this process to thwart host antiviral activities by targeting LUBAC or NEMO ubiquitination. For instance, the NS3 protein of HCV interacts with LUBAC, competitively impeding LUBAC's binding to NEMO, inhibiting NEMO's linear ubiquitination and NF-κB activation, aiding HCV's immune evasion[291].

Sumoylation, a post-translational modification closely linked to ubiquitination, regulates diverse cellular functions, including antiviral responses[292-294]. Viral proteins can exploit sumoylation to evade host defenses. For example, the human adenovirus protein E1B-55K enhances the interaction between the SUMO-targeted ubiquitin ligase (STUbL) RNF4 and the antiviral factor Daxx, counteracting Daxx's antiviral effect by promoting its proteasomal degradation[295].

The ubiquitin−proteasome system presents a promising target for developing host-directed antiviral agents. Exploiting viruses' reliance on this pathway and its role in protein homeostasis may lead to novel broad-spectrum antiviral strategies, such as proteolysis-targeting chimera (PROTAC) technology[296]. This approach not only addresses drug resistance but also targets traditionally undruggable proteins. Additionally, ubiquitin-specific proteases (USPs) modulate type I interferon production, with antiviral USPs (USP2b, USP3, USP18, USP25, UL36USP, and HAUSP) and proviral USPs (USP4, USP13, USP15, and USP17) playing crucial roles in antiviral immunity[297]. Understanding how viruses manipulate the ubiquitin−proteasome system is essential for designing targeted antiviral interventions.

8. Other host-directed targets

Successful viral infection involves a series of steps, including entry into host cells, release of the viral genome from endosomes, and intracellular trafficking for replication. During these processes, various cellular factors are exploited for viral purposes (Fig. 15). These factors include ion channels, transporters, receptors, and membrane components that facilitate virus entry, such as Ca^{2+} channels, K^+ channels, Cl^- channels, endo-lysosomal two-pore channels (TPCs), hepatic sodium/bile acid cotransporter (NTCP), purinergic receptors, and the tetraspanin CD151[298-303]. Cell-based assays have identified HBV entry inhibitors that target NTCP, including propranolol, progesterone, vanitaracin A, proscillaridin A, NTI-007, and fasiglifam (Table S1)[304-308].

Certain membrane components, such as heparan sulfate proteoglycans (HSPGs) and 6-*O*-sulfated chondroitin sulfate proteoglycans (CSPGs), can serve as non-selective attachment sites for virus entry. These proteoglycans play roles in various viral infections by facilitating entry through electrostatic interactions between the negatively charged heparan sulfate moieties of HSPGs and the positively charged amino acids on virus surfaces[309-311]. As a result, interventions that target HSPGs or competitively bind to virion particles have demonstrated antiviral effects[312,313]. Targeting HSPGs could potentially offer a broad-spectrum antiviral approach due to the non-specific nature of the interaction between HSPGs and viruses. However, this approach may be associated with challenges such as low performance and systemic toxicity. Similarly, certain antiviral chemicals like salicylanilide niclosamide act through electrostatic interactions, independent of a direct target. This compound blocks virus entry by acidifying the micro-environment[314]. While this method represents a novel approach to antiviral defense, it is limited by the issues related to non-specific interactions.

Viruses exploit various proteins that facilitate post-entry processes such as internalization, fusion, trafficking, and uncoating. These proteins include membrane components from lysosomes (lysosome-associated membrane glycoprotein 3 (LAMP3)), cell surfaces (lymphocyte antigen 6E (LY6E), cytohesin-2 (CYTH2), and AP-2 complex subunit mu (AP2M1)), and the Golgi apparatus (acyl-CoA-binding domain-containing protein-3 (ACBD3))[315-319].

The endoplasmic reticulum (ER) plays a critical role in maintaining intracellular homeostasis and is a prime target during viral infections due to the substantial production of viral proteins. Viruses often exploit the ER to aid in their replication and assembly processes. Chemical or genetic interventions target various aspects of ER functions. These interventions can affect the ER translocon complex (SEC61), ER chaperone (HSPA5), ER cotranslational modification machinery (collagen proline hydroxylation), and ER proteostasis[320-323]. Targeting ER functions has proven to be an effective strategy for antiviral therapy, highlighting the significance of the ER in viral replication.

During virus egress from host cells, certain membrane constituents can be integrated into virion particles. These components can either enhance viral infectivity, such as tissue factor (TF), or conversely, trap virions on the cell surface, like transmembrane protein 106A (TMEM106A), potentially restricting their spread[324,325].

Specific cellular architectures or their associated proteins and components of the cytoskeleton play crucial roles in host-virus interactions. Structures like filopodia, tight junction (TJ) proteins, and the protein tubulin provide physical support for virus attachment, intracellular trafficking, and virion budding[326-328]. In some instances, these structures and proteins can even act as cellular receptors for virus entry, as seen with TJ proteins like claudin-1 and occludin in the context of HCV infection[327].

While targeting these factors shows promise for developing antiviral agents, their highly conserved biological functions in host

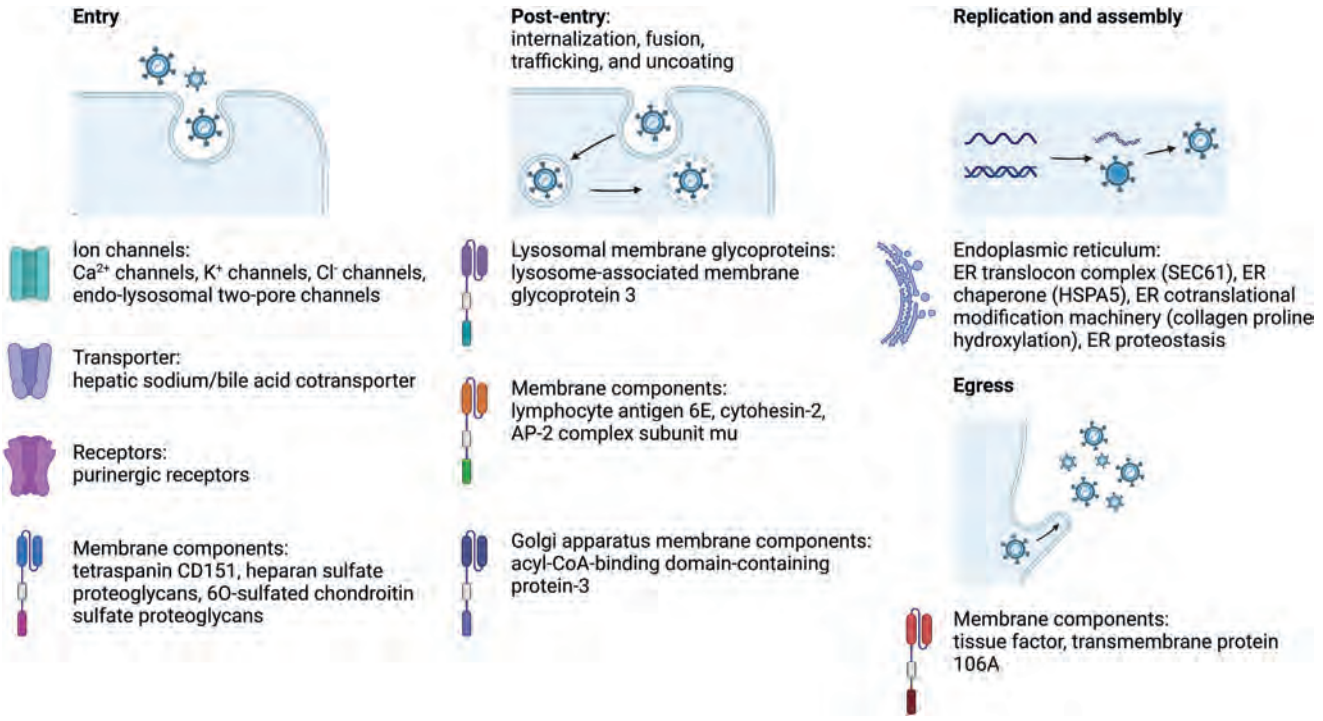

Figure 15 Other host-directed antiviral targets from this review and their roles in the process of viral infection. (Created in BioRender. Gu, X. (2025) https://BioRender.com/z08y439).

cells present challenges. One potential approach is using prodrugs that are selectively activated in virus-infected cells, such as combretastatin peptide hybrids[326]. This strategy allows for targeted drug delivery, reducing off-target effects. However, when utilizing prodrugs, factors like hydrolytic stability, permeability, and metabolic clearance rate must be carefully considered, as they can impact the efficacy and safety of these compounds. Thoughtful design and optimization of prodrugs are essential to ensure their stability, efficient activation in target cells, and appropriate clearance from the body to minimize potential side effects.

9. Chemistry strategies that can be employed to reduce the toxicity of drugs targeting these host factors

Host proteins, being more conserved than viral proteins, exhibit lower susceptibility to mutation, rendering drugs targeting host proteins less prone to resistance—an essential attribute for developing broad-spectrum antivirals, given the significant challenge of resistance faced by current antiviral treatments.

Despite numerous host factors playing pivotal roles in the viral life cycle, only a limited subset has been explored as antiviral targets, primarily due to concerns raised by HDAs. Targeting host proteins can potentially lead to unintended toxicity issues, as these proteins may be vital for specific cellular functions. Design strategies focusing on regions that interact with viral proteins or nucleic acids without disrupting other host protein interactions can enhance the precision of drug design. For instance, employing structure-based drug design using crystal structure information enables the development of drugs that selectively bind to viral proteins, thereby reducing non-specific interactions with host proteins. Analyzing interactions between viral and host proteins can help identify key proteins shared among closely related coronaviruses, aiding in the screening of drug candidates with potent antiviral effects.

To mitigate the toxicity of drugs targeting host proteins, several chemistry strategies can be employed: 1) Covalent inhibitors enhance drug efficacy by forming covalent bonds with the target, potentially reducing the required dosage and frequency of administration, thereby minimizing host toxicity. The nucleophilic nature of proteins, with abundant hydroxyl, sulfhydryl, amino, and other groups, allows them to interact effectively with electrophilic groups present in covalent binding compounds like Michael receptors, halogens, carbonyls, and isocyanogens, leading to altered protein conformation and subsequent inhibition of protein activity[329]. 2) The development of PROTAC molecules offers a method to selectively degrade host proteins, thereby reducing drug toxicity. Comprising target protein-specific ligands and E3 ubiquitin ligase recruitment ligands connected by linkers, PROTAC molecules facilitate the ubiquitination and subsequent degradation of the target protein[330]. 3) Addressing the functional deficiency of RNA binding proteins, which underlies various diseases, poses a challenge with conventional drugs. Inspired by targeted protein degradation techniques, a novel approach for the targeted degradation of RNA-binding proteins, known as RNA-PROTAC, has been developed[331]. Its successful application in anti-tumor research is anticipated to inspire innovative ideas for antiviral drug design. 4) Ribonuclease targeting chimeras (RIBOTACs) represent a novel strategy for degrading RNA by selectively binding to RNAs, particularly those forming complex secondary and tertiary structures, and subsequently activating the ribonuclease L (RNase L). 5) Utilizing synthetic carbohydrate receptors (SCRs) to disrupt the interaction between viruses and host cell membrane proteins offers a method to reduce viral infectivity, thereby inhibiting the virus life cycle. SCRs, potent inhibitors of highly glycosylated envelope viruses, exhibit a dual mode of action by impeding viral invasion and aiding in viral clearance through interference with sugar molecules in the viral envelope. 6) The combination of HDAs and DAAs results in additive inhibition throughout the disease progression compared to individual agents. In the realm of pharmaceutical chemistry, the design of dual-target inhibitors or degraders can effectively reduce drug side effects. These strategies employ diverse mechanisms to decrease drug toxicity to the host while maintaining robust viral inhibition, offering a range of pathways for the development of broad-spectrum antiviral drugs.

10. The role of artificial intelligence in identifying and validating host proteins as antiviral targets

Artificial intelligence (AI) demonstrates significant promise in identifying and validating host proteins as targets for antiviral therapies, playing a crucial role in drug repurposing and the development of innovative antiviral treatments. AI technology excels in analyzing vast datasets to unveil hidden connections among existing drugs, disease targets, and potential therapies. This capability expedites the identification of drug targets, particularly during sudden infectious disease outbreaks, and empowers research into diseases with intricate mechanisms like cancer, neurodegenerative disorders, and autoimmune conditions. AI also proved invaluable in drug repurposing by uncovering new indications for existing drugs through the analysis of novel associations between drugs and diseases. This approach leverages dosage and safety data of known drugs, expediting clinical trials and substantially reducing development timelines and costs. AI accelerates drug repurposing through various strategies such as virtual screening, target identification, structure-based drug design, and natural language processing. Machine learning (ML) algorithms facilitate swift drug development, including the repurposing of existing drugs, to identify new or approved antiviral medications capable of inhibiting pathogens like SARS-CoV-2. The application of AI in structural biology, drug repurposing, and development, particularly in the context of COVID-19 research, offers a comprehensive view of AI's applications and inspires researchers to harness its potential in combating the pandemic. AI and ML-based methodologies for drug repurposing encompass network algorithms applied to knowledge maps containing relationships among diverse medical entities (*e.g.*, diseases, drugs, proteins) to pinpoint pertinent host protein targets or regions within host interaction networks that can be targeted. AI also contributes to the structural elucidation of SARS-CoV-2 proteins, a critical step in drug discovery. By predicting the structures of infectious proteins, AI identifies potential drugs that could effectively target these proteins, proposing new chemical compounds for further evaluation as potential therapeutics. Moreover, AI employs quantitative structure-activity relationship (QSAR) modeling to establish quantitative mathematical models correlating chemical structure with biological activity, repurposing them for other disease conditions. The utilization of AI in determining drug dosages, assessing administration effects, predicting bioactive substances, and monitoring drug release aids in identifying bioactive compounds tailored to specific disease-associated targets[332].

11. Discussion

For viral infections, direct-acting agents remain pivotal in treatment, with HDAs serving as a valuable complementary strategy rather than a sole alternative. As an adjunct approach, HDAs can help control viral replication, mitigate resistance development, and offer symptomatic relief by modulating dysregulated host responses, particularly in cases of hyper-inflammation.

In theory, any host factor playing a specific role in physiological conditions could be a potential target for HDAs, given viruses' reliance on cellular machinery for proliferation. However, due to technological constraints and the intricacies of the host genome, many of these factors remain undiscovered, representing a key objective in future antiviral research. Recent advancements have unveiled numerous host factors involved in viral infection through genomic, transcriptomic, and proteomic analyses, alongside high-throughput screening and chemical or genetic interventions. It is crucial to discern whether observed changes are causal or consequential and whether they stem from virus propagation, host defense mechanisms, or limitations in screening methods, a consideration that should always be borne in mind and may explain many undisclosed or misidentified targets[333]. Moreover, the credibility of genomic, transcriptomic, and proteomic analyses would be strengthened by independent research findings, clinical reports, and consistent, thorough investigations. Despite certain cellular factors initially deemed undruggable, such as transcription factors, kinases, and miRNAs, technological and scientific progress has overturned this perception, emphasizing the importance of recognizing both current and future "undruggable" targets.

Innovative solutions beyond traditional targeted therapies have emerged, including the synthetic lethality strategy, PROTACs, lysosome-targeting chimeras (LYTACs), alternative regulation of upstream or downstream effectors, topology-matching design, genome modification technology, and the development of supportive databases and computational methods[334-344]. These advancements promise a wider array of options for combating diseases and broadening the spectrum of antiviral targets.

The intricate and dynamic relationship between viruses and their hosts is shaped by the selective pressures and defensive mechanisms of each entity. Viruses have evolved diverse strategies to evade host immunity, such as encoding homologous proteins and RNAs that counteract or mimic host functions and exploiting cellular factors for viral propagation. Notably, under prolonged inhibition, resistant viruses may switch to different isoforms or entirely bypass the initially dependent pathway[185,345]. This dynamic host-virus interplay appears to be a pervasive phenomenon, potentially reflecting a delicate balance between host and parasites. The adaptability of viruses to exploit alternative pathways underscores the challenges in developing effective antiviral therapies and the significance of comprehending the intricate interplay between viruses and hosts.

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Author contributions

Xiaoxia Gu and Weiguang Sun conceived the review topic. Xiaoxia Gu drafted and edited the manuscript. Mengzhu Zheng, Ya Gao, Shuang Lin, Xiaotian Zhang, Chunmei Chen, Hucheng Zhu, Weiguang Sun, and Yonghui Zhang revised the manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

Appendix A. Supporting information

Supporting information to this article can be found online at https://doi.org/10.1016/j.apsb.2025.03.011.

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