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Review

The role of cholesterol 25-hydroxylase in viral infections: Mechanisms and implications

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ABSTRACT

Viral infections pose significant threats to human health, causing various diseases with varying severity. The intricate interactions between viruses and host cells determine the outcome of infection, including viral replication, immune responses, and disease progression. Cholesterol 25-hydroxylase (CH25H) is an enzyme that catalyzes the conversion of cholesterol to 25-hydroxycholesterol (25HC), a potent antiviral molecule. In recent years, increasing evidence has highlighted the critical involvement of CH25H in modulating immune responses and influencing viral infections. Notably, the review discusses the implications of CH25H in viral pathogenesis and the development of therapeutic strategies. It examines the interplay between CH25H and viral immune evasion mechanisms, highlighting the potential of viral antagonism of CH25H to enhance viral replication and pathogenesis. Furthermore, it explores the therapeutic potential of targeting CH25H or modulating its downstream signaling pathways as a strategy to control viral infections and enhance antiviral immune responses. This comprehensive review demonstrates the crucial role of CH25H in viral infections, shedding light on its mechanisms of action in viral entry, replication, and immune modulation. Understanding the complex interplay between CH25H and viral infections may pave the way for novel therapeutic approaches and the development of antiviral strategies aimed at exploiting the antiviral properties of CH25H and enhancing host immune responses against viral pathogens. In the current review, we tried to provide an overview of the antiviral activity and importance of CH25H in viral pathogenesis.

1. Introduction

CH25H and 25HC are vital elements in cellular cholesterol metabolism [1]. CH25H cross-talks with the immune system and represents interferon (IFN) stimulation [2]. IFN is one of the primary innate immunity parts in virus infections, and plenty of viral immune evasion mechanisms are developed for the IFN pathway [3]. CH25H, an interferon-inducible gene, has garnered increasing attention due to its potent antiviral properties. It participates in cholesterol metabolism and is integral to the host's immune defense against viral pathogens. By producing 25HC, CH25H exerts a broad spectrum of antiviral effects, interfering with viral entry, replication, and immune evasion mechanisms. Upon infection, the innate immune system induces rapid interferons (IFNs) production, translating hundreds of interferon-stimulated genes (ISGs) to limit virus replication. CH25H, a conserved ISG gene that was identified recently, encodes an endoplasmic reticulum (ER) associated enzyme to produce the lipid 25HC [4–6]. A plethora of studies have demonstrated a tight relationship between cellular metabolism and signaling pathways activated by viral infections in immune cells called immunometabolism [7,8]. Different viruses for multiple ends have altered metabolism and related signaling pathways in innate and acquired immune systems [9,10].

Viruses manipulate cellular lipids and membranes to benefit the viral life cycle in different steps of replication [11,12]. Frequent outbreaks of viral infections in the last decades highlighted the importance of antiviral treatment and research [13,14]. A broad antiviral effect of 25HC

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and its enzyme CH25H has been shown in numerous studies [8]. The conducted studies introduced CH25H antiviral effects in different steps of viral infections, for instance, entrance [15], penetration [16], or replication [17]. In this regard, we tried to provide an overview of the antiviral properties and importance of CH25H in viral pathogenesis and replication. This review aims to provide a comprehensive overview of the mechanistic role of CH25H in viral infections, focusing on its impact on viral entry, replication, and host immune defense mechanisms. Understanding the intricate interactions between CH25H and viral infections has the potential to unravel novel therapeutic strategies against viral pathogens and enhance host immune responses. In summary, understanding the mechanistic role of CH25H in viral infections is crucial for unraveling the intricate dynamics between viruses and host cells. This review aims to provide a comprehensive analysis of the involvement of CH25H in viral entry, replication, and immune modulation, shedding light on its implications for the development of novel therapeutic strategies against viral pathogens and the enhancement of host immune responses.

2. The CH25H biogenesis

Cholesterol constitutes approximately 20% of the lipids present in the plasma membrane and serves as a pivotal regulator of cellular homeostasis. It plays many vital roles in maintaining the integrity, fluidity, and functionality of the cell membrane. One of its significant functions is the formation and maintenance of specialized microdomains called lipid rafts. These lipid rafts, rich in cholesterol and sphingolipids, serve as platforms for the organization and clustering of various signaling molecules, receptors, and proteins, thereby influencing crucial cellular processes such as signal transduction, protein trafficking, and cell adhesion. Additionally, cholesterol contributes to the synthesis of steroid hormones, bile acids, and vitamin D, highlighting its essential role in regulating numerous physiological and biochemical pathways within the cell [18,19]. Cholesterol biosynthesis produces crucial intermediate compounds - isoprenoids and oxysterols - that play important roles in cellular processes. Isoprenoids are required for protein prenylation, which facilitates protein attachment to cell membranes and is important for various activities, including vesicular transport and signal transduction. Oxysterols, generated through cholesterol oxidation, regulate cellular functions and maintain cholesterol homeostasis. They also serve as intermediates in bile acid synthesis and modulate the immune system. Understanding the significance of these intermediates in cellular processes enhances our knowledge of cholesterol metabolism's broader implications on cellular homeostasis and physiological functions [20]. Oxysterols are significantly involved in maintaining cholesterol homeostasis through their ability to act as readily transportable forms of sterol. They are crucial in regulating cholesterol synthesis within cells [21]. Oxysterols are derived from the diet, especially from foods high in cholesterol, or synthesized within various tissues and cells through specific cholesterol hydroxylases or autooxidation processes.

One notable oxysterol is 25HC, produced from cholesterol by the enzyme CH25H [22,23]. Different enzymes are required to synthesize 25HC in the mitochondria or the endoplasmic reticulum (ER) [24]. Furthermore, it has been discovered that oxysterols, including 25HC, can elicit both pro-inflammatory and anti-inflammatory immune responses while also exhibiting the ability to suppress viral infections across a wide range of pathogens [25,26]. A study investigating the regulatory mechanisms of CH25H expression revealed an intriguing finding: 25HC itself can activate CH25H expression, establishing a positive feedback loop. The effects of 25HC were observed to be mediated by liver X receptors (LXRs), which serve as receptors for 25HC. Additionally, 25HC was found to modulate cholesterol metabolism through its interaction with Sterol Regulatory Element Binding Proteins (SREBPs) [27]. Extensive reviews on the production and regulation of 25HC have been conducted in other studies [28,29].

3. The 25HC interaction with immune responses

The intricate interplay between the immune system and 25HC has garnered significant attention recently. 25HC, an oxysterol derived from cholesterol, exhibits diverse immunomodulatory effects and is crucial in regulating immune responses. This comprehensive section aims to provide an overview of the current understanding of the interaction between 25HC and immune responses, encompassing its pro-inflammatory and anti-inflammatory effects and its implications in immune regulation and host defense [7]. 25HC plays a significant role in modulating immune responses, exhibiting both pro-inflammatory and anti-inflammatory properties. Several studies have investigated the interaction between 25HC and immune responses, shedding light on its diverse immunomodulatory effects [7,30,31]. Despite oxysterol's distinctive role in metabolism, studies have also mentioned its significance in immunity. Macrophages and dendritic cells rapidly generate CH25H in response to a wide range of TLR (toll-like receptor) ligands and IFN [2]. The expression of CH25H is upregulated in macrophages in response to the activation of TLRs [32,33]. 25HC has also been found to regulate the production of IgA by B cells [34], inhibit the release of interleukin-1 (IL-1) family cytokines [35], affect B cell migration, and control monocyte differentiation to macrophages [36]. Like mediators of the immune system, 25HC dysregulation is involved in immunopathological conditions such as atherosclerosis which is partly related to the expression of inflammatory cytokines, including IL-8 [36,37]. Furthermore, 25HC participates in pro-inflammatory Th1 lymphocyte trafficking and is involved in neuroinflammation through activating the inflammasome component, NLRP3 (NLR family pyrin domain containing 3) [38–40].

The host's innate immune system is essential for the initial recognition of invading viruses. On the other hand, due to the regulatory roles of many of the intermediates in the cholesterol biosynthetic in innate and adaptive immunity (as reviewed comprehensively in [41,42] and broadly antiviral activity of 25HC, further investigation into the molecular mechanism of action of 25HC, could lead to the development of potential antiviral treatments. In summary, 25-HC is a potent modulator of immune responses that can influence immune cell activation, differentiation, and migration. Through its interaction with nuclear receptors, 25-HC can regulate the expression of genes involved in immune responses, including cytokines, chemokines, and immune cell receptors. Furthermore, 25-HC has emerged as a key regulator of antiviral immune responses, suggesting its potential as a therapeutic target for viral infections. The interaction between 25-hydroxycholesterol and immune responses is a complex and dynamic process. 25HC exerts pro-inflammatory and anti-inflammatory effects, activating and regulating immune activation. Moreover, it plays a significant role in immune cell development, polarization, and host defense against viral pathogens. Understanding the mechanisms underlying the immunomodulatory properties of 25HC provides valuable insights into immune regulation and offers potential avenues for therapeutic interventions targeting immune-related disorders and viral infections.

3.1. CH25H and viral infections

CH25H has emerged as a key player in the host defense against viral infections. As an ISG, CH25H is induced in response to viral detection, primarily by activating pattern recognition receptors (PRRs) [7,43]. This induction is part of the innate immune response, which aims to restrict viral replication and dissemination [43]. The enzymatic activity of CH25H leads to the conversion of cholesterol into 25HC, a soluble metabolite with potent antiviral properties. The role of CH25H in viral infections is multifaceted and encompasses various mechanisms to counteract viral pathogens. One of the primary functions of CH25H is the direct inhibition of viral replication [7,44]. 25HC, the product of CH25H activity, has been shown to disrupt viral envelope integrity, thereby preventing viral entry, assembly, and release [7]. This antiviral

effect has been observed against enveloped viruses such as influenza, HIV, and herpesviruses. By targeting the viral envelope, CH25H restricts the ability of viruses to establish productive infections. The upregulation of CH25H leads to the amplification of this antiviral state by stimulating the expression of ISGs [7]. Consequently, CH25H aids in establishing a hostile environment for viral replication and spread within the infected host.

As many viral pathogens manipulate cellular metabolic pathways, it has been imperative to study oxysterols and synthetic LXR ligands on interactions between host and pathogens [41]. Several studies have observed that suppressing cholesterol metabolism or reducing cellular cholesterol could reduce viral entry and replication [45,46]. This consequently results in identifying sterol biosynthesis downregulation via IFNs in a way that helps protect the cell [47]. A variety of those studies demonstrated that inhibition of SREBP pathways and activation of LXRs are potential mechanisms for inhibiting virus replication, including HIV (human immunodeficiency virus) and HCV (hepatitis C virus) [45,48,49]. The interaction of 25HC and CH25H by the immune system and immunologic functions makes these two components a reasonable choice for antiviral research. In this regard, there is a variety of studies for the evaluation of the 25CH antiviral effects. CH25H can induce its antiviral impacts in a wide range of viral families and different viral life cycle stages. Some In-vitro studies evaluated this inhibitory effect. The summary of the conducted research is available in Table 1. Furthermore, this fact needs to consider that viral infections can also manipulate CH25H levels, while there are limited studies in this field (Table 2).

The therapeutic potential of CH25H in viral infections has garnered significant interest. Strategies to enhance CH25H expression or exogenous administration of 25HC or its analogs have been proposed as potential antiviral therapies. However, several challenges must be addressed, including optimizing delivery methods, determining appropriate dosages, and carefully evaluating possible side effects. In conclusion, CH25H is vital in the host's defense against viral infections. Its induction in response to viral detection and subsequent production of 25HC contributes to the inhibition of viral replication and the modulation of host immune responses. The broad-spectrum antiviral activity of CH25H and 25HC against enveloped and non-enveloped viruses highlights their significance in combating viral infections. Further research is needed to fully understand the underlying mechanisms and explore the therapeutic potential of CH25H-based interventions in managing viral diseases Fig. 1.

3.2. Coronaviridae

Coronaviruses (CoVs) are a group of zoonotic viruses belonging to the *Coronaviridae* family, characterized by a single-stranded positive-sense RNA genome. They were first identified in the 1960 s and can cause various animal and human diseases [67,68]. These viruses are divided into four subfamilies: alpha (α), beta (β), gamma (γ), and delta (δ) [69]. In December 2019, an outbreak of acute atypical respiratory infections occurred in Wuhan, Hubei Province, China. A novel coronavirus was identified as the causative agent of these infections and was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [69,70]. Studies have demonstrated the potent antiviral effects of 25HC against coronaviruses, including SARS-CoV and SARS-CoV-2. 25HC inhibits viral replication by disrupting viral entry and interfering with different stages of the viral life cycle. It has been shown to block viral fusion with host cell membranes by depleting cholesterol and inhibiting the activity of cholesterol-dependent viral entry receptors. The illnesses caused by SARS-CoV-2 later became known as Coronavirus Disease 2019 (COVID-19) [71]. As of February 22, there have been nearly 400 million reported cases worldwide, with approximately 5.9 million reported deaths [72]. Numerous studies have focused on antiviral treatments for COVID-19 [73–76]. In this regard, in vitro studies have examined the potential antiviral role of cholesterol CH25H against SARS-CoV-2 [50,51]. Following SARS-CoV-2 infection, CH25H upregulation has been observed in human organoid models and lung epithelial cells, potentially leading to the production of 25CH. This mechanism involves the blockade of membrane fusion of SARS-CoV-2 from infected cells through

Table 1
A summary of conducted research on the inhibitory mechanism of CH25H on different viruses.

Virus features		Study features			Inhibition of viral infection			Ref.
Family	Name	First author	Year	Study setting	Mechanism	Interacted viral protein	Inhibition stage in viral life cycle	
Herpesviridae	KHSV	Serquiña	2021	*In-vitro*	Inhibition of viral gene expression	-	Replication	[17]
Herpesviridae	EBV	Serquiña	2021	*In-vitro*	Inhibition of viral gene expression	-	Replication	[17]
Coronaviridae	PDCoV	Ke	2021	*In-vitro*	Inhibition viral entry	NC	Entry	[15]
Coronaviridae	SARS-CoV-2	Wang	2020	*In-vitro*	Depleting membrane cholesterol	Spike	Entry	[50]
Coronaviridae	SARS-CoV-2	Zang	2020	*In-vitro*	Inhibition membrane fusion	Spike	Entry	[51]
Paramyxoviridae	BPIV3	Lv	2019	*In-vitro*	Inhibition synthesis Viral RNA	-	Replication	[52]
Rhabdoviridae	Rabies virus	Yuan	2019	*In-vitro*	Inhibition of viral penetration	NP, PP	Entry	[53]
Coronaviridae	PEDV	Zhang	2019	*In-vitro*	Inhibition of viral penetration	NC	Entry	[54]
Reoviridae	MRV	Doms	2018	*In-vitro*	Inhibition viral Un-coating	Outer capsid	Entry, Replication	[55]
Rotaviridae	Rotavirus	Civra	2018	*In-vitro*	Inhibition cholesterol recycling	-	Penetration	[16]
Arteriviridae	PRRS	Ke	2017	*In-vitro*	Inhibition of viral penetration	NSP1α	Entry	[56]
Herpesviridae	PRV	Wang	2017	*In-vitro*	Inhibition viral entry	gB	Attachment	[57]
Herpesviridae	KHSV	Serquiña	2017	*In-vitro*	Inhibition of a post entry step	-	-	[58]
Flaviviridae	ZIKV	Li	2017	*In-vitro*	Inhibition of a post entry step	-	Entry	[59]
Arenaviridae	Lassa virus	Ranjan	2016	*In-vitro*	Decreased G1 glycosylation	G1	-	[6]
Flaviviridae	HCV	Brey	2015	*In-vitro* *In-vivo*	Inhibition membrane web formation	-	Replication	[60]
Flaviviridae	HCV	Chen	2014	*In-vitro*	Inhibition NS5A dimer formation	NS5A	Replication	[61]
Rotaviridae	Rotavirus	Civra	2014	*In-vitro*	-	-	-	[62]
Papillomaviridae	HPV-16							
Picornaviridae	HRV							

SARS-CoV-2: Severe acute respiratory coronavirus −2, BPIV3: Bovine parainfluenza virus type 3, MRV: Mammalian orthoreovirus, PRRS: porcine reproductive and respiratory syndrome (Betaarterivirus suid 1), NSP: Nonstructural protein, NP: Nucleoprotein, PP: Phosphoprotein, NC: Nucleocapsid, PDCoV: Porcine delta coronavirus, PRV: Pseudorabies virus, PEDV: Porcine epidemic diarrhea virus, KHSV: Kaposi's sarcoma-associated herpesvirus, ZIKV: Zika virus, EBV: Epstein-Barr virus, HCV: Hepatitis C virus, HPV: Human papilloma virus, HRV: Human rhinoviruses

Table 2
A summary of conducted research for the inhibitory mechanism of viral infections on CH25H.

Virus features		Study features			Virus effect on CH25H	Ref.
Family	Name	First author	Publication year	Study setting	Mechanism	
Hepadnaviridae	HBV	Song	2019	*In-vitro/ In-vivo*	Inhibition of CH25H expression by STAT1-Tyr701 phosphorylation	[63]
Flaviviridae	ZIKV	Magoro	2019	*In-vitro*	Increasing 25HC expression by increasing IL-1β, TNFα, and IL-6	[64]
Arteriviridae	PRRS	Dong	2018	*In-vitro*	NSP1β and NSP11 downregulate the CH25H expression	[65]
Herpesviridae	HSV-1	You	2017	*In-vitro*	UL41 protein downregulates the expression of CH25H	[66]

HBV: Hepatitis B virus, PRRS: porcine reproductive and respiratory syndrome (Betaarterivirus suid 1), ZIKV: Zika virus, HSV-1: Herpes simplex virus 1, NSP: non-structural protein,

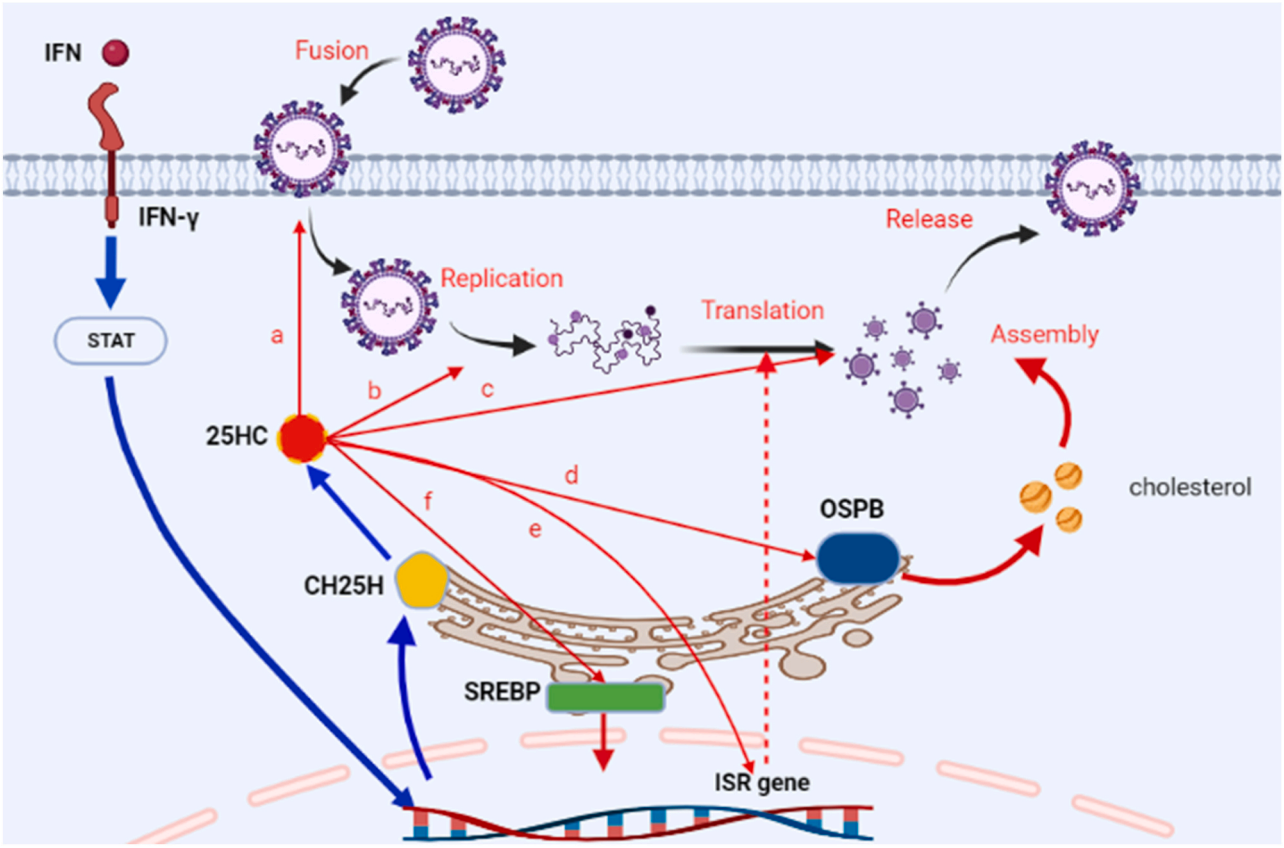

Fig. 1. The antiviral activities of 25HC are broad and encompass multiple mechanisms. The expression of CH25H can be triggered through interferon receptor (IFNR) signaling, leading to the production of 25HC by converting cholesterol. Extensive research has demonstrated that CH25H and 25HC play significant roles in combating viral infections through various means: a) Inhibition of virus adsorption and entry by reducing the cholesterol levels of plasma membrane lipids. b) Suppression of viral genome replication. c) Antagonism of prenylation is crucial for the replication and assembly of viral and endogenous proteins. d) Interaction with oxysterol-binding proteins to modify cholesterol distribution. e) Activation of gene expression associated with the integrated stress response (ISR) in macrophages, resulting in increased oxidative stress and suppression of translation. f) Regulation of inflammation, innate immunity, and adaptive immunity. Overall, the collective actions of CH25H and 25HC encompass a wide range of antiviral mechanisms, offering a robust defense against viral infections.

cholesterol depletion. The cholesterol depletion is triggered by 25CH and acyl-CoA: cholesterol acyltransferase (ACAT) in the ER [50]. Additionally, it has been suggested that 25CH may interfere with the membrane fusion of SARS-CoV-2 by blocking cholesterol export [51]. However, limited information is available regarding the inhibitory effects of CH25H on other coronaviruses [15,54]. Overall, the conducted studies converge on the potential antiviral role of CH25H and 25CH. Despite promising molecular aspects, further studies are urgently needed to understand the potential uses of CH25H or 25CH as antiviral agents.

The antiviral and immunomodulatory properties of 25HC offer potential therapeutic implications in the context of coronaviruses. Strategies to enhance endogenous 25HC production or exogenous administration of 25HC or its analogs have been proposed as potential therapeutic interventions against COVID-19. By harnessing the antiviral and immunomodulatory effects of 25HC, it may be possible to limit viral replication, attenuate excessive inflammation, and enhance the host immune response against coronaviruses. However, further research is needed to determine the optimal dosage, timing, and delivery methods of 25HC-based therapeutics. 25-Hydroxycholesterol emerges as a critical regulator in the host-virus interaction, particularly in the context of coronaviruses. Its potent antiviral properties, impact on viral entry and replication, and modulation of the host's innate immune response make it a promising target for therapeutic interventions against coronaviral infections. Future studies aimed at elucidating the precise mechanisms of action and conducting clinical trials will further enhance our understanding of the therapeutic potential of 25HC in combating coronaviruses.

3.3. Herpesviridae

Herpesviridae is a family of large double-stranded DNA, enveloped viruses that cause persistent infection within the immune-competent host. Based on their genome organization, biological characteristic, and cellular tropism, the *herpesvirinae* family is classified into three subfamilies: Alpha-, Beta-, and Gammaherpesviruses [77,78]. HSV-1 (herpes simplex virus type 1) is one of this family's most widespread human members and is often associated with cold sores. It can also cause herpes keratitis and herpes simplex encephalitis [79,80].

This study provides important insights into the antiviral activity of 25-hydroxycholesterol (25HC) against two oncogenic Gammaherpesviruses, Kaposi's sarcoma herpesvirus (KSHV) and Epstein-Barr virus (EBV). The researchers discovered that viral microRNAs expressed during latent and lytic infection of KSHV downregulate cholesterol biogenesis, preventing the production of 25HC, a cholesterol derivative. Building upon this finding, the study aimed to elucidate the transcriptomic changes and antiviral mechanisms associated with 25HC treatment [17]. The researchers conducted RNA sequencing (RNA-Seq) analysis on primary endothelial cells. They found that 25HC treatment inhibited KSHV gene expression, particularly the critical latency-associated nuclear antigen (LANA) and replication and transcription activator (RTA) genes.

Furthermore, the study demonstrated that 25HC treatment induced the expression of interferon-stimulated genes (ISGs) and various inflammatory cytokines, such as interleukin 8 (IL-8) and IL-1α [17]. These findings suggest that 25HC exerts its antiviral effects by activating innate immune responses. Importantly, the study expanded its investigation to include EBV, another oncogenic virus [17]. The researchers found that 25HC inhibited EBV infection of primary B cells by suppressing viral genes, including latent membrane protein 1 (LMP-1), and inducing apoptosis. RNA-Seq analysis revealed the induction of IL-1 and IL-8 pathways in both primary endothelial cells and B cells upon 25HC treatment. The study also explored the regulation of CH25H, the enzyme responsible for converting cholesterol to 25HC. They observed that type I IFN induced CH25H expression in human B cell-enriched peripheral blood mononuclear cells (PBMCs), suggesting a potential role of CH25H in antiviral immune responses [17]. Overall, this study provides significant insights into the antiviral mechanisms of 25HC against KSHV and EBV infections. It demonstrates that 25HC inhibits viral gene expression, induces innate immune responses, and activates inflammatory cytokine pathways. The findings shed light on the interplay between cholesterol metabolism and viral infections and highlight the broad-spectrum antiviral potential of 25HC. The study's results contribute to our understanding of how modified forms of cholesterol, like 25HC, can combat multiple viral infections, including KSHV, EBV, and potentially other viruses, with potential implications for the development of novel antiviral therapies [17].

A recent study discovered a new mechanism of antiviral action for oxysterols against HSV-1 beyond their previously proposed role in viral entry inhibition [81]. The findings suggest that oxysterols, particularly 25HC and 27HC, exhibit potent antiviral activity and can enhance the host immune response by modulation of IL-6 secretion. This highlights the multifaceted nature of oxysterols in antiviral defense and expands our understanding of their potential therapeutic applications against HSV-1 infections [81]. The study's findings pave the way for further investigations into the antiviral mechanisms of oxysterols and their potential as therapeutic agents in combating viral infections [81]. All in all, 25HC plays a significant role in the pathogenesis of herpesvirus infections. Its potent antiviral effects, impact on viral replication and latency, and modulation of host immune responses make it an attractive target for therapeutic interventions against herpesviruses. Understanding the precise mechanisms underlying the antiviral and immunomodulatory properties of 25HC in herpesvirus infections will pave the way for developing novel therapeutic strategies that aim to control viral replication, prevent viral reactivation, and enhance host immune responses.

3.4. Flaviviridae

The Flavivirus genus comprises many small, single-stranded, positive-sense RNA viruses that can replicate within the cytoplasm of vertebrate and arthropod hosts [82]. Primary hosts are generally mammals and birds that can develop asymptomatic to severe infection and sometimes fatal hemorrhagic fever or neurological diseases. Some of the major pathogens affecting humans are yellow fever (YF), dengue (DEN), Japanese encephalitis (JE), West Nile (WN), tick-borne encephalitis (TBE), and (ZIK) viruses (ZIK). The other members of this group cause economically significant diseases in domestic animals and wild animals [83,84]. The CH25H can induce inhibition of replication and entry in Falaviviridae members [59–61]. In this regard, there are limited studies about the role of the CH25H on Falaviviridae members. Conducted studies mostly focused on HCV and ZIKV.

The HCV NS5A is a major element of the polymerase complex and is an essential factor in virus replication and assembly [85]. A study by Chen et al. shows that CH25H can inhibit the NS5A during HCV replication [61]. In another study, researchers investigated the role of interferon-inducible CH25H in inhibiting hepatitis C virus (HCV) replication. They found that CH25H, known to produce 25HC, exerted broad antiviral activity. Interestingly, the antiviral function of CH25H against HCV was not exclusively mediated by 25HC, as CH25H mutants lacking hydroxylase activity still displayed antiviral effects against HCV.

Further investigations revealed that CH25H interacted with the NS5A protein of HCV, inhibiting its dimer formation, which is crucial for viral replication. These findings unveil a novel mechanism through which CH25H restricts HCV replication, involving both 25HC-dependent and independent events. This highlights the multifaceted antiviral functions of CH25H in combating HCV infection [86]. The recent study focused on the role of interferon-inducible CH25H in restricting HCV replication. The researchers found that CH25H expression, triggered by type I interferon, was significantly upregulated in HCV-positive liver biopsies and HCV-infected primary human hepatocytes. Transient expression of CH25H in human hepatoma cells inhibited HCV infection, independent of the viral genotype, and required CH25H's enzymatic activity. The antiviral effect of CH25H was primarily observed at the level of RNA replication, affecting subgenomic replicons of different HCV genotypes. Additionally, electron microscopy revealed that the production of 25HC by CH25H inhibited the formation of the membranous web, the site of HCV replication independent of RNA replication. These findings highlight the crucial role of CH25H and its enzymatic product, 25HC, in restricting HCV replication by interfering with viral entry and the formation of the replication factory [87]. In addition, it seems to be inhibitory effects of CH25H are not limited to NS5B. It has been suggested that CH25H can inhibit the membrane web production by NS4B in infected cells [60].

Considering the importance of cholesterol hemostasis in HCV replication and tumorigenesis, it could be reasonable to suggest the CH25H and 25HC as important research items for further evaluation of HCV therapeutic agents. Meanwhile, conducted studies about the ZIKV represent controversial effects of CH25H. The CH25H can inhibit viral entry [59], while another study by Magoro et al. revealed that ZIKV infection could increase 25HC expression levels due to increasing IL-1β, TNFα, and IL-6 [64]. This finding represents the importance of further investigations into the function of the CH25H in Falaviviridae replication, especially the ZIKV, and HCV. In sum, 25HC emerges as a crucial player in flaviviridae infections, displaying potent antiviral properties and influencing host lipid metabolism and immune responses. The interplay between 25HC and flaviviridae viruses provides new insights into the complex interactions between viral pathogens and host lipid pathways. Harnessing the antiviral and immunomodulatory functions of 25HC may open avenues for the development of novel therapeutic strategies against flaviviridae infections. Further research is warranted

to unravel the precise mechanisms of action and evaluate the therapeutic potential of 25HC in combating these medically significant viral pathogens.

3.5. Reoviridae

The Reoviridae family contains a large family of viruses with genomes composed of 9–12 double-stranded RNA segments that infect various hosts such as vertebrates, invertebrates, plants, fungi, and prokaryotes [88,89]. This family has nine genera, including Aquareoviruses and pathogens infecting animals and plants [90,91]. The Reovirus, Rotavirus, and Orbivirus genera have been extensively studied in recent years due to their ability to cause disease in animals, including humans [90]. Rotavirus (RV) is the most commonly associated etiologic agent with severe infectious acute gastroenteritis in children and major cause of infant death in developed countries [92]. Approximately 128,000 deaths have been attributed to it after introducing vaccines to children under five years of age [93–95]. There are limited studies about the CH25H in Reoviridae replication (Table 1). Doms and colleagues [55] suggested the inhibitory role of CH25H in MRV by inhibiting the un-coting stage. Furthermore, the researchers also found that 27HC, another naturally occurring oxysterol, demonstrates remarkable antiviral activity against HPV-16, HRoV, and HRhV.

In contrast, other oxysterols with pathophysiological relevance, such as 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol, displayed weaker antiviral effects. These findings suggest that the endogenous production of oxysterols, including 25HC and 27HC, can play a crucial role in the host's defense against a wide range of viral infections [96]. Modulating the production of these oxysterols may serve as a primary strategy for the host to counteract viral infections. Additionally, the results indicate that 25HC and 27HC could be explored as potential therapeutic agents for treating HPV-16, HRoV, and HRhV infections, offering new possibilities for developing targeted antiviral strategies [96]. Some studies also suggested the inhibitory effects of CH25H in rotaviruses [16,62]. These data about the rotavirus and other reoviruses seem to be primary at the current time, and furfur studies are critical for a clear conclusion about the inhibitory stage of CH25H in the Rotavirus replication cycle. For enveloped viruses, it has been observed that 25HC inhibits membrane fusion, likely by affecting membrane characteristics such as hydrophobicity or cholesterol aggregation. However, the mechanisms by which 25HC restricts infection by non-enveloped viruses are poorly understood. In this study, the researchers focused on examining whether 25HC restricts the infection of mammalian reovirus [97]. The study results showed that treatment with 25HC restricted the infection of reovirus prototype strains, precisely type 1 Lang and type 3 Dearing. However, it was observed that 25HC did not limit the infection of reovirus infectious subvirion particles (ISVPs), which are capable of penetrating either at the cell surface or within early endosomal membranes [97]. The researchers also found that treatment with 25HC affected the trafficking of reovirus particles to late endosomes and delayed the uncoating process of the virus. These findings suggest that 25HC inhibits the efficiency of cellular entry of reovirus virions, which rely on specific endosomal membrane dynamics for effective membrane penetration [97]. The exact mechanisms by which 25HC modulates these processes are yet to be fully understood.

In summary, the introduction describes the inhibitory effects of 25HC, produced by CH25H, on mammalian reovirus infection. It highlights the broad antiviral properties of CH25H and its product 25HC, discusses their impact on enveloped and non-enveloped viruses, and presents the specific effects of 25HC on reovirus entry and uncoating. These findings contribute to understanding the mechanisms by which 25HC restricts viral infections and the potential therapeutic implications of targeting this pathway [97].

4. Potential therapeutic role of CH25H in viral infections

The emerging understanding of CH25H and its product, 25HC, has shed light on their potential therapeutic implications in the context of viral infections. CH25H, an ISG, is induced upon viral detection as part of the innate immune response. It catalyzes the conversion of cholesterol into 25HC, which exhibits broad-spectrum antiviral properties against various viral pathogens [7]. This has raised the prospect of harnessing the antiviral effects of CH25H for therapeutic purposes.

One of the key advantages of targeting CH25H in viral infections is its ability to inhibit a wide range of viruses. 25HC has been shown to have antiviral activity against enveloped viruses, including influenza, HIV, and herpesviruses. It disrupts viral envelope integrity, inhibiting viral entry, assembly, and release. Additionally, 25HC can induce the production of IFNs and stimulate the expression of ISGs, further enhancing the antiviral state of infected cells. This multifaceted antiviral activity makes CH25H an attractive target for therapeutic interventions against viral infections [7,98]. Although the exact mechanisms are still being elucidated, CH25H-mediated inhibition of non-enveloped viruses appears to involve modulation of cellular processes related to viral entry and uncoating. These findings highlight the potential of CH25H as a therapeutic target for a broader range of viral infections, including those caused by non-enveloped viruses [97,99,100]. Manipulating CH25H expression or administering exogenous 25HC represents a potential therapeutic strategy against viral infections. Augmenting CH25H expression or providing exogenous 25HC could enhance the host's natural antiviral defenses, limiting viral replication, spread, and pathogenesis. This approach could be particularly beneficial when limited or no specific antiviral treatments are available.

However, several challenges must be addressed for translating CH25H-based therapies into clinical applications. First, optimizing the delivery and stability of exogenous 25HC in vivo is essential to ensure effective antiviral activity. Determining the appropriate timing and duration of CH25H induction or 25HC administration will also be crucial for maximizing therapeutic efficacy. Furthermore, potential off-target effects and long-term consequences of modulating CH25H activity should be thoroughly evaluated.

As previously indicated, a significant hurdle in realizing the therapeutic potential of 25CH lies in the delivery method. However, progress has been made in addressing this challenge, particularly concerning the therapeutic application of 25CH within the respiratory system. Notably, nanoparticle-based 25CH delivery methods hold promise as an effective strategy for 25CH-based treatments in the context of COVID-19. Nevertheless, it is paramount to underscore that this challenge remains a central consideration for the safe application of 25CH in other organs. A pivotal aspect inherent to therapeutic applications is the determination of an appropriate dosage. Like any other substance, 25CH can potentially yield harmful effects when administered in high doses [101]. Simultaneously, as evidenced by dose-dependent effects observed in in-vitro antiviral studies, identifying the optimal dosage for each specific virus emerges as a crucial juncture during the preclinical stages of development [102].

Using 25CH as a potential therapeutic application necessitates a comprehensive evaluation of its associated side effects. As previously indicated, 25CH demonstrates the capability to initiate antiviral defenses. This antiviral response is orchestrated through the IFN pathway and the provocation of an inflammatory reaction. Notably, the inflammation elicited by 25CH may serve a dual purpose. On the one hand, it plays a pivotal role in constraining viral infections.

Conversely, this inflammation could potentially induce detrimental effects. Research has elucidated that the inflammation caused by 25CH is a critical factor contributing to cognitive impairment and neuropsychiatric disorders. A significant consideration in employing 25CH for therapeutic intentions is the potential interplay between synergistic or antagonistic effects with other components or pathways. For instance, while the antiviral efficacy of 25CH can be augmented by the EK1

peptide [103], it is also known that 25CH can induce antagonist effects on oxysterols [104]. Moreover, it is imperative to acknowledge that the viral realm might wield resistance mechanisms against the CH25H pathway. Studies have highlighted that during the latency phase of infection, the KHSV virus can effectively inhibit this pathway by utilizing miRNAs [105].

In conclusion, the therapeutic potential of CH25H in viral infections is an exciting area of research. The ability of CH25H and its product, 25HC, to exert broad-spectrum antiviral effects against both enveloped and non-enveloped viruses highlights their importance in host defense. Exploiting the antiviral properties of CH25H through various strategies holds promise for developing novel therapeutic approaches to combat viral infections. In the realm of understanding CH25H and 25CH, certain knowledge gaps persist. These gaps present inherent challenges that must be addressed for the prospective utilization of 25CH as a therapeutic avenue. Overcoming these challenges can grant future investigations a more precise vantage point. A paramount concern is comprehending the accurate dynamics of interaction and signaling pathways governing 25CH induction and its efficacious mechanisms. Equally pivotal is developing a secure and efficient mode for delivering 25CH to various organs. Additionally, a spectrum of other challenges necessitates careful consideration. This entails determining the optimal and safe dosage of 25CH for invoking antiviral effects in diverse viral infections and ascertaining the duration and efficacy of such therapeutic interventions within both animal models and clinical contexts. The collective constellation of these challenges underlines the imperative for forthcoming research endeavors within this domain. Further studies are needed to unravel the precise mechanisms of CH25H-mediated antiviral activity and to address the challenges associated with its clinical implementation.

5. Conclusion

In conclusion, the role of CH25H in viral infections is an expanding field of research with significant implications. CH25H, as an interferon-stimulated gene (ISG), plays a multifaceted role in the antiviral defense against a wide range of viral pathogens. By producing its enzymatic product, 25HC, CH25H exerts potent antiviral effects by interfering with various stages of the viral life cycle, including viral entry, replication, and assembly. Moreover, 25HC has been demonstrated to modulate host immune responses by promoting the expression of interferon-stimulated genes and regulating immune cell functions. The mechanisms underlying the antiviral actions of CH25H and 25HC are diverse and context-dependent. They may involve cholesterol depletion, inhibition of membrane fusion, modulation of lipid rafts, and activation of innate immune pathways. Additionally, CH25H and 25HC exhibit broad-spectrum antiviral activity against various viral families, encompassing enveloped viruses like coronaviruses, flaviviruses, and herpesviruses. Future studies should focus on unraveling the precise molecular mechanisms by which CH25H and 25HC exert their antiviral effects. Investigating their interactions with viral proteins, cellular receptors, and signaling pathways will provide valuable insights into their mode of action. Given their potent antiviral properties, exploring the therapeutic potential of CH25H and 25HC is crucial. Strategies aimed at enhancing endogenous CH25H expression or administering exogenous 25HC or its analogs hold promise for developing novel antiviral therapies. However, careful evaluation of dosage, delivery methods, and potential side effects is necessary. Investigating the clinical relevance of CH25H and 25HC in viral infections is critical for translating these findings into practical applications. Assessing their expression patterns, levels, and correlations with disease progression in patient samples will provide valuable insights into their diagnostic and prognostic significance. Understanding the impact of CH25H and 25HC on viral pathogenesis is also important. Exploring how viral pathogens modulate CH25H expression and function will illuminate the complex interplay between viruses and the host immune response. In summary, the role of CH25H and 25HC in viral infections is a fascinating area of research with broad implications. Further investigation into their mechanisms, therapeutic potential, role in viral pathogenesis, and clinical relevance will advance our understanding of their significance in combating viral infections and pave the way for developing novel antiviral strategies.

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Parastoo Yousefi: Conceptualization, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing. **Ali Gholami** and **Mohsen Mehrjo:** Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Mandana Akhavan** and **Mohammad Hossein Razizadeh:** Formal analysis, Writing – original draft. **Sajad Karampoor** and **Alireza Tabibzadeh:** Conceptualization, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

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Consent for publication

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