VIRULENCE
2025, VOL. 16, NO. 1, 2474188
https://doi.org/10.1080/21505594.2025.2474188

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

OPEN ACCESS Check for updates

Distinctive function of Tetraspanins: Implication in viral infections

Yuzhi Zhang[a,b,c], Chengwei Pan[d], Sijie Wang[a,b,c], Yidan Zhou[a,b,c], Jiawei Chen[a,b,c], Xiaoyu Yu[a,b,c], Ruining Peng[a,b,c], Nu Zhang[a,b,c], and Hui Yang [a,b,c]

[a]School of Life Sciences, Northwestern Polytechnical University, Xi'an, Shaanxi, China; [b]Engineering Research Center of Chinese Ministry of Education for Biological Diagnosis, Treatment and Protection Technology and Equipment, Xi'an, China; [c]Research Center of Special Environmental Biomechanics & Medical Engineering, Northwestern Polytechnical University, Xi'an, Shaanxi, China; [d]Department of Mechanical Engineering, University of Victoria, Victoria, BC, Canada

ABSTRACT

Harboring four transmembrane domains in their structural hallmark, Tetraspanins (Tspans) are a family of glycoproteins with pivotal functions in a variety of biological and cellular processes. Through interacting laterally with each other or specific membrane proteins, Tspans organize tetraspanin-enriched microdomains (TEMs), modulating cellular signaling, adhesion, fusion, and proliferation. An abundance of evidence has identified the multiple functions in the progression of cancer as well as the underlying molecular mechanisms. Recently, plenty of studies have focused on the utilities of Tspans by pathogens for infection, especially the infection of viruses. The expression of Tspans correlates with the phase of viral infection, the type of virus, and targeted therapies. In particular, perturbations of Tspans in host cells can affect viral attachment, intracellular trafficking, translation, virus assembly, and release. In this review, we summarize and provide a historical overview of the discovery and characterization of various kinds of virus infection and highlight their diversity and complexity, along with the virus life cycle. Furthermore, we examined the current understanding of how various Tspans are involved in the regulatory mechanisms underlying viral infection. This review aims to offer a comprehensive understanding of the targeting of Tspans for therapeutic intervention in infections caused by diverse pathogens.

ARTICLE HISTORY
Received 31 October 2024
Revised 3 February 2025
Accepted 25 February 2025

KEYWORDS
Tetraspanins; virus infection; targeted therapy; regulatory mechanism

Introduction

The Tetraspanins (Tspans) are a group of transmembrane proteins with molecular masses ranging mainly from 20 to 30 kDa and widely expressed in many species [1]. They were first discovered on the surface of human leukocytes and characterized by a conserved superfamily with four transmembrane glycoproteins [2]. The distinguished multi-transmembrane structure consists of four highly hydrophobic transmembrane domains (TM1-TM4), which delimit two extracellular loops (SEL and LEL), one intracellular loop (IL), and short cytoplasmic *N*- and C-terminal tails (Figure 1). The IL and transmembrane domains include multiple conserved polar residues. The two extracellular loops, equipped with glycosylation sites, contain 20–28 amino acid residues and 76–131 amino acid residues, respectively [3]. The large extracellular loops (LEL or EC2) consist of a variable region and a conserved region that contains 2–6 constant cysteine residues: CCG (Cys-Cys), PXSC (Phe-X-Ser-Cys), and EGC (Glu-Gly-Cys) [4]. These cysteine residues are linked to form disulfide bonds, which correctly fold and stabilize the LEL. The above structural features are necessary for the interaction of Tspans with other transmembrane proteins, such as integrins or other signaling molecules. The crystal structural model of the LEL of CD81, a well-characterized member of the Tetraspanins family, has provided critical insights into the structure of this region, highlighting its importance in Tspans' functional roles [5].

Tspans can laterally associate with themselves, other Tspans, or other ligand proteins on the cell surface to form Tetraspanin-enriched-microdomains (TEMs or TERMs) and serve as structural and functional components of the plasma membrane. To date, most ligand proteins found in TEMs can be classified into four major groups: members of the immunoglobulin and integrin superfamilies; proteins with Ig domains; intracellular adaptor molecules such as syntenin; intracellular signaling molecules, such as heterotrimeric G proteins, phosphoinositide 4-kinase (PI4K) and activated protein kinase C (PKC). The interactions formed

CONTACT Hui Yang kittyyh@nwpu.edu.cn; Nu Zhang zhangnu@nwpu.edu.cn

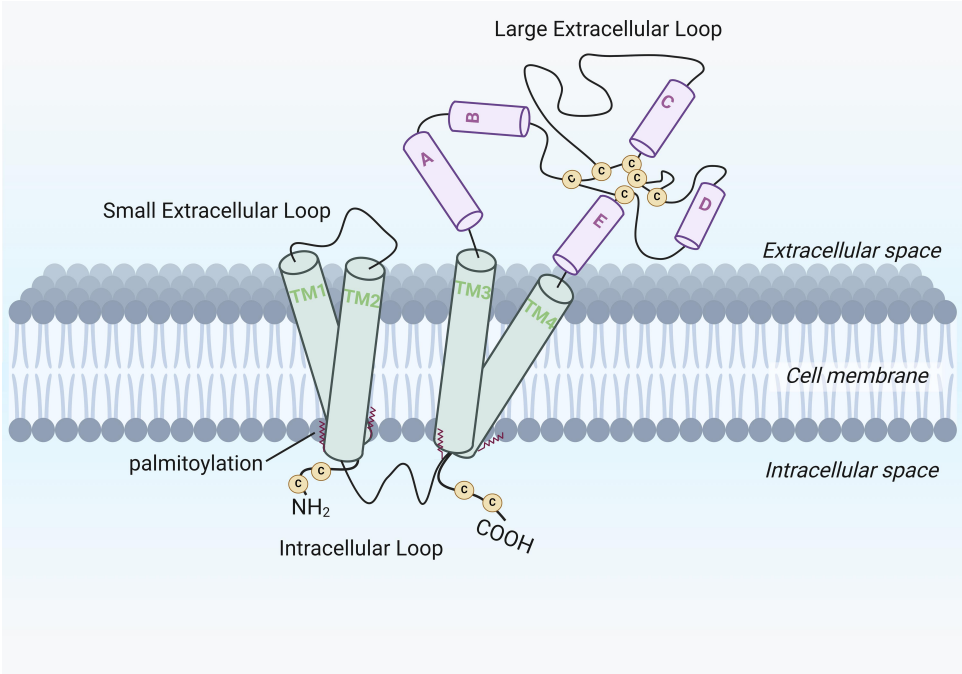

Figure 1. Schematic of typical Tspans structure. Tspans are comprised of four highly hydrophobic transmembrane domains (TM1-TM4), which delimit two extracellular loops (SEL and LEL), one intracellular loop (IL), and short cytoplasmic *N*- and C-terminal tails. The LEL domain consists of a variable region including a signature CCG motif and a conserved region that contains six constant cysteine residues. Multiple juxta membrane cysteine residues are heavily palmitoylated.

by TEMs are indirect and can be determined by co-immunoprecipitation using mild detergents that preserve the tetraspanin–tetraspanin interactions. Thereby, the specific TEMs are not only involved in various normal cellular processes, such as cell signaling, cell adhesion, migration, proliferation, and differentiation, but also fundamental immune functions. In particular, as members of Tspans, CD9, CD63, CD81, CD82, and CD151 have been involved in viral infection [4,6–9].

Viruses are microscopic intracellular parasites that cannot complete most aspects of vital functions by themselves outside the host cell. According to their external structure, viruses can be divided into two categories: enveloped viruses, which have a lipid membrane wrapped around the protein coat that facilitates viral invasion, and non-enveloped viruses, which lack the membrane [10]. Viruses hijack healthy host cells, exploiting their resources and reproductive machinery for rapid replication. This life cycle of viral infection often leads to the death of the cell and, in some cases, illness. Cutting-edge research has unveiled the intricate process and precise mechanisms that viruses employ to infiltrate host cells and initiate infections. Given the essential structure, broad tissue distribution, and specific localization, it is unsurprising that Tspans are involved in multiple stages of viral infection, from the initial cell membrane attachment to the viral particle release and syncytium formation. As described above, the functional TEMs comprised diverse Tspans in the plasma membrane might serve as platforms for virus infection [11], particularly for viruses that directly utilize Tspans as receptors. Similarly, viral enveloped proteins accumulate in TEMs and induce large assemblies of viral transmembrane proteins to facilitate efficient budding. Here, we summarize and compare comprehensive progress in our understanding of the central role of Tspans in the life cycles of related viruses. We aim to provide an integrated understanding of the Tspans' role in the life cycles of viruses and how they affect their pathogenesis.

The specific characteristics and roles of Tspans

CD81

Oren et al. identified a murine monoclonal antibody (mAb) that induced a reversible anti-proliferative effect on a human lymphoma cell line [12]. The protein that interacted with this mAb was exactly CD81 (TAPA-1), which was identified using cell transfection and functional screening methods and cDNA libraries. CD81 is a 26 kDa cell surface protein and is expressed in various types of cells. Initially, CD81 was part of the

B lymphocyte surface receptor complex (CD19/CD21/CD81/Leu13), which generates hyperactive B cells in response to antigens, thereby triggering downstream signaling pathways [13,14]. Additionally, 5A6, a mAb against CD81, has been shown to effectively inhibit the growth of B-cell lymphoma *in vitro* and trigger the intrinsic immune cytotoxic mechanism *in vivo* [15]. Apart from its impact on B cell proliferation, the importance of CD81 in the immune system has also attracted more attention. It has been revealed that the CD81-deficient mice undergo normal *T*- and conventional B-cell development, although CD19 expression on B cells is reduced [16]. Thus, CD81 is not essential for lymphocyte development, and the underlying reason for this has been speculated to be the recruitment of other members of the Tspans family to compensate for the absence of CD81. The reintroduction of CD81 into CD81-deficient B cells restored normal levels of CD19, indicating that the reduction of CD19 expression is owing to the lack of CD81. Moreover, the interaction between CD81 and EWI-2 contributes to the recruitment of α-actin to T cell immune synapses [17].

CD82

CD82 (KAI1/CD82) was originally discovered in research on T-cell activation [18]. Subsequent studies have shown that CD82 acts as a suppressor of tumor metastasis, which may be a potential biomarker for the metastasis of solid malignant tumors [19]. Ubiquitously expressed CD82 functions as a protein scaffold, modulating both cell-matrix and cell–cell pro-adhesiveness, and suppressing cell migration and cell invasion by interacting with a variety of integrin complexes [20]. For instance, CD82 binds the α4β1 integrins, which accumulate at the cell membrane and result in bone marrow homing of progenitor cells [21,22]. In terms of regulation of the immune function, CD82 is highly expressed in multiple immune cells and plays various roles in immune cell responses, including facilitating antigen presentation [23], modulating T-cell polarization [24], and supporting macrophage activation [25]. It has been revealed that macrophage CD82 supports CpG-dependent TLR9 trafficking and signaling. Additionally, CD82 specifically controls TLR9-mediated NF-kB activation and TNF-α production, which is critical for inflammatory cytokine production [26].

CD63

CD63 is a 30–60 kDa member of the tetraspanin protein family. Following endocytosis, CD63 resides in late endosomes/multivesicular bodies (MVBs) and lysosomes via the endosomal route, hence CD63 is also known as lysosome-associated membrane protein 3 (LAMP-3). An increasing number of studies indicated that transport of CD63 may occur through incorporation into intraluminal vesicles (ILVs) or the AP-3 pathway [27]. In this process, the elaborate trafficking and distribution of CD63 must be tightly modulated. CD63 exhibits extensive cellular and tissue distribution and was initially identified as a protein expressed on the cell surface of activated blood platelets [28]. Subsequently, it has been implicated in numerous cellular functions, mainly involved in fundamental cellular processes, such as cell trafficking and endocytosis [29]. It was later revealed that following platelet activation and granule exocytosis, CD63 is transported to the plasma membrane and is associated with the αIIbβ3-CD9 complex [30]. In dendritic cells, CD63 interacts with MHC-II molecules through the endosomal system and triggers an immune response by presenting antigens [31]. Moreover, the function of CD63 in T-lymphocytes is related to the regulation of the chemokine receptor (CXCR) [32]. It was observed that CD63-mediated costimulation significantly induces the activation and proliferation of T cells, accompanied by strong production of IL-2 [33]. It is noteworthy that the specific interactions between Tspans and defined β1 integrins (e.g. α4β1, α3β1, and α6β1) have been demonstrated by immunoprecipitation, suggesting that novel and specific complexes consisting of CD63, CD81, phosphatidylinositol 4-kinase (PI4K), and integrin (α3β1) may play a key role in cell motility [34–36]. Finally, investigations using soluble recombinant LEL domains to identify the specific function of individual Tspans have confirmed that CD63 LEL plays a direct role in monocyte fusion [37].

CD151

The tetraspanin CD151 was first identified in platelet and endothelial cells by using a monoclonal antibody against human acute myeloid leukemia cells [38]. CD151 (PETA-3/SFA-1) is widely expressed in various cells and tissues, including epithelium, endothelium, muscle, renal glomeruli, proximal and distal tubules, and Schwann cells. As with other Tspans, CD151 interfaces with several integrins in cell membranes [39]. Earlier studies have shown that CD151 modulates cell motility and adhesion through interplay with laminin-binding integrins such as α3β1 or α6β4 [40]. However, unlike other tetraspanin–integrin interactions reported previously, CD151 is capable of maintaining a robust association with α3β1, α6β4, and α7β1 integrin

heterodimers, even in stringent detergents [39,41]. Furthermore, the contribution of CD151 to adhesion-dependent signaling is implicated in its capacity to recruit signaling kinases into proximity to integrin complexes, such as type II phosphatidylinositol 4-kinase (PI4-k) and protein kinase C (PKC) [42]. Both anti-CD151 and anti-α3 antibodies showed a significant reduction of neutrophil motility, suggesting a functionally important role for the CD151-α3β1-PtdIns 4-kinase complexes in cell migration [41]. Meanwhile, CD151 deficiency causes suppressed migration activity in T cells, leading to decreased inflammation in a colitis model [43].

CD9

CD9 is a ubiquitously expressed protein that is localized within the endothelial cells, some leukocytes, and diverse tumoral cells [44]. This molecule was originally recognized as a lymphohematopoietic marker [45], belonging to an evolutionarily conserved family of proteins. According to the cell type in which it is located or the molecules it associates with, CD9 is involved in multiple biological processes such as cell adhesion, motility, growth, and sperm-egg fusion [46]. For instance, CD9 is predominantly expressed and localized in the oocyte microvilli, which was reported to play a pivotal function in fertilization [47]. In addition, previous studies have demonstrated that the presence of an anti-CD9 antibody strongly impairs *in vitro* fertilization, and it has been observed that CD9-deficient female mice were affected by infertility [48]. By injection of exogenous human CD9, mouse CD9 or CD81 mRNA at oocytes, the infertility of CD9-/- mouse was partially or completely reversed. In CD9-/- eggs, the high expression level of mouse CD81 mRNA injection is similar to that of endogenous mouse CD9, implying that CD81 may provide functional compensation to some extent for CD9 function in sperm-egg fusion. Furthermore, like other members of the Tspans, CD9 is also associated with integrin adhesion receptors. It was reported that pro- and anti-migratory functions of CD9 were attributed to its modulatory activity toward integrin complexes [49]. Importantly, the tetraspanin CD9 and CD81 have been identified as indispensable for membrane fusion up to date [50]. The anti-CD9 and anti-CD81 mAbs substantially hindered the fusion of murine myoblasts and triggered early myotube degeneration [51], suggesting that the CD9 and CD81 molecules play a pivotal role in muscle cell fusion.

A brief overview indicates that Tspans are found in virtually all tissues (CD81, CD82, CD63, CD151, and CD9). Although their functions overlap in many ways, different molecules have specific functions in specific biological processes or disease contexts. CD82 plays an important role in inhibiting cancer cell invasion and metastasis, whereas CD63 is more involved in regulating cell phagocytosis and extracellular vesicle formation [27]. Additionally, CD9 participates in cellular adhesion, motility, and fertility, specifically in sperm-egg fusion. CD81 has been extensively studied for its role in immune cell regulation, including B cell activation and proliferation. Although their biological roles are varied, Tspans share the ability to interact with a wide array of membrane-bound receptors and integrins, forming complex networks of protein–protein interactions that fine-tune cellular behavior [4]. This versatility makes Tspans key players in research on cancer progression, immune response, cell migration, and membrane fusion.

Tspans in viral entry

Viral entry is a cascade of coordinated events that starts with binding to target cells. This entry process represents the initial stage of viral infection in a virus life-cycle, leading to either the intact virus entering the uninfected host-cell, or introducing its genetic material into the cell. An increasing number of studies performed with the entry of multiple viruses into different host cells have painted an overall picture of the viral entry process. The manner in which a virus enters a cell varies depending on its type, as each type of virus binds variously to these receptors [52], and some viruses require more than one receptor. The pathway by which a virus successfully hijacks the host cell is heavily determined by the initial interaction between the virus and the cell surface receptor. Conceptually, as the glycoproteins are located on the cell surface, the Tspans are likely to incorporate into intact viruses. Subsequent studies do demonstrate that they function in the context of extracellular viruses, namely at the virus cell entry stage [53]. Although the interactions between the virus and cell surface at the molecular level are extremely complex and variable, many essential tetraspanin receptors for the virus entry into cells have been identified [54].

Tspans in enveloped virus entry

One of the first characterized discoveries of a tetraspanin interacting with a virus was the identification of CD81 as a necessary ligand for Hepatitis C virus (HCV) [55]. The E1 and E2 envelope proteins of HCV associate to form a glycoprotein complex located on the outermost layer of the virus that drives

entry into the target cells [56]. In Pileri's research, by screening a cDNA expression library from a subclone of a human T-cell lymphoma line, it was identified that CD81 serves as an E2-binding receptor for HCV. Since its identification is associated with E2, the role of CD81 in HCV entry has been confirmed in numerous studies. Indeed, antibodies targeting the LEL of CD81, as well as a recombinant, soluble form of CD81 LEL, effectively suppress the entry of HCVpp [57,58], HCVcc [59,60], serum-derived HCV [61] and HCV infection *in vivo* [62]. Furthermore, studies demonstrating the anti-CD81 mAb generated by genetic immunization can specifically block HCV infection in different genotypes during the post-binding step of the viral entry process [63], underscore the relevance of targeting CD81 for the prevention of HCV infection. In addition to CD81, several other host proteins, including Scavenger Receptor class B type I (SR-BI), claudin-1 (CLDN1), occludin (OCLN), and Epidermal Growth Factor Receptor (EGFR) are extensively studied entry factors during the HCV entry [64].

In T lymphocytic cells, the knockdown of CD81 and CD9 enhanced human immunodeficiency virus type 1 (HIV-1) entry and promoted syncytia formation, whilst CD81 and CD9 overexpression had an opposing effect [65]. This result indicated CD81 and CD9 as negative regulators of HIV-1 invasion (Figure 2). Separately, a CD63ΔN mutant hindered the T-cell tropic HIV-1 (X4 hIV-1) entry by suppression of CXCR4 surface expression [32]. The N-linked glycans-portions of the CD63 have the ability to form a complex with CXCR4 exclusively at the Golgi apparatus [66]. This intricate interaction can shift the destination of CXCR4 from the plasma membrane to late endosomes/lysosomes, consequently reducing receptor availability on the cell surface and rendering T lymphocytes less susceptible to infection. Meanwhile, CD81 was identified as a putative host factor exploited by influenza A virus (IAV) via genome-wide small interfering RNA (siRNA) screen and proteomic analyses [67]. Following IAV binding, internalized viral particles underwent IAV fusion within CD81-positive endosomes. Their findings also revealed a relevance between IAV fusion within CD81-positive endosomes and infected cells expressing viral proteins, indicating that CD81 directs IAV to endosomes before its uncoating. Notably, during the IAV entry process, CD81 had no impact on the virus binding, internalization, or trafficking to early endosomes. This finding differs significantly from previous studies on HCV and HIV-1 entry [67]. A report using knockout cell lines indicated that the cell-surface complexes comprised CD9, the virus receptor, and a virus-activating protease enable MERS-CoV pseudoviruses to enter cells rapidly and efficiently [68]. Similarly, aberrant expression of CD9 in COVID-19 patients from nasopharyngeal samples was also observed [69]. Given the predominance distribution of CD82 within T cells and B cells, CD82 was found to bind Human T cell leukemia virus type 1 (HTLV-1) Env proteins (Figure 2) [70]. Also, HTLV-1 Gag protein showed a strong co-localization accompanied by CD82-enriched membrane domains at the plasma membrane of T cells, which may contribute to the mobilization of nascent virions for trafficking to sites of intercellular junction [71]. Subsequent studies revealed that, unlike HTLV-1, the HIV-1 Gag did not co-localize, co-isolate, or immunoprecipitate with CD82, suggesting this interplay is specific.

Tspans in non-enveloped virus entry

The entry of human papillomaviruses (HPV) is a multi-step process that sequentially involves engagement with different receptors. Papillomaviruses are more difficult to culture *in vitro*, which limits the progress of related studies. Therefore, pseudo viruses have been the most widely used tool in HPV infection studies and immunogenicity evaluations [72]. Initially, HPV attaches to proteoglycans such as heparan sulfate proteoglycans (HSPGs) and laminin-332 (previously named laminin-5) as primary attachment sites. Subsequently, they transfer to secondary receptor molecules for entry into the host cells [73]. In previous reports, a strong co-localization of virions with two members of the Tspans, CD151 and CD63, on the plasma membrane of infected HeLa cells and primary keratinocytes was detected by confocal fluorescence microscopy [74,75]. This co-localization suggests the biological relevance of CD151 for HPV infection. However, the initial binding of HPV to the cell (e.g. HeLa or HaCaT) surface is independent of CD151 (Figure 2). Subsequent experiments using total internal reflection (TIRF) microscopy for live-cell imaging observed the lateral movement of HPV16 in association with CD151 before internalization. Most notably, only viruses associated with CD151 disappeared from the TIRF field, whilst after the CD151-depleted, viruses still remained on the plasma membrane. Antibodies or siRNA directed against CD151 significantly impaired HPV uptake and disassembly. These observations and data support that the correlation of HPV with CD151 at the cell surface is a precondition for viral internalization. Another tetraspanin that was shown to play a role in HPV infection and of epithelial cells is CD9. Specifically, the diminished expression of CD9 can support infection by

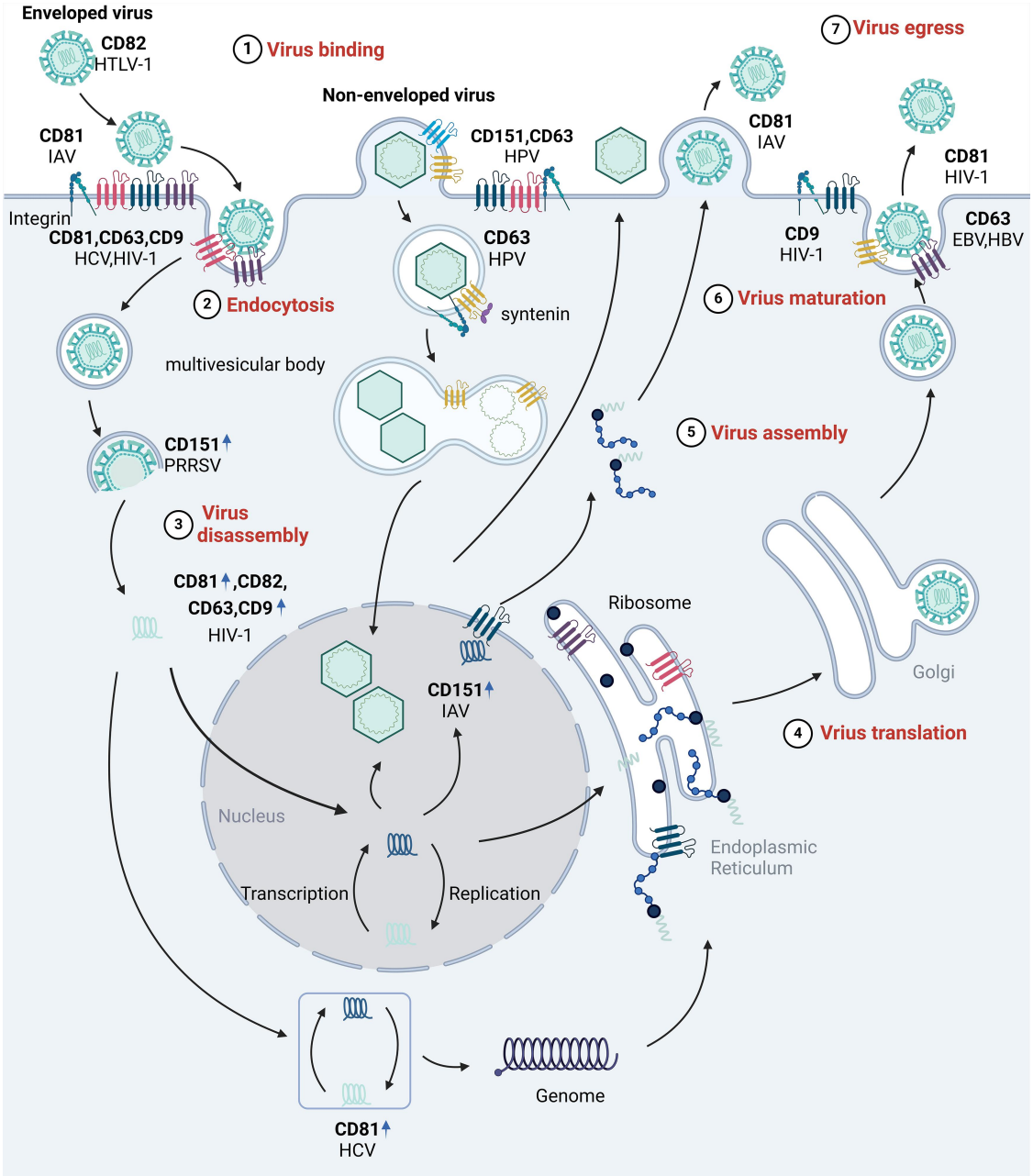

Figure 2. Diagram showing the role of Tspans during virus infection. (1) Tspans on the cell membranes serve as virus-specific receptors, engaging with proteins on the virus surface. (2) Tspans trigger viral internalization through endocytosis or fusion. (3) Tspans support the disassembly of the virus. In the case of some viruses, the downregulation of CD151 perturbs HPV disassembly. (4) altering Tspans has an impact on virus replication, ultimately resulting in the efficient delivery of viral genomes into the cytoplasm or nucleus. (5) Tspans mediate the assembly of new viral particles within host cells. (6) spatial aggregation of Tspans and viruses assists in virus maturation. (7) the resulting high concentration of virus envelope components enables efficient budding and release.

modulating the activity of ADAM17 sheddase and the downstream phosphorylation of ERK. These observed effects are correlated with the assembly of the HPV entry platform [76]. However, it is important to note that pseudo viruses may not fully mimic the HPV life cycle. Delineation of these events will provide deeper insights into the mechanisms underlying HPV16 infection.

Tspans in viral replication

After the viral genome has been successfully uncoated, they exploit the machinery and metabolism of a host cell to initiate the process of replication, creating multiple copies of themselves. In this stage, the RNA or DNA viral genomic information eventually completes the manufacture of its proteins through transcription

and translation [77]. Once all the necessary components for the mature virion formation gather at a particular site in the cell, viral particles are assembled by the synthetic viral proteins and become productive viruses. Generally, it is a broad conception that Tspans are involved in the intracellular trafficking of internalized viruses and assist in defining virus assembly. The exact regulatory processes by which these proteins function were detected in infected host cells.

Tspans in enveloped virus replication

In addition to regulating several virus entry processes, CD81 also participates in the post-entry events of the virus life cycle. Given that HCV enters cells through clathrin-mediated endocytosis and low pH-dependent fusion with endosomes, Sharma and co-workers investigated the effects of low pH and CD81 on HCV infectivity [78]. Their finding showed that at the early stage of the entry process, CD81 drives HCV to undergo low-pH-dependent fusion, further suggesting CD81 may be both necessary and sufficient to enable HCV E1E2 glycoprotein to fuse with acidic intracellular compartments. Furthermore, the replication of HCV was markedly affected by force silencing or expression of CD81, indicating its role in the efficient replication of the HCV genome. In their study, the CD81 expression level was positively correlated with the HCV RNA replication but inversely affected the viral protein synthesis. This converse relationship further suggests that CD81 might direct HCV RNA to the replication process [79]. In this aspect, TEMs composed of CD81/CD82/CD63 in T cells have been demonstrated to play a comparable role in HIV-1 assembly [80] and CD81 directly or indirectly interacts with HIV-core proteins (Gag) in HIV-1 producing cells (Figure 2). Meanwhile, the function of CD63 was also fully investigated during HIV-1 replication. The CD63-specific siRNA not only significantly reduces the level of the early HIV protein Tat, but also leads to a decrease in the late protein p24, thereby inhibiting the late trafficking event of HIV-1 in $CD4^+$ T cells, dendritic cells, and macrophages [81–83]. These findings revealed that CD63 exerts a dual function both in the early stages of HIV-1 replication and the late stages of viral protein assembly. Nevertheless, the molecular signaling mechanisms, especially those that might contribute to early or late events in the HIV replication cycle, remain to be fully elucidated. Likewise, a recent study reported that CD9 and ITGA3 are specifically upregulated by HIV-1 infection in the macrophage, thereby supporting viral replication [84]. Based on the high expression level of CD151 in the human respiratory tract, Qiao and his colleagues [85] performed with CD151 knockout mice in an *in vivo* model of IAV infection reported that CD151 silencing may not exert an impact on IAV entry and nuclear transport, but nuclear export and subsequent assembly of viral progenies was restrained (Figure 2). For porcine reproductive and respiratory syndrome virus (PRRSV), as one of its entry mediators, CD151 also suppressed PRRSV replication indirectly through microRNA regulation (Figure 2) [86].

Tspans in non-enveloped virus replication

Upon association and co-internalization between viral particles and the Tspans CD151 and CD63, the endocytosed HPV is trafficked toward perinuclear vesicles that contain CD63. Additionally, a recent study has established that CD63 silencing leads to decreased infectivity of HPV16, HPV18, and HPV31 in different keratinocyte cell lines. Interestingly, CD63 knockdown did not affect virus binding or virus internalization. Instead, CD63 silencing led to the loss of viral major capsid protein epitope L1–7 during intracellular HPV trafficking. The essentiality of CD63 and syntenin in HPV intracellular trafficking was also demonstrated by Linda and colleagues [87]. These researches supported the notion that CD63 controls post-endocytic HPV trafficking. As of now, there is limited knowledge about HPV replication, except for the fact that CD63 and syntenin facilitate the trafficking of the HPV particles in host cells as a complex. The co-localization of endocytosed HPV with Tspans probably suggests a potential involvement of Tspans in the replication of HPV.

Tspans in viral egress

Once the viral proteins and viral genomes are accumulated, and the producer cells have exhausted all their resources to generate the viral progeny, viruses must be released from the infected host cells to spread the infection. The presence or absence of a viral envelope is a critical factor in the viral life cycle that determines the cellular exit strategies of enveloped from non-enveloped viruses. As for non-enveloped viruses, the virus particles are released from the infected cell via cell lysis, a process that dismantles its membrane and ultimately leads to cell death either through apoptosis or necrosis. Thus, no specific egress mechanism is essential [88]. By contrast, the complex series of events that facilitate the enveloped viral egress are typically orchestrated at the plasma membrane by viral transmembrane proteins interacting with the inner structure proteins. Conceptually, virus budding involves the

deformation of a cellular membrane, resulting in the envelopment of the forming bud by one or more lipid bilayers [89]. This membrane scission occurs, enabling the enveloped particle to escape into the extracellular space.

Since Tspans were shown as a critical host factor in the cell membrane, numerous researches have been dedicated to exploring the underlying mechanisms that drive the virus egress in association with Tspans.

Tspans in enveloped virus egress

While it is well established that CD81 acts as a central regulator in HCV entry and replication by an interplay with the other factors in proximity, not much is known regarding its involvement in HCV budding, egress, and transmission. Studies on HCV egress noted that anti-CD81 mAbs inhibited both cell-free and cell-to-cell viral infection [90]. Meanwhile, assembled viral particles are released from the cell through a CD81-independent secretory pathway (Figure 2) [91]. Interestingly, another report on HCV transmission highlights that the coexistence of the CD81-independent and CD81-dependent routes can directly infect new cells through cell-to-cell transmission [92]. Overall, the involvement of CD81 in HCV transmission remains largely controversial. It has been observed that during the egress of IAV, CD81 was recruited to the virus budding sites on A549 cells (human lung carcinoma cells), and in particular, incorporated with individual virions at the specific sub-viral locations. Additionally, when CD81 levels were ablated via siRNA knockdown, the budding process was impaired, resulting in virions being failed to detach from the plasma membrane [67]. Specifically, the decrease in the number of released virus particles, without a concurrent reduction in the number of assembling virions upon CD81 knockdown, indicates that CD81 may be engaged in a late stage of the virus budding process, potentially influencing the final scission step. This was accompanied by a remarkable drop in IAV progeny virion release, even when the uncoating defect was compensated. In conjunction with the above mentioned, CD81 serves as a marker and an important component in the entry and release of infectious IAV particles.

In a previous study, the HIV-1 Env and Gag proteins in T cells were found to strongly colocalize with CD63 and CD81 and, to a lesser extent, with CD9. The observation was made using immunoprecipitation and cryoimmunoelectron microscopy (CEM) revealed the incorporation of CD63 and CD81 into virions budding at the plasma membrane. This confirms that TEMs are essential for the HIV-1 egress event in T cells [93]. This is also supported by the fact that downregulating CD9 reduced the production of HIV-1 particles [84].

Through elegant immunofluorescence studies, Ninomiya et al. showed that CD63, an exosome-associated protein, co-localizes with HBV protein components in infected HepG2.2.15 cells [94]. CD63-depleted cells substantially result in a significant reduction in the levels of large hepatitis B surface antigens (LHBs) and a decreased infectivity of the released HBV particles. This suggests that CD63 plays a crucial role in enhancing the efficient production and release of mature HBV particles. Conversely, its inhibition induces the secretion of less infectious virus particles [94]. Interestingly, CD63 affects Epstein–Barr virus (EBV) latent membrane protein 1-mediated intracellular signaling including MAPK/ERK and NF-κB activation [95]. This intriguing mechanism could potentially be applied to the trafficking of LHBs to exosomal membranes, supporting the production and release of mature HBV particles.

Tspans in non-enveloped virus egress

Currently, no association between Tspans and HPV release has been identified. Pyeon et al. confirmed that by using small molecule inhibitors of HPV infection, the events in cell cycle progression through the early stage mitosis are essential for successful HPV infection [96]. For instance, nuclear envelope breakdown, cytoplasmic microtubule reorganization, and subnuclear structure change modulate efficiently HPV life cycle. Further studies will be required to clarify the specific role of Tspans during the budding of HPV or other naked virions.

Targeting Tspans in viral disease

The studies presented in the preceding sections combining molecular and biochemical analyses have highlighted the critical roles of distinct Tspans in regulating the infections caused by diverse viruses, as listed in Table 1. Thus, it appears to be highly relevant to consider tetraspanin protein as a promising therapeutic target for viral infection.

So far, the role of Tspans in the field of viruses can be divided into two distinct types: the direct interplay between Tspans and virus or viral-expressed proteins (e.g. CD81 with HCV E2), and the indirect interplay by mediated host cell functions through TEMs [105]. In the latter scenario, this is largely attributed to the involvement of TEMs in natural cellular processes of the organism such as adhesion, internalization,

Table 1. Effects of tetraspanin functions in various virus life cycles.

Virus	Tetraspanin	Process	References
HCV	CD81	Binding the E2 protein Promoting the RNA replication	[55,97,98]
HIV-1	CD81	Inhibiting the invasion Interacts with the Gag protein	[65] [80]
	CD63	replication cycle and late viral protein assembly	[84]
	CD9	Promoting the RNA replication	[99]
	TEMs (CD81/ CD82/CD63)	Virus assembly Virus egress from T cells	[100] [101]
IAV	CD151	Nuclear export and assembly of virion	[7,85]
	CD81	Virion release	[67]
PRRSV	CD151	Virus entry mediator	[102]
HPV	CD151, CD63	Component of virus entry	[7,103]
HTLV-1	CD63	Virus trafficking	[87]
	CD82	Binding the Gag protein	[104]
HBV	CD63	Enhancing the virus production and release	[94]
CoV	CD9	Virus entry	[68]

vesicular transport, or exocytosis. However, despite the broad tissue distribution across various tissues, the multitude of diverse cellular functions, and the fact that some have been described since the 1990s, their clinical potential remains unfulfilled. Generally, there are three primary approaches available for targeting Tspans: mAb, siRNA, and soluble EC2 loops. Other tools such as exosomes are also being discussed.

Monoclonal antibodies

Theoretically, anti-tetraspanin mAbs primarily focus on suppressing the physical interaction with partner proteins. Different usage of mAbs targeting CD9, CD63, CD81, or CD151 has been raised to investigate diverse tetraspanin functions. These research efforts have shown promising results *in vivo*. Previously, it has been identified that the murine IgG1 antibody 5A6, which specifically targets CD81, is equally effective in inhibiting the growth of B cell lymphoma in a xenograft model as marketed rituximab [106], alternative anti-CD81 mAbs have been developed since then, and they are recognized as exceptional tools commonly used to manipulate CD81 molecules, particularly during the invasion of CD81-dependent virus entry into cells. Anti-CD81 mAbs can prevent HCV infection, at least in part, by blocking the binding of the HCV E2 protein [62,63]. However, to our knowledge, there are currently no anti-CD81 mAbs in clinical development [107].

In the realm of both infectious and chronic diseases, mAbs targeting CD151 are commercially available and possess remarkable potential for preventing metastatic diseases [59]. Considering the involvement of CD151 in the evasion of IAV viruses, it implied that intervention of anti-CD151 mAbs is more likely a therapeutic agent against IAV.

Yet, not all anti-tetraspanin mAbs exert an antagonist effect. In this regard, a few anti-tetraspanin antibodies promote tetraspanin-dependent functions rather than inhibit them. As described above, anti-CD81 mAbs could trigger HIV-1 Env-associated viral entry in primary human T cells [108]. Therefore, the utilization of anti-tetraspanin mAbs as a therapeutic method in viral infections should be cautiously approached due to their diverse and virus-specific consequences.

Moreover, even though Tspans are relatively tiny molecules on the cell surface (approximately 100 extracellular amino acids), mAbs targeting the same tetraspanin can exhibit a variety of distinct characteristics [109]. For example, some anti-CD151 mAbs may be capable of binding entire proteins, whilst others recognize only an epitope of the CD151 molecules exposed when CD151 is not associated with laminin-binding integrins [110,111]. Likewise, the antibody specifically targeting CD81 and CD9 recognizes a diverse epitope and presents an equally potent capacity. The unique 5A6 antibody is precisely attributed to the fact that it recognizes a specific epitope on the LEL of CD81, thereby inducing anti-metastatic activity [112]. Therefore, it remains to be seen whether mAbs combined with different epitopes will show a sufficient degree of prophylactic efficacy.

Recombinant soluble LEL

Previous research has established that recombinant forms of the LEL domains of Tspans have the potential to influence various physiological functions, such as sperm egg fusion and fertilization [113,114]. In addition, recombinant soluble LELs also have been used effectively to investigate and analyze the mechanisms of different Tspans in viral infection processes. Typically, recombinant soluble LEL targeting CD81 leads to decreased infectivity of HCV [115]. During infection, soluble LEL of CD81 competitively binds to the HCV E2 protein, thereby preventing the virus from interacting with endogenous CD81 LEL. The specific binding site for their interactions has been mapped to a hydrophobic patch in the variable sub-loop region of the tetraspanin, which comprises Ile^{182}, Phe^{186}, Asn^{184}, and Leu^{162} [116]. In a related study, it was demonstrated that a small peptide analog including residues 176–189 exhibits moderate inhibitory activity against the CD81-LEL-HCV-E2 interaction [117]. Some small molecule inhibitors, such as benzyl salicylate and fexofenadine of the interaction between the CD81 LEL and the HCV protein E2 have been tested and designed.

However, it remains unclear whether maximal efficacy can be achieved using small molecule inhibitors *in vivo*, as the majority of HCV studies performed to date have been conducted in cell cultures [118]. All existing results support that soluble LELs may inhibit the uptake of virions.

Apart from the direct interference of HCV E2-CD81 binding, it has been observed that various soluble LELs derived from CD9, CD63, CD81, and CD151 possess the remarkable ability to effectively inhibit CCR5-tropic HIV-1 infection in macrophages [119]. This intriguing finding suggests that it is the intricate network of the entire TEM, encompassed by multiple Tspans rather than individual Tspans, that plays a critical role during this process. Curiously, the mechanism of inhibition of HIV-1 infection is affected by anti-CD63 mAbs but not by antibodies to other Tspans. An alternative explanation for these results is that CD63 might be involved in a more direct manner in viral infection, potentially providing a rationale for the selectivity observed with mAbs.

Collectively, it is evident that recombinant soluble LELs derived from various Tspans have emerged as promising tools for disrupting tetraspanin-associated viral infection. Although the mechanism of the molecules discussed afore has not been fully elucidated, it is speculated that soluble LELs have the capacity to disrupt lateral interactions crucial for the assembly and maintenance of TEMs.

Gene ablation

The utilization of genetic manipulation to safeguard host cells is an effective method in preventing viral infections, as numerous researchers have confirmed its protective efficacy and its association with RNAi technology. For *in vitro* experiments, RNAi methods encompass siRNA, shRNA, and CRISPR-Cas9 for the targeted knockdown of specific Tspans. During the invasion of viral infection, the CD63 knockdown in macrophages inhibited HIV replication and assembly events [82], CD151 siRNA can hinder HPV uptake [7], while siRNA against CD81 regulated IAV fusion within the endosomes [67]. Additional RNAi studies have shown that the knockdown of CD81 significantly reduced susceptibility to HCV infection more effectively than soluble CD81 [59]. Generally, tetraspanin-specific RNAi techniques can be therapeutically beneficial in infectious diseases by antagonizing crucial functions of Tspans or associated proteins, ultimately leading to diminished viral infection (for example, fusion, uptake, assembly, and budding). Nevertheless, the specific mechanism of viral infection responding to these siRNA molecules that target individual Tspans is not always beneficial. For instance, CD9 and CD81 siRNA have been reported to increase HIV-1 entry and syncytia formation. Similarly, CD81 knockdown also impacts IAV budding, which results in a reduction in progeny IAV [67].

To date, tetraspanin-deficient mice are viable with relatively minor defects, suggesting the potential capacity for family members to mutually compensate [120,121]. Nevertheless, the targeted strategies for gene knockout caused by viruses are rarely reported, and siRNA or shRNA remains an effective prophylactic measure to combat the virus. In an *in vivo* model, infected mice with the CD151 gene knockout show a significant down-regulation tendency in the virus titer, leading to an improved survival rate. This robust host antiviral response and inflammasome activation are attributed to the essential role of CD151 in resisting infection, which relies on nuclear export signaling [85].

Despite these promising results, there are still some inherent uncertainties and difficulties associated with therapeutics that target Tspans. On the one hand, in the context of viral infections, individual Tspans do not consistently function as agonists or antagonists, even within the same viral system. Hence, either inhibition or enhancement may pose a challenge to the development of effective therapeutic strategies. On the other hand, due to the close interaction between the members of the tetraspanin family, more than one tetraspanin is often involved in the same disease background. During the assembly stage of HIV infection, the aggregation of TEMs composed of CD81, CD82, and CD63, along with viral structural proteins, has been observed [80], indicating the compensation and redundancy provided by non-targeted Tspans. Over the years, multiple methods with highly specific targeting and conformational modification have been developed, aiming to circumvent the related therapeutic problems. In addition, it is worth mentioning that non-protein elements of TEMs, such as palmitoylation or cholesterol, may represent a prospective treatment strategy for virus elimination [105,122]. Finally, the extensive distribution of numerous Tspans across various cells and tissue types potentially creates a challenge in achieving tetraspanin targeting [121]. In this case, the development of cell-specific delivery systems would be imperative to fully harness the advantages of tetraspanin function in preventing viral infection.

Conclusion and future perspectives

There are substantial consequences resulting from infection with any virus. The complexity and unique features

of the Tspans have led to the discovery of multiple pathways of host-virus interactions. As is evident from studies reviewed herein, it is clear that Tspans can mount vigorous responses to several acute and chronic viral infections, including IAV, HCV, and HPV infections as discussed here. In many situations, this triggers a series of normal host-cell processes involved in tetraspanin or TEMs, such as fusion, endocytosis, and intracellular trafficking, to enter cells, replication, and budding. In parallel, the role of diverse Tspans involved in the specific stages of viral infection was identified. The balance between therapeutic strategies and responses contributing to organic damage and immunopathology is crucial in all host responses to viral infections. Clearly, further knowledge is still required concerning direct tetraspanin-mediated interactions with viral infection and the consequences of prevention measures targeted by Tspans. Numerous insights into the potential role of Tspans in viral infections have been revealed, spanning studies at the cellular level to investigations in mouse models; however, treatment needs remain largely unmet. Different tetraspanin functions and molecular interactions are increasingly studied over time, broadening the treatment options for infectious diseases as well as the possibilities for intervention. Elucidating the intricate interactions between Tspans and viruses, and understanding how these interactions are modulated by Tspans holds the key to the development of targeted therapeutics. Therefore, future studies aimed at shedding light on these aspects are of utmost importance. The modeling of spatiotemporal interactions mediated by Tspans and TEMs is now being conducted in numerous laboratories, indicating that the scaffolding properties of Tspans contribute to the formation, stabilization, and dynamics of signal transduction complexes at the plasma membrane. Moreover, effective therapy strategies aiming at Tspans or TEMs are urgently to be harnessed.

Acknowledgements

All figures were created with BioRender.com.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the National Natural Science Foundation of China (12002285), and Fundamental Research Funds for the Central Universities (D5000220026).

Data availability statement

Data availability is not applicable to this article as no new data were created in this study.

Author contributions

H. Y. and N. Z. conceived and outlined the review. Y. Z. and N. Z. wrote the manuscript and designed the figures. C. P., J. C., S. W., and YD. Z. coordinated the revision and manuscript preparation. X. Y. and R. P. analyzed the literature. All authors read, edited, and approved the final manuscript.

ORCID

Hui Yang http://orcid.org/0000-0001-7567-3048

References

[1] Veenbergen S, Van Spriel AB. Tetraspanins in the immune response against cancer. Immunol Lett. 2011;138(2):129–136. doi: 10.1016/j.imlet.2011.03.010
[2] Boucheix C, Rubinstein E. Tetraspanins. Cell Mol Life Sci. 2001;58(9):1189–1205. doi: 10.1007/PL00000933
[3] Maecker HT, Todd SC, Levy S. The tetraspanin superfamily: molecular facilitators. The FASEB J. 1997;11(6):428–442. doi: 10.1096/fasebj.11.6.9194523
[4] Monk PN, Partridge LJ. Tetraspanins – gateways for infection. Infect Disord Drug Targets. 2012;12(1):4–17. doi: 10.2174/187152612798994957
[5] Kitadokoro K. CD81 extracellular domain 3D structure: insight into the tetraspanin superfamily structural motifs. Embo J. 2001;20(1):12–18. doi: 10.1093/emboj/20.1.12
[6] Van Spriel AB, Figdor CG. The role of tetraspanins in the pathogenesis of infectious diseases. Microbes Infect. 2010;12(2):106–112. doi: 10.1016/j.micinf.2009.11.001
[7] Scheffer KD, Berditchevski F, Florin L. The tetraspanin CD151 in papillomavirus infection. Viruses. 2014;6(2):893–908. doi: 10.3390/v6020893
[8] Fast LA, Lieber D, Lang T, et al. Tetraspanins in infections by human cytomegalo- and papillomaviruses. Biochem Soc Trans. 2017;45(2):489–497. doi: 10.1042/BST20160295
[9] Ooi YS, Stiles KM, Liu CY, et al. Genome-wide RNAi screen identifies novel host proteins required for alphavirus entry. PLOS Pathog. 2013;9(12):e1003835. doi: 10.1371/journal.ppat.1003835
[10] Cohen FS. How Viruses Invade Cells. Biophys J. 2016;110(5):1028–1032. doi: 10.1016/j.bpj.2016.02.006
[11] Martin F, Roth DM, Jans DA, et al. Tetraspanins in viral infections: a fundamental role in viral biology? J Virol. 2005;79(17):10839–10851. doi: 10.1128/JVI.79.17.10839-10851.2005
[12] Oren R, Takahashi S, Doss C, et al. TAPA-1, the target of an antiproliferative antibody, defines a new family of transmembrane proteins. Mol Cell Biol. 1990;10(8):4007–4015. doi: 10.1128/MCB.10.8.4007
[13] Matsumoto AK, Martin DR, Carter RH, et al. Functional dissection of the CD21/CD19/TAPA-1/

Leu-13 complex of B lymphocytes. J Exp Med. 1993;178(4):1407–1417. doi: 10.1084/jem.178.4.1407
[14] van Zelm MC, Smet J, Adams B, et al. CD81 gene defect in humans disrupts CD19 complex formation and leads to antibody deficiency. J Clin Invest. 2010;120(4):1265–1274. doi: 10.1172/JCI39748
[15] Vences-Catalan F, Kuo CC, Rajapaksa R, et al. CD81 is a novel immunotherapeutic target for B cell lymphoma. J Exp Med. 2019;216(7):1497–1508. doi: 10.1084/jem.20190186
[16] Maecker HT, Levy S. Normal lymphocyte development but delayed humoral immune response in CD81-null mice. J Exp Med. 1997;185(8):1505–1510. doi: 10.1084/jem.185.8.1505
[17] Fooksman DR, Vardhana S, Vasiliver-Shamis G, et al. Functional anatomy of T cell activation and synapse formation. Annu Rev Immunol. 2010;28(1):79–105. doi: 10.1146/annurev-immunol-030409-101308
[18] Gaugitsch HW, Hofer E, Huber NE, et al. A new superfamily of lymphoid and melanoma cell proteins with extensive homology to schistosoma mansoni antigen Sm23. Eur J Immunol. 1991;21(2):377–383. doi: 10.1002/eji.1830210219
[19] Dong JT, Lamb PW, Rinker-Schaeffer CW, et al. KAI1, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2. Science. 1995;268(5212):884–886. doi: 10.1126/science.7754374
[20] Hall A, Fontelonga T, Wright A, et al. Tetraspanin CD82 is necessary for muscle stem cell activation and supports dystrophic muscle function. Skelet Muscle. 2020;10(1):34. doi: 10.1186/s13395-020-00252-3
[21] Saito-Reis CA, Balise VD, Pascetti EM, et al. Tetraspanin CD82 regulates S1PR1-mediated hematopoietic stem and progenitor cell mobilization. Stem Cell Reports. 2021;16(10):2422–2431. doi: 10.1016/j.stemcr.2021.08.009
[22] Termini CM, Cotter ML, Marjon KD, et al. The membrane scaffold CD82 regulates cell adhesion by altering α4 integrin stability and molecular density. MboC. 2014;25(10):1560–1573. doi: 10.1091/mbc.e13-11-0660
[23] Jones EL, Wee JL, Demaria MC, et al. Dendritic cell migration and antigen presentation are coordinated by the opposing functions of the Tetraspanins CD82 and CD37. J Immunol. 2016;196(3):978–987. doi: 10.4049/jimmunol.1500357
[24] Kim SH, Park A, Yu J, et al. CD82 promotes CD8+ T cell immune responses by mediating T cell polarization and immunological synapse formation. J Immunol. 2021;206(1_Supplement):.14.03–.14.03. doi: 10.4049/jimmunol.206.Supp.14.03
[25] McGowan ENS, Wong O, Jones E, et al. Tetraspanin CD82 restrains phagocyte migration but supports macrophage activation. iScience. 2022;25(7):104520. doi: 10.1016/j.isci.2022.104520
[26] Khan NS, Lukason DP, Feliu M, et al. CD82 controls CpG-dependent TLR9 signaling. FASEB J. 2019;33(11):12500–12514. doi: 10.1096/fj.201901547R
[27] Rous BA, Reaves BJ, Ihrke G, et al. Role of adaptor complex AP-3 in targeting wild-type and mutated CD63 to lysosomes. MboC. 2002;13(3):1071–1082. doi: 10.1091/mbc.01-08-0409
[28] Modderman PW. Cluster report: CD631989. 1042.
[29] Pols MS, Klumperman J. Trafficking and function of the tetraspanin CD63. Exp Cell Res. 2009;315(9):1584–1592. doi: 10.1016/j.yexcr.2008.09.020
[30] Israels SJ, McMillan-Ward EM. CD63 modulates spreading and tyrosine phosphorylation of platelets on immobilized fibrinogen. Thromb Haemost. 2005;93(2):311–318. doi: 10.1160/TH04-08-0503
[31] Levy S, Shoham T. The tetraspanin web modulates immune-signalling complexes. Nat Rev Immunol. 2005;5(2):136–148. doi: 10.1038/nri1548
[32] Yoshida T, Kawano Y, Sato K, et al. A CD63 mutant inhibits T-cell tropic human immunodeficiency virus type 1 entry by disrupting CXCR4 trafficking to the plasma membrane. Traffic. 2008;9(4):540–558. doi: 10.1111/j.1600-0854.2007.00700.x
[33] Pfistershammer K, Majdic O, Stockl J, et al. CD63 as an activation-linked T cell costimulatory element. J Immunol. 2004;173(10):6000–6008. doi: 10.4049/jimmunol.173.10.6000
[34] Sincock PM, Mayrhofer G, Ashman LK. Localization of the transmembrane 4 superfamily (TM4SF) member PETA-3 (CD151) in normal human tissues: comparison with CD9, CD63, and alpha5beta1 integrin. J Histochem Cytochem. 1997;45(4):515–525. doi: 10.1177/002215549704500404
[35] Berditchevski F, Zutter MM, Hemler ME. Characterization of novel complexes on the cell surface between integrins and proteins with 4 transmembrane domains (TM4 proteins). Mol Biol Cell. 1996;7(2):193–207. doi: 10.1091/mbc.7.2.193
[36] Sugiura T, Berditchevski F. Function of α3β1–tetraspanin protein complexes in tumor cell invasion. Evidence for the role of the complexes in production of matrix metalloproteinase 2 (MMP-2). J Cell Biol. 1999;146(6):1375–1389. doi: 10.1083/jcb.146.6.1375
[37] Tachibana I, Hemler ME. Role of transmembrane 4 superfamily (TM4SF) proteins CD9 and CD81 in muscle cell fusion and myotube maintenance. J Cell Biol. 1999;146(4):893–904. doi: 10.1083/jcb.146.4.893
[38] Ashman LK, Aylett GW, Mehrabani PA, et al. The murine monoclonal antibody, 14A2. H1, identifies a novel platelet surface antigen. Br J Haematol. 1991;79(2):263–270. doi: 10.1111/j.1365-2141.1991.tb04531.x
[39] Baldwin G, Novitskaya V, Sadej R, et al. Tetraspanin CD151 regulates glycosylation of (alpha)3(beta)1 integrin. J Biol Chem. 2008;283(51):35445–35454. doi: 10.1074/jbc.M806394200
[40] Haeuw JF, Goetsch L, Bailly C, et al. Tetraspanin CD151 as a target for antibody-based cancer immunotherapy. Biochem Soc Trans. 2011;39(2):553–558. doi: 10.1042/BST0390553
[41] Yauch RL, Berditchevski F, Harler MB, et al. Highly stoichiometric, stable, and specific association of integrin α3β1 with CD151 provides a Major Link to Phosphatidylinositol 4-kinase, and May regulate cell migration. Mol Biol Cell. 1998;9(10):2751–2765. doi: 10.1091/mbc.9.10.2751
[42] Wright MD, Moseley GW, van Spriel AB. Tetraspanin microdomains in immune cell signalling and malignant disease. Tissue Antigens. 2004;64(5):533–542. doi: 10.1111/j.1399-0039.2004.00321.x

[43] Zelman-Toister E, Bakos E, Cohen S, et al. CD151 regulates T-Cell migration in health and inflammatory bowel disease. Inflamm Bowel Dis. 2016;22(2):257–267. doi: 10.1097/MIB.0000000000000621
[44] Charrin S, Le Naour F, Oualid M, et al. The major CD9 and CD81 molecular partner. Identification and characterization of the complexes. J Biol Chem. 2001;276 (17):14329–14337. doi: 10.1074/jbc.M011297200
[45] Boucheix C, Benoit P. CD9 antigen: will platelet physiology help to explain the function of a surface molecule during hematopoietic differentiation? Nouv Rev Fr Hematol. 1988;30(4):201–202.
[46] Brosseau C, Colas L, Magnan A, et al. CD9 tetraspanin: a new pathway for the regulation of inflammation? Front Immunol. 2018;9:2316. doi: 10.3389/fimmu.2018.02316
[47] Miyado K, Yamada G, Yamada S, et al. Requirement of CD9 on the egg plasma membrane for fertilization. Science. 2000;287(5451):321–324. doi: 10.1126/science.287.5451.321
[48] Kaji K, Oda S, Miyazaki S, et al. Infertility of CD9-deficient mouse eggs is reversed by mouse CD9, human CD9, or mouse CD81; polyadenylated mRNA injection developed for molecular analysis of sperm-egg fusion. Dev Biol. 2002;247(2):327–334. doi: 10.1006/dbio.2002.0694
[49] Powner D, Kopp PM, Monkley SJ, et al. Tetraspanin CD9 in cell migration. Biochem Soc Trans. 2011;39 (2):563–567. doi: 10.1042/BST0390563
[50] Jankovicova J, Frolikova M, Palenikova V, et al. Expression and distribution of CD151 as a partner of alpha6 integrin in male germ cells. Sci Rep. 2020;10 (1):4374. doi: 10.1038/s41598-020-61334-2
[51] Charrin S, Latil M, Soave S, et al. Normal muscle regeneration requires tight control of muscle cell fusion by tetraspanins CD9 and CD81. Nat Commun. 2013;4 (1):1674. doi: 10.1038/ncomms2675
[52] Maginnis MS. Virus-receptor interactions: the key to cellular invasion. J Mol Biol. 2018;430(17):2590–2611. doi: 10.1016/j.jmb.2018.06.024
[53] Hantak MP, Qing E, Earnest JT, et al. Tetraspanins: architects of viral entry and exit platforms. J Virol. 2019;93(6):e01429–01417. doi: 10.1128/JVI.01429-17
[54] Yamauchi Y, Helenius A. Virus entry at a glance. J Cell Sci. 2013;126(Pt 6):1289–1295. doi: 10.1242/jcs.119685
[55] Pileri P, Uematsu Y, Campagnoli S, et al. Binding of hepatitis C virus to CD81. Science. 1998;282:938–941. doi: 10.1126/science.282.5390.938
[56] Torrents de la Peña A, Sliepen K, Eshun-Wilson L, et al. Structure of the hepatitis C virus E1E2 glycoprotein complex. Science. 2022;378(6617):263–269. doi: 10.1126/science.abn9884
[57] Bartosch B, Vitelli A, Granier C, et al. Cell entry of hepatitis C virus requires a set of co-receptors that include the CD81 tetraspanin and the SR-B1 scavenger receptor. J Biol Chem. 2003;278(43):41624–41630. doi: 10.1074/jbc.M305289200
[58] Bartosch B, Dubuisson J, Cosset FL. Infectious hepatitis C virus pseudo-particles containing functional E1-E2 envelope protein complexes. J Exp Med. 2003;197 (5):633–642. doi: 10.1084/jem.20021756
[59] Wakita T, Pietschmann T, Kato T, et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med. 2005;11(7):791–796. doi: 10.1038/nm1268
[60] Zhong J, Gastaminza P, Cheng G, et al. Robust hepatitis C virus infection in vitro. Proc Natl Acad Sci USA. 2005;102(26):9294–9299. doi: 10.1073/pnas.0503596102
[61] Lavillette D, Tarr AW, Voisset C, et al. Characterization of host-range and cell entry properties of the major genotypes and subtypes of hepatitis C virus. Hepatology. 2005;41(2):265–274. doi: 10.1002/hep.20542
[62] Meuleman P, Hesselgesser J, Paulson M, et al. Anti-CD81 antibodies can prevent a hepatitis C virus infection in vivo. Hepatol (Baltim, Md). 2008;48 (6):1761–1768. doi: 10.1002/hep.22547
[63] Fofana I, Xiao F, Thumann C, et al. A novel monoclonal anti-CD81 antibody produced by genetic immunization efficiently inhibits hepatitis C virus cell-cell transmission. PLOS ONE. 2013;8(5):e64221. doi: 10.1371/journal.pone.0064221
[64] Mailly L, Baumert TF. Hepatitis C virus infection and tight junction proteins: the ties that bind. Biochim Biophys Acta Biomembr. 2020;1862(7):183296. doi: 10.1016/j.bbamem.2020.183296
[65] Gordon-Alonso M, Yanez-Mo M, Barreiro O, et al. Tetraspanins CD9 and CD81 modulate HIV-1-induced membrane fusion. J Immunol. 2006;177(8):5129–5137. doi: 10.4049/jimmunol.177.8.5129
[66] Yoshida T, Ebina H, Koyanagi Y. N-linked glycan-dependent interaction of CD63 with CXCR4 at the golgi apparatus induces downregulation of CXCR4. Microbiol Immunol. 2009;53(11):629–635. doi: 10.1111/j.1348-0421.2009.00167.x
[67] He J, Sun E, Bujny MV, et al. Dual function of CD81 in influenza virus uncoating and budding. PloS Pathog. 2013;9(10):e1003701. doi: 10.1371/journal.ppat.1003701
[68] Earnest JT, Hantak MP, Li K, et al. The tetraspanin CD9 facilitates mers-coronavirus entry by scaffolding host cell receptors and proteases. PLOS Pathog. 2017;13(7):e1006546. doi: 10.1371/journal.ppat.1006546
[69] Islam A, Khan MA, Ahmed R, et al. Transcriptome of nasopharyngeal samples from COVID-19 patients and a comparative analysis with other SARS-CoV-2 infection models reveal disparate host responses against SARS-CoV-2. J Transl Med. 2021;19(1):32. doi: 10.1186/s12967-020-02695-0
[70] Pique C, Lagaudriere-Gesbert C, Delamarre L, et al. Interaction of CD82 tetraspanin proteins with HTLV-1 envelope glycoproteins inhibits cell-to-cell fusion and virus transmission. Virology. 2000;276 (2):455–465. doi: 10.1006/viro.2000.0538
[71] Mazurov D, Heidecker G, Derse D. HTLV-1 gag protein associates with CD82 tetraspanin microdomains at the plasma membrane. Virology. 2006;346(1):194–204. doi: 10.1016/j.virol.2005.10.033
[72] Wu X, Nie J, Wang Y. Pseudotyped virus for papillomavirus. Adv Exp Med Biol. 2023;1407. doi: 10.1007/978-981-99-0113-5_5

[73] Florin L, Sapp M, Spoden GA. Host-cell factors involved in papillomavirus entry. Med Microbiol Immunol. 2012;201(4):437–448. doi: 10.1007/s00430-012-0270-1

[74] Spoden G, Freitag K, Husmann M, et al. Clathrin- and caveolin-independent entry of human papillomavirus type 16—involvement of tetraspanin-enriched microdomains (TEMs). PLOS ONE. 2008;3(10):e3313. doi: 10.1371/journal.pone.0003313

[75] Scheffer KD, Gawlitza A, Spoden GA, et al. Tetraspanin CD151 mediates papillomavirus type 16 endocytosis. J Virol. 2013;87(6):3435–3446. doi: 10.1128/JVI.02906-12

[76] Mikulicic S, Fritzen A, Scheffer K, et al. Tetraspanin CD9 affects HPV16 infection by modulating ADAM17 activity and the ERK signalling pathway. Med Microbiol Immunol. 2020;209(4):461–471. doi: 10.1007/s00430-020-00671-5

[77] Rohrmann GF. Baculovirus Molecular Biology [Internet]. 4th ed. National Center for Biotechnology Information; 2019.

[78] Sharma NR, Mateu G, Dreux M, et al. Hepatitis C virus is primed by CD81 protein for low pH-dependent fusion. J Biol Chem. 2011;286(35):30361–30376. doi: 10.1074/jbc.M111.263350

[79] Zhang YY, Zhang BH, Ishii K, et al. Novel function of CD81 in controlling hepatitis C virus replication. J Virol. 2010;84(7):3396–3407. doi: 10.1128/JVI.02391-09

[80] Grigorov B, Attuil-Audenis V, Perugi F, et al. A role for CD81 on the late steps of HIV-1 replication in a chronically infected T cell line. Retrovirology. 2009;6(1):28. doi: 10.1186/1742-4690-6-28

[81] Chen H, Dziuba N, Friedrich B, et al. A critical role for CD63 in HIV replication and infection of macrophages and cell lines. Virology. 2008;379(2):191–196. doi: 10.1016/j.virol.2008.06.029

[82] Li G, Endsley MA, Somasunderam A, et al. The dual role of tetraspanin CD63 in HIV-1 replication. Virol J. 2014;11(1):23. doi: 10.1186/1743-422X-11-23

[83] Fu E, Pan L, Xie Y, et al. Tetraspanin CD63 is a regulator of HIV-1 replication. Int J Clin Exp Pathol. 2015;8(2):1184–1198.

[84] Kruize Z, Cobos Jimenez V, Martinez FO, et al. CD9 and ITGA3 are regulated during HIV-1 infection in macrophages to support viral replication. Virology. 2021;562:9–18. doi: 10.1016/j.virol.2021.07.002

[85] Qiao Y, Yan Y, Tan KS, et al. CD151, a novel host factor of nuclear export signaling in influenza virus infection. J Allergy Clin Immunol. 2018;141(5):1799–1817. doi: 10.1016/j.jaci.2017.11.032

[86] Wu J, Peng X, Zhou A, et al. MiR-506 inhibits PRRSV replication in MARC-145 cells via CD151. Mol Cell Biochem. 2014;394(1–2):275–281. doi: 10.1007/s11010-014-2103-6

[87] Grassel L, Fast LA, Scheffer KD, et al. The CD63-Syntenin-1 complex controls Post-endocytic trafficking of oncogenic human Papillomaviruses. Sci Rep. 2016;6(1):32337. doi: 10.1038/srep32337

[88] Ws R. Virus life cycle. Molecular virology of human pathogenic viruses. 2017;2017:31–45. doi: 10.1016/b978-0-12-800838-6.00003-5

[89] Welsch S, Muller B, Krausslich HG. More than one door – budding of enveloped viruses through cellular membranes. FEBS Lett. 2007;581(11):2089–2097. doi: 10.1016/j.febslet.2007.03.060

[90] Brimacombe CL, Grove J, Meredith LW, et al. Neutralizing antibody-resistant hepatitis C virus cell-to-cell transmission. J Virol. 2011;85(1):596–605. doi: 10.1128/JVI.01592-10

[91] Feneant L, Levy S, Cocquerel L. CD81 and hepatitis C virus (HCV) infection. Viruses. 2014;6(2):535–572. doi: 10.3390/v6020535

[92] Catanese MT, Loureiro J, Jones CT, et al. Different requirements for scavenger receptor class B type I in hepatitis C virus cell-free versus cell-to-cell transmission. J Virol. 2013;87(15):8282–8293. doi: 10.1128/JVI.01102-13

[93] Jolly C, Sattentau QJ. Human immunodeficiency virus type 1 assembly, budding, and cell-cell spread in T cells take place in tetraspanin-enriched plasma membrane domains. J Virol. 2007;81(15):7873–7884. doi: 10.1128/JVI.01845-06

[94] Ninomiya M, Inoue J, Krueger EW, et al. The Exosome-associated Tetraspanin CD63 contributes to the efficient assembly and infectivity of the hepatitis B virus. Hepatol Commun. 2021;5(7):1238–1251. doi: 10.1002/hep4.1709

[95] Cheerathodi M, Nkosi D, Cone AS, et al. Epstein-Barr virus LMP1 modulates the CD63 interactome. Viruses. 2021;13(4):675. doi: 10.3390/v13040675

[96] York SB, Sun L, Cone AS, et al. Zika virus hijacks extracellular vesicle tetraspanin pathways for cell-to-cell transmission. mSphere. 2021;6(3):e0019221. doi: 10.1128/mSphere.00192-21

[97] Flint M, Maidens C, Loomis-Price LD, et al. Characterization of hepatitis C virus E2 glycoprotein interaction with a putative cellular receptor, CD81. J Virol. 1999;73(8):6235–6244. doi: 10.1128/JVI.73.8.6235-6244.1999

[98] Wünschmann S, Medh JD, Klinzmann D, et al. Characterization of hepatitis C virus (HCV) and HCV E2 interactions with CD81 and the low-density lipoprotein receptor. J Virol. 2000;74(21):10055–10062. doi: 10.1128/jvi.74.21.10055-10062.2000

[99] Thali M. The roles of tetraspanins in HIV-1 replication. Curr Top Microbiol Immunol. 2009;339:85–102. doi: 10.1007/978-3-642-02175-6_5

[100] Tippett E, Cameron PU, Marsh M, et al. Characterization of tetraspanins CD9, CD53, CD63, and CD81 in monocytes and macrophages in HIV-1 infection. J Leukoc Biol. 2013;93(6):913–920. doi: 10.1189/jlb.0812391

[101] Khurana S, Krementsov DN, de Parseval A, et al. Human immunodeficiency virus type 1 and influenza virus exit via different membrane microdomains. J Virol. 2007;81(22):12630–12640. doi: 10.1128/JVI.01255-07

[102] Shanmukhappa K, Kim JK, Kapil S. Role of CD151, a tetraspanin, in porcine reproductive and respiratory syndrome virus infection. Virol J. 2007;4(1):62. doi: 10.1186/1743-422X-4-62

[103] Finke J. Structural and functional characterization of the HPV16 entry platform on the cell surface. Ph.D. Thesis, Universitäts-und Landesbibliothek Bonn. 2020.

[104] Mazurov D, Heidecker G, Derse D. The inner loop of tetraspanins CD82 and CD81 mediates interactions with human T cell lymphotrophic virus type 1 gag protein. J Biol Chem. 2007;282(6):3896–3903. doi: 10.1074/jbc.M607322200
[105] Hassuna N, Monk PN, Moseley GW, et al. Strategies for targeting tetraspanin proteins: potential therapeutic applications in microbial infections. BioDrugs. 2009;23(6):341–359. doi: 10.2165/11315650-000000000-00000
[106] Robert J-MH, Amoussou NG, Mai HL, et al. Tetraspanins: useful multifunction proteins for the possible design and development of small-molecule therapeutic tools. Drug Discov Today. 2021;26(1):56–68. doi: 10.1016/j.drudis.2020.10.022
[107] Bailly C, Thuru X. Targeting of tetraspanin CD81 with monoclonal antibodies and small molecules to combat cancers and viral diseases. Cancers (Basel). 2023;15(7):2186. doi: 10.3390/cancers15072186
[108] Sims B, Farrow AL, Williams SD, et al. Tetraspanin blockage reduces exosome-mediated HIV-1 entry. Arch Virol. 2018;163(6):1683–1689. doi: 10.1007/s00705-018-3737-6
[109] Geary SM, Cambareri AC, Sincock PM, et al. Differential tissue expression of epitopes of the tetraspanin CD151 recognised by monoclonal antibodies. Tissue Antigens. 2001;58(3):141–153. doi: 10.1034/j.1399-0039.2001.580301.x
[110] Yauch RL, Kazarov AR, Desai B, et al. Direct extracellular contact between integrin α3β1 and TM4SF protein CD151. J Biol Chem. 2000;275(13):9230–9238. doi: 10.1074/jbc.275.13.9230
[111] Serru V, Le Naour F, Billard M, et al. Selective tetraspan–integrin complexes (CD81/α4β1, CD151/α3β1, CD151/α6β1) under conditions disrupting tetraspan interactions. Biochem J. 1999;340(1):103–111. doi: 10.1042/bj3400103
[112] Abu-Saleh N, Kuo CC, Jiang W, et al. The molecular mechanism of CD81 antibody inhibition of metastasis. Proc Natl Acad Sci USA. 2023;120(26):e2305042120. doi: 10.1073/pnas.2305042120
[113] Takeda Y, Tachibana I, Miyado K, et al. Tetraspanins CD9 and CD81 function to prevent the fusion of mononuclear phagocytes. J Cell Biol. 2003;161(5):945–956. doi: 10.1083/jcb.200212031
[114] Higginbottom A, Takahashi Y, Bolling L, et al. Structural requirements for the inhibitory action of the CD9 large extracellular domain in sperm/oocyte binding and fusion. Biochem Biophys Res Commun. 2003;311(1):208–214. doi: 10.1016/j.bbrc.2003.09.196
[115] Flint M, von Hahn T, Zhang J, et al. Diverse CD81 proteins support hepatitis C virus infection. J Virol. 2006;80(22):11331–11342. doi: 10.1128/JVI.00104-06
[116] Drummer HE, Wilson KA, Poumbourios P. Identification of the hepatitis C virus E2 glycoprotein binding site on the large extracellular loop of CD81. J Virol. 2002;76(21):11143–11147. doi: 10.1128/jvi.76.21.11143-11147.2002
[117] Dhanasekaran M, Baures PW, VanCompernolle S, et al. Structural characterization of peptide fragments from hCD81–lel. J Pept Res. 2003;61(2):80–89. doi: 10.1034/j.1399-3011.2003.00038.x
[118] New C, Lee ZY, Tan KS, et al. Tetraspanins: Host factors in viral infections. Int J Mol Sci. 2021;22(21):11609. doi: 10.3390/ijms222111609
[119] Ho S-H, Martin F, Higginbottom A, et al. Recombinant extracellular domains of tetraspanin proteins are potent inhibitors of the infection of macrophages by human immunodeficiency virus type 1. J Virol. 2006;80(13):6487–6496. doi: 10.1128/jvi.02539-05
[120] Hemler ME. Tetraspanin functions and associated microdomains. Nat Rev Mol Cell Biol. 2005;6(10):801–811. doi: 10.1038/nrm1736
[121] Hemler ME. Targeting of tetraspanin proteins–potential benefits and strategies. Nat Rev Drug Discov. 2008;7(9):747–758. doi: 10.1038/nrd2659
[122] Masciopinto F, Campagnoli S, Abrignani S, et al. The small extracellular loop of CD81 is necessary for optimal surface expression of the large loop, a putative HCV receptor. Virus Res. 2001;80(1–2):1–10. doi: 10.1016/s0168-1702(01)00245-3