Published in final edited form as:

Nat Rev Drug Discov. 2008 September ; 7(9): 747–758. doi:10.1038/nrd2659.

Targeting of tetraspanin proteins — potential benefits and strategies

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Abstract

The tetraspanin transmembrane proteins have emerged as key players in malignancy, the immune system, during fertilization and infectious disease processes. Tetraspanins engage in a wide range of specific molecular interactions, occurring through the formation of tetraspanin-enriched microdomains (TEMs). TEMs therefore serve as a starting point for understanding how tetraspanins affect cell signalling, adhesion, morphology, motility, fusion and virus infection. An abundance of recent evidence suggests that targeting tetraspanins, for example, by monoclonal antibodies, soluble large-loop proteins or RNAi technology, should be therapeutically beneficial.

There are 33 mammalian tetraspanin proteins^{1,2}, which are listed in the HUGO Gene Nomenclature Committee web site (see Further Information) when searching for the term “TSPAN”. Mammalian tetraspanins, as well as tetraspanins from other species, are transmembrane proteins that are abundantly expressed and are defined by their similarity in size (20–30 kDa protein core), topology (small and large outer loops, short N-terminal and C-terminal tails, four transmembrane domains) and shared structural features (FIG. 1a). These include characteristic disulphide bonds and a CCG motif in the large outer loop, hydrophilic residues within transmembrane domains, and membrane-proximal palmitoylation sites^{3–6}. The large outer loop — called the large extracellular loop (LEL) or extracellular domain 2 (EC2) — can be further divided into a constant region, containing conserved A, B and E helices, and a variable region containing sites for specific protein–protein interactions^{3,4,7}. Structural data from uroplakin tetraspanins (UPK1A, UPK1B)⁸ and from molecular models of tetraspanins^{9,10} indicate close packing of the four transmembrane domain helices and an overall rod-shaped structure (FIG. 1b), which is suitable for the docking of partner proteins⁸. In support of this model, tetraspanin extracellular domains, transmembrane domains^{11–13} and intracellular membrane-proximal cysteines¹⁴ can all be in close contact with neighbouring proteins. Other similarly sized proteins also contain four transmembrane domains; for example, the L6 family proteins, the connexins and the PMP22/EMP/MP20/Claudin superfamily of proteins. Although these other proteins may be tetraspans, they are not members of the tetraspanin family because they lack sequence homology and key structural features of tetraspanins.

At least a few different tetraspanins are expressed on nearly all cell and tissue types¹. Details regarding human tetraspanin expression on specific normal and malignant cell and tissue types can be found at Oncomine Research (see Further Information) and elsewhere^{15–17}. Genetic evidence in a number of species, including fungi, worms, flies, mice and humans,

confirms that tetraspanins exert a wide-ranging influence on the nervous system, immune system, tumours, infectious disease processes, fertilization, and development in skin and other organ systems¹⁸⁻²³. Besides determining cell morphology, tetraspanins also modulate cell motility, invasion, fusion, adhesion strengthening, signalling and protein trafficking^{5,17,19}. This Review will discuss the functions of specific tetraspanins, their key molecular interactions and their prospects as therapeutic targets.

Tetraspanin microdomains

Tetraspanins protrude only 4–5 nm from the plasma membrane^{7,8}. They do not typically serve as cell-surface receptors, although the hepatitis C virus protein E2 can bind to CD81 (REF. 24), the FimH protein in uropathogenic bacteria binds to tetraspanin UPK1A²⁵, and ligands for tetraspanin CD9 have been suggested²⁶. Tetraspanins are best known for their ability to organize laterally into tetraspanin-enriched microdomains (TEMs). Initially, TEMs were defined biochemically based on the tendency of tetraspanin proteins and their partners to remain associated under non-stringent detergent conditions^{23,27}. Later, they were characterized by immunoelectron microscopy as units, with an area of ~0.2 μm^2 and 0.6–0.7 μm spacing, sometimes appearing on the plasma membrane²⁸. The presence of gangliosides and cholesterol^{27,29,30} helps to explain the resistance of TEMs to solubilization with detergent, while suggesting similarity to lipid rafts¹⁹. However, evidence involving cholesterol depletion, detergent solubilization, sucrose density analyses and palmitoylation-site mutation^{19,23,31-36} clearly establishes TEMs as discrete biochemical entities, which is in contrast to the rather ambiguously defined lipid rafts.

At the core of TEMs are tetraspanins engaging in direct protein–protein interactions with both transmembrane and intracellular proteins, including the immunoglobulin superfamily members EWI-2 (also known as IGSF8, CD316) and EWI-F (also known as C9P-1, FPRP), Claudin 1, epidermal growth factor receptor (EGFR) membrane-bound ligands, integrins and Syntenin-1, to carry out various functions (BOX 1). These primary complexes include tetraspanin homodimers, which have been captured by covalent crosslinking³⁷ and by protein crystallization⁷. Tetraspanins also form primary complexes with several other types of molecules (TABLE 1).

Primary complexes then join into a network of looser secondary interactions, which include not only multiple distinct tetraspanins, and many other transmembrane proteins^{19,38}, but also signalling enzymes such as conventional protein kinase C (PKC) isoforms^{39,40} and phosphatidylinositol 4-kinase^{41,42}. Of the more than 100 tetraspanin partners that have been identified, most of them fall into the category of ‘secondary’ interacting partners, mainly because direct association has not been demonstrated and/or because association is lost under more stringent detergent conditions such as 1% Triton X-100 and digitonin, as compared with resistance to milder detergents such as 1% Brij 96 and 1% Brij 99 (REF. 19).

Notably, tetraspanins and many of their partner proteins (such as integrins, EWI proteins and Claudin-1) undergo protein palmitoylation, which does not contribute to primary interactions but helps to stabilize secondary interactions within TEMs³²⁻³⁴. Among the 8 β and 18 α subunits that comprise the integrin family, the only subunits that undergo

palmitoylation are α_3 , α_6 , α_7 and β_4 . Interestingly, it is these same subunits that form the most robust associations with tetraspanins. A practical consequence of the enrichment for palmitoylated proteins within TEMs is that metabolic labelling with ^3H -palmitate is a particularly useful tool for studying TEMs^{43–45}.

Cholesterol can physically and functionally associate with TEMs^{30,46}. Treatment of cells with methyl- β -cyclodextrin to deplete cholesterol reduces CD81 oligomerization⁴⁶, and with more extensive treatment intact TEMs can be released into the media in microvesicles^{14,31}. In a natural process that may be related, multivesicular bodies assemble and are then shed to form 50–100 nm vesicles, known as exosomes⁴⁷. It remains to be determined whether tetraspanins have a functional role in the production or maintenance of exosomes or other types of microvesicles. In one instance, exosomes have been shown to deliver a pro-angiogenic stimulatory signal that was dependent on the presence of the tetraspanin TSPAN8 (previously known as CO-029, TM4SF3)⁴⁸, emphasizing that exosome tetraspanins can be functionally active.

Targeting tetraspanin-dependent functions

So far, there has been negligible drug discovery targeting of tetraspanins, perhaps because cell-surface tetraspanins do not typically interact with soluble ligands or counter-receptors on apposing cells. In addition, many of the 33 mammalian tetraspanins have been only minimally studied. However, as more reagents are generated (such as monoclonal antibodies (mAbs), small interfering RNAs (siRNAs) and transgenic mouse models) and new assay systems become available, further examples of tetraspanins as potential therapeutic targets should emerge. Nonetheless, several tetraspanins have already been implicated in a number of normal and pathological processes with the potential to be targeted therapeutically (TABLE 2).

Normal physiology and non-infectious diseases

Tumour progression

Tetraspanin CD151 may contribute to tumour progression at multiple levels. For example, CD151 expression is significantly increased in prostate cancer compared with benign prostate hyperplasia⁴⁹. Moreover, higher levels of CD151 are associated with poor prognosis in lung and prostate cancers^{49,50}, and overexpression of CD151 promotes metastasis in colon carcinoma and fibrosarcoma cells⁵¹. In addition, CD151 expression is increased in a subset of human breast cancer samples, particularly those of high grade and/or triple negative basal-type⁴⁵. Consistent with CD151 having a functional role in cancer development, its ablation from basal-like breast cancer cells impairs both ectopic and orthotopic growth in xenograft models⁴⁵. The mechanistic consequences of CD151 ablation include subcellular redistribution of α_6 integrins, reorganization of integrin partner proteins and altered migration and invasion accompanied by diminished adhesion-dependent signalling through the Rho GTPase subfamily member RAC1 and focal adhesion kinase FAK⁴⁵. Interestingly, anti-CD151 antibodies have been shown to inhibit spontaneous and experimental metastasis and tumour cell intravasation, but not tumour growth, in chick

embryo and mouse xenograft models, or in epidermoid carcinoma, fibrosarcoma or colon carcinoma cells⁵¹⁻⁵³.

Primary tumour growth and metastasis are strongly supported by CD151, not only when it is present on tumour cells but also when present in the host animal. Lewis lung carcinoma cells implanted into CD151-null mice show markedly reduced primary tumour growth⁴⁴ through a mechanism that may involve diminished tumour angiogenesis in the host mouse (see below). Furthermore, host CD151 contributes to experimental metastasis. For example, in CD151-null mice, lung melanoma colonies are diminished in number but not in size, suggesting that CD151 affects the initiation of metastatic growth but not proliferation (Y. Takeda *et al.*, personal communication). In all of these studies, CD151 mostly acts in concert with laminin-binding integrins^{44,45}. There may be an advantage in targeting CD151, rather than the laminin-binding integrins to which it associates, as the $\alpha_3\beta_1$ and $\alpha_6\beta_4$ integrins are needed for normal development of skin and kidney and other tissues in 129Sv and/or C57BL6 mice⁵⁴⁻⁵⁶. By contrast, CD151 is not needed for normal development in those mouse strains^{44,57}, although it can affect kidney function when mice are at least partly bred into the FVB mouse strain⁵⁸, and it may contribute to normal human kidney and skin development⁵⁹. It remains to be seen whether CD151 targeting can ablate tumour progression without adversely affecting normal physiology.

Apart from CD151, the tetraspanin TSPAN8 is also associated with tumour progression. Overexpression of TSPAN8 supports pancreatic adenocarcinoma metastasis^{60,61}, and TSPAN8 is increased on hepatocellular carcinomas that are poorly differentiated and prone to intrahepatic spreading⁶². In addition, TSPAN8 is upregulated in oesophageal carcinoma and promotes invasion and migration in oesophageal carcinoma cell lines⁶³.

Tetraspanins may also act as tumour suppressors. For example, the presence of CD9 (REFS 64-66) impairs invasion, metastasis and/or survival in many cancer cell types. CD9 may also suppress primary growth of colon carcinomas⁶⁷, whereas TSPAN13 (previously known as NET-6, TM4SF13) can suppress breast carcinoma proliferation and invasion *in vitro*⁶⁸, and CD63 may suppress melanoma growth and metastasis⁶⁹. Although the latter result has been called into question owing to a cell-line contamination⁷⁰, another report shows that knockdown of CD63 elevates the invasive potential of melanoma cells⁷¹, consistent with CD63 having suppressor function. CD82 (previously known as KAI1) is perhaps the most well-known tetraspanin tumour suppressor⁷². It was initially reported to suppress prostate cancer metastasis⁷³. Now, at least 11 different tumour types show an inverse correlation between CD82 expression and tumour invasion and/or metastasis⁷². Tumour suppression activity could be related to CD82-induced redistribution and/or downregulation of other crucial cell-surface molecules including gangliosides and EGFR⁷⁴, urinary plasminogen activator receptor (UPAR)⁷⁵ and the $\alpha_6\beta_1$ integrin⁷⁶.

Angiogenesis

CD151-null mice are defective in angiogenesis *in vivo*, in assays involving implantation of corneal pellets, subcutaneous Matrigel plugs and subcutaneously injected tumour cells⁴⁴. However, vascular development appears normal in these mice^{44,57,58}, emphasizing that the angiogenesis defect may be restricted to pathological conditions. In support of a pro-

angiogenic role for CD151, overexpression of CD151 promotes neovascularization and improves blood perfusion in a rat hindlimb ischaemia model, probably by a mechanism that involves activation of the phosphatidylinositol 3-kinase (PI3K)–AKT signalling pathway⁷⁷. The absence of CD151 does not affect angiogenesis in an *in vivo* oxygen-induced retinopathy model⁴⁴, perhaps because retinal vascularization depends more on a specialized astrocytic template⁷⁸ and less on a typical laminin-containing basement membrane. CD151, which is abundant at endothelial cell–cell junctions, supports several *in vitro* endothelial cell functions that are of relevance to angiogenesis. These include endothelial cell invasion, chemotactic migration, cable formation, Matrigel contraction, tube formation, sprouting, and signalling through AKT and RAC1⁴⁴.

Another tetraspanin, TSPAN8, has also been linked to angiogenesis. When TSPAN8 is expressed on a rat carcinoma cell line, it stimulates angiogenesis *in vivo*, with TSPAN8 itself also becoming upregulated on newly sprouting endothelium⁴⁸.

Oocyte fertilization

When CD9 and CD81 are absent from oocytes, sperm–egg fusion is completely eliminated⁷⁹. Tetraspanin absence does not affect the initial sperm binding but prevents subsequent sperm–egg fusion events⁷⁹. Oocyte CD9 appears to function by optimizing the length, thickness, density and radius of curvature for oocyte microvilli, which may play an active role in capturing sperm cells⁸⁰. Oocyte CD9 associates closely with its major protein partners (EWI-2 and EWI-F)⁸⁰, which is relevant for two reasons. First, EWI-2 can drive the localization of CD9 and CD81 into filopodia⁸¹. Second, EWI proteins may associate directly with ezrin-radixin-moesin proteins, thereby linking CD9 and other TEM proteins to the actin cytoskeleton⁸². So, CD9–EWI protein complexes may work together in regulating oocyte microvilli structure and function. During sperm–egg fusion in humans, tetraspanins CD9, CD81 and CD151 may all have a role, with CD9 controlling the molecular organization of key proteins, such as integrin $\alpha_6\beta_1$, on the oocyte surface⁸³. With respect to inhibiting fertilization, tetraspanins may be the most attractive targets yet described⁸⁴. Indeed, multiple strategies have already been used to target oocyte tetraspanins and to inhibit fertilization (see below).

Immune cell functions

Leukocyte subsets — T and B lymphocytes, granulocytes, monocytes, macrophages, dendritic cells and natural killer cells — each express eight or more different tetraspanins¹⁵. These tetraspanins associate directly or indirectly with numerous molecules — for example, T-cell receptors, B-cell receptors, major histocompatibility complex (MHC) class I, MHC class II, CD2, CD4, CD8, CD19, EWI-2, $\alpha_4\beta_1$ and $\alpha_6\beta_1$ integrins — that have crucial roles in the functions of immune cells³⁸. Excellent reviews highlight the extensive and sometimes apparently conflicting roles played by tetraspanins on immune cells^{15,18,38}. Because of these conflicting roles, and because of the unpredictability of targeting immune cells in general, it is not yet clear whether tetraspanin targeting on immune cells will be a productive endeavour. Nonetheless, a brief overview of some possibilities are discussed.

Antibody crosslinking of several tetraspanins (for example, CD9, CD53, CD81 and CD82) can co-stimulate T cells, suggesting positive effector roles³⁸. However, mouse knockout models of four different tetraspanins (CD81, CD37, TSPAN32 and CD151) exhibit T-cell hyperproliferation in response to *in vitro* stimulation, which is consistent with endogenous tetraspanins exerting negative regulatory functions^{57,85-87}. These tetraspanins may down-modulate T-cell triggering, either by recruitment of a negative regulatory phosphatase⁸⁸ or by sequestering key molecules into TEMs.

On B cells, tetraspanin CD81 is required for molecular organization and efficient collaboration between the B cell receptor and its partners (CD21, CD19 and various signalling enzymes)¹⁸. Also, CD81 is required for maturation and surface expression of CD19⁸⁹, a molecule with a key role during B-cell activation. In addition, CD81 associates with the $\alpha_4\beta_1$ integrin on B cells, thereby strengthening $\alpha_4\beta_1$ integrin adhesion under shear flow conditions⁹⁰. Other tetraspanins also have key roles on B cells. For example, CD9 suppresses the motility of intraperitoneal B1 cells⁹¹, and CD37 supports T-cell dependent B-cell responses, especially at suboptimal doses of antigen⁸⁶.

Tetraspanins may also contribute to antigen presentation. There is extensive evidence for tetraspanin associations with MHC class I and MHC class II molecules^{18,38}, whereas CD81 (on T cells and antigen presenting cells) and CD82 (on T cells) both distribute into immune synapses^{92,93}. Furthermore, multiple tetraspanins associate with a subset of MHC class II molecules that may have distinct antigen presenting properties³⁵, and lateral associations between heterologous MHC class II molecules (I-A and I-E) depend on CD9 (REF. 94). However, there is not yet definitive evidence for a functional role for specific tetraspanins during antigen presentation. It is thought that alternative tetraspanins are able to compensate for the effects of removing individual tetraspanins.

Platelet functions

Tetraspanin CD151 is essential for normal platelet function. Platelets from CD151-null mice show defective platelet aggregation, impaired spreading on fibrinogen and delayed clot retraction⁹⁵. CD151 functions by physically associating with $\alpha_{IIb}\beta_3$, the major platelet integrin, leading to impaired outside-in (that is, post-ligand occupancy) signalling by the integrin⁹⁵. Another tetraspanin, TSPAN32 (previously known as TSSC6, PHEMX), also associates with $\alpha_{IIb}\beta_3$ integrin and modulates outside-in signalling, with TSPAN32-null mouse platelets similarly showing impaired clot retraction, aggregation and spreading on fibrinogen⁹⁶. In addition, TSPAN32 is required for stable platelet thrombus formation during vascular injury⁹⁶. Tetraspanin CD63 also associates with $\alpha_{IIb}\beta_3$ integrin and may regulate platelet spreading and signalling on fibrinogen⁹⁷. However, the functions of $\alpha_{IIb}\beta_3$ integrins in CD63-null platelets have not yet been evaluated. The $\alpha_{IIb}\beta_3$ integrin is a well-established therapeutic target with antagonists widely used to treat coronary thrombosis⁹⁸. It remains to be determined whether integrin-associated tetraspanins, which so far have a less dramatic role, will also be useful targets in platelet thrombus formation.

Several other studies, involving mAbs, suggest additional functional roles for various tetraspanins on platelets and other types of myeloid cells¹⁵. However, interpretations are complicated by expression of the immunoglobulin- γ receptor Fc γ RIIIa and other Fc receptors

on myeloid cells, which leads to antibody crosslinking and dual signalling effects⁹⁹. Nonetheless, modulation of Fc receptor signalling by tetraspanins may be relevant to immune disorders⁹⁹.

Other non-infectious disease functions

CD151-null mice show significantly impaired wound healing in a skin incision model, with deficient re-epithelialization being accompanied by laminin basement membrane disorganization and failure to upregulate α_6 and β_4 integrin subunits at the wound site¹⁰⁰. CD81 has a key role during experimental spinal-cord injury in rats. Intraspinal infusion of an anti-CD81 antibody enhanced motor function, and spared tissue loss at the lesion site¹⁰¹. It is suggested that the anti-CD81 antibody inhibits microglial proliferation and/or macrophage recruitment at the injury site¹⁰¹.

Infectious diseases

HIV-1

Tetraspanins are involved at multiple steps in HIV-1 infection. After HIV-1 particles are captured by dendritic cells they are sorted through compartments containing tetraspanins (CD9, CD81, CD82), which redistribute into an 'infectious synapse', thus enabling transfer of virus particles to CD4⁺ T cells¹⁰². Alternatively, virus particles endocytosed by dendritic cells into tetraspanin-enriched compartments are released within vesicles resembling exosomes, which have an enhanced capability to infect CD4⁺ T cells¹⁰³. Although it has not yet been demonstrated whether tetraspanins are required for transfer of virus particles from dendritic cells to CD4⁺ T cells, they do have a role during HIV-1-induced membrane fusion of CD4⁺ T cells. Anti-CD9 and anti-CD81 mAbs enhance syncytia formation in T lymphoblasts, apparently by blocking (that is, reversing) the inherent syncytium suppressing effect that is exerted by endogenous T-cell CD9 and CD81 molecules¹⁰⁴.

Tetraspanins may also have an active role during HIV-1 infection of macrophages. For example, the entry of HIV-1 R5-tropic virus is inhibited by anti-CD63 mAb¹⁰⁵, and both R5-tropic and X4-tropic viruses can be potently inhibited by soluble LEL/EC2 domains from multiple tetraspanins (CD63, CD81, CD82, CD53 and CD151)¹⁰⁶. Once inside macrophages, HIV-1 assembles within a novel type of intracellular membrane domain containing CD81, CD9 and CD53, with CD63 then being recruited and incorporated into virions¹⁰⁷. Finally, HIV-1 egress may occur through TEMs, as key endosomal sorting components (TSG101 and VPS28), involved in HIV-1 budding, are recruited to TEMs upon virus expression²⁸. However, it remains to be seen whether specific tetraspanins are required for virus assembly and egress. In this regard, despite CD63 being involved in macrophage infection^{105,106}, and being present at sites of virus assembly and budding¹⁰⁸, it is not required for virus production in human macrophages¹⁰⁹. As a consequence of assembly in tetraspanin-enriched compartments, newly released HIV-1 particles show incorporation of CD63 and multiple other tetraspanins. By an unknown mechanism, these embedded tetraspanins have the potential to down-modulate subsequent HIV-1 infection at a post-attachment entry step¹¹⁰.

Hepatitis C virus

Hepatitis C virus (HCV) binding to CD81 is necessary, but not sufficient for HCV infection¹¹¹. Early studies focused on direct binding of the virus E2 glycoprotein to a site in the soluble LEL of CD81 (REF. 24). However, later studies show that the affinity of E2 for CD81 is not predictive of HCV infection and therefore potentially misleading. For example, CD81 mutations that substantially reduce E2 binding have minimal effect on HCV infection¹¹², whereas those reducing HCV entry do not correspondingly reduce E2 binding¹¹³. Nonetheless, CD81 is required for infection and remains an attractive target. Multiple CD81 molecules, together with various other known and unknown hepatocyte surface proteins, are suggested to engage in a number of low-affinity interactions with HCV, which are necessary for the initial steps in virus binding^{112,114}. Claudin-1, a membrane protein that physically associates with CD81 (REFS 14,115), may collaborate with CD81 during a later step in virus entry^{115,116}. Although Claudin-1 is typically found in tight junctions, tetraspanin–claudin complexes are not in tight junctions^{14,117}, which suggests that HCV might engage non-junctional claudins.

Other infectious disease pathologies

Antibodies to CD82 and CD81 block human T-cell lymphotropic virus type 1 (HTLV-1)-induced syncytium formation in T lymphoblastoid cells¹¹⁸. Overexpression of CD82 also inhibited syncytium formation, in addition to cell-to-cell virus transmission¹¹⁹. Both the HTLV-1 Gag protein, which directs virion assembly and release at the plasma membrane, and the Env protein, interact with tetraspanins CD82 (REFS 119,120) and CD81 (REF. 119). Hence, TEMs may participate both in HTLV-1-induced syncytia formation as well as virus assembly and release. Studies of the canine distemper virus (CDV) show that transfection of NIH-3T3 (mouse fibroblasts) or MBDK (bovine kidney) cells with CD9 is sufficient to enable infection¹²¹. Later studies show that CD9 acts not as a virus receptor, but as a regulator of CDV haemagglutinin-dependent cell–cell fusion¹²². These examples of tetraspanins facilitating virus-dependent fusion events are consistent with CD9 and CD81 regulating other types of cell–cell fusion, such as seen for gametes⁷⁹, myoblasts¹²³, monocytes during osteoclastogenesis¹²⁴, and monocytes forming multi-nucleated giant cells¹²⁵. CD9 may also support feline immunodeficiency virus infection. Again, the tetraspanin does not affect virus binding, but instead contributes to virus assembly and/or release¹²⁶.

CD81 (but not CD9) is required in hepatocytes to allow invasion by sporozoites from malaria-causing parasites *Plasmodium yoelii* and *Plasmodium falciparum*¹²⁷. CD81 functions together with cholesterol in the context of TEMs to enable *Plasmodium* sporozoite invasion⁴⁶. No interaction between CD81 and sporozoite proteins has been observed, which suggests that CD81 may be playing a co-receptor role¹²⁷. Tetraspanin CD9 is a co-receptor for diphtheria toxin. CD9 does not bind directly to the toxin, but forms a membrane complex with the diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor; HB-EGF), to increase the number of effective binding sites¹²⁸.

Strategies for targeting tetraspanins

Tetraspanins might be targeted using mAbs, recombinant soluble LELs, RNA interference (RNAi) or other approaches (FIG. 2).

Monoclonal antibodies

As of 2007, over 400 therapeutic mAbs had entered commercially sponsored clinical development¹²⁹. Although this list includes mAbs that target tetraspanin-associated proteins, such as epithelial cell adhesion molecule (EPCAM) and EGFR, mAbs targeting tetraspanins themselves are not listed¹²⁹. However, a few promising *in vivo* results have so far been observed. For example, mAb targeting of CD81 improves recovery from spinal injury in rats¹⁰¹, intrauterine injection of an anti-CD9 mAb increased embryo implantation¹³⁰, intra-tumour injection of anti-CD9 mAbs inhibited *in vivo* colon carcinoma growth⁶⁷, and a modified anti-CD37 antibody depleted human B-cell chronic lymphocytic leukaemia (B-CLL) cells and improved survival in a mouse xenograft model¹³¹. Results listed in TABLE 2 indicate the potential for additional mAb targeting of a number of tetraspanins in different clinical settings.

Anti-CD81 mAbs can block HCV infection at least in part by blocking binding of the HCV E2 protein^{113,114}. However, in most of the other examples listed in TABLE 2, it is not clear how anti-tetraspanin mAbs alter cell functions. Theoretically, anti-tetraspanin mAbs could block lateral associations with partner proteins. However, such an effect has not yet been well documented. It is more likely that treatment of cells with multivalent anti-tetraspanin mAbs causes formation of aggregates of TEM proteins, leading to disruption of TEMs and/or down-modulation of the target tetraspanin, perhaps together with key associated proteins. Indeed, it is suggested that the ability of anti-tetraspanin mAbs to perturb large patches of TEM proteins probably explains why mAb treatment effects often exceed the magnitude of effects seen upon knockdown or knockout of individual tetraspanin proteins. For example, anti-CD63 mAb strongly inhibits HIV-1 entry into macrophages¹⁰⁵, but RNAi ablation of CD63 had no effect on HIV-1 infectivity and virus assembly¹⁰⁹. Similarly, anti-CD81 antibodies affect numerous cellular functions¹³² such as lymphocyte proliferation¹³³ and thymocyte development in fetal organ culture¹³⁴, but CD81-null mice show relatively mild phenotypes, with normal lymphocyte proliferation and thymic development^{85,135}.

Another mode of action is that the mAb may exert an agonist effect. In this regard, a few anti-tetraspanin antibodies enhance rather than inhibit tetraspanin-dependent functions. For example, an anti-CD151 antibody can stimulate cell adhesion, thereby preventing de-adhesion and immobilizing tumour cells⁵³. In other examples, anti-CD9 and anti-CD81 antibodies may stimulate cell–cell fusion, either by an agonist effect or by blocking inherent anti-fusion properties of CD9 and CD81 (REFS 104,125). In addition, anti-CD9 mAbs amplify the inherent tumour suppressor function of CD9 (REF. 67).

Despite tetraspanins being relatively small molecules on the cell surface (only ~100 extracellular amino acids), antibodies to the same tetraspanin can have a range of different properties¹³⁶. For example, some anti-CD151 antibodies recognize total protein, whereas others selectively recognize an epitope exposed only when CD151 is not bound to laminin-

binding integrins¹³⁶⁻¹³⁸. Similarly, epitopes defined by anti-CD81 and anti-CD9 mAbs vary widely^{46,139,140}, which is partly due to differential shielding of epitopes. As a practical benefit, specialized anti-tetraspanin mAbs may have utility in diagnosing the molecular state of tetraspanins in intact cells. For example, antibodies that selectively detect unbound CD151 might provide information regarding integrin association¹³⁶⁻¹³⁸, whereas an anti-CD9 antibody might also provide information regarding integrin activation and/or association¹⁴⁰. A low-affinity mAb that selectively binds clustered CD9 can be used to assess the changes in the state of homo-oligomeric CD9 clustering on the cell surface¹⁴¹. Similarly, an antibody with preference for oligomerized CD81 can provide clues regarding the cell-surface organization of CD81 (REF. 46).

Besides blocking, clustering or otherwise disrupting specific molecular targets, mAbs also can trigger the removal of target cells. This can be achieved directly by the stimulation of apoptosis in carcinoma cell lines¹⁴² or in myotubes¹²³ with specific anti-CD81 and/or anti-CD9 antibodies. Alternatively, antibody-coated cells may undergo complement-dependent cytotoxicity (CDC; lysis due to complement fixation) or antibody-dependent cell-mediated cytotoxicity (ADCC). In the latter case, apoptosis is triggered after antibody-coated cells are recognized by Fc receptors of natural killer cells. In this regard, a modified anti-CD37 antibody triggered efficient ADCC-dependent *in vivo* removal of B-CLL cells¹³¹. In fact, the therapeutic efficacy of anti-CD37 may surpass that of rituximab (an anti-CD20 antibody)¹³¹, which is currently used in the treatment of human B-CLL. Finally, mAbs can be coupled to a lethal substance, such as a toxin, radioisotope or nanoparticle, which can then be used to ablate cells bearing the mAb target molecule. Although an iodine[131]-labelled anti-CD37 mAb yielded promising results in patients with B-cell lymphoma¹⁴³, there has been no recent continuation of these studies. It remains to be seen whether other tetraspanins will show the sufficient degree of disease-type, cell-type or tumour specificity needed for this approach.

Soluble EC2 loops

Recombinant soluble LEL/EC2 of tetraspanin CD9 inhibited sperm–egg fusion when pre-incubated with oocytes but not sperm cells. These results suggest that CD9 may act in *cis* on the oocyte surface, whereas sperm lack a *trans* acting counter-receptor for CD9 (REF. 144). Subsequent studies confirmed these results using recombinant soluble LELs from CD9 and CD81, with CD63 soluble LEL as a negative control¹⁴⁵. In other studies, human (but not mouse) CD9 soluble LEL inhibited blood monocyte fusion¹²⁵, and soluble LELs from CD9 and CD151 disrupted endothelial cell interactions with leukocytes¹⁴⁶. Recombinant soluble LELs have also been used effectively to inhibit virus functions. Multiple soluble LELs (from CD63, CD9, CD81 and CD151) potently inhibit CCR5-tropic HIV-1 infection of macrophages¹⁰⁶. The soluble LEL proteins appear to act on target cells by interfering with the uptake of virions¹⁰⁶. In several of these studies, mutant soluble LELs, or soluble LELs from another species, function less effectively, thus serving as important controls. Together, these results, obtained in several different settings, emphasize the utility of soluble LEL reagents. Curiously, HIV-1 infection of macrophages is inhibited by multiple soluble LELs (CD63, CD9, CD81 and CD151) but only by a single anti-tetraspanin mAb (anti-CD63, but not anti-CD9 or anti-CD81). One interpretation of these results is that CD63 plays a more

direct role in virus infection, thus explaining mAb selectivity. By contrast, soluble LELs from multiple other tetraspanins may interfere with endogenous lateral homo-tetraspanin and heterotetraspanin oligomerizations, thereby disrupting TEMs, which indirectly impairs the function of CD63.

Recombinant soluble LEL protein from CD81 also inhibits HCV infectivity. In this case, the mechanism is more obvious. During infection, the HCV E2 protein binds directly to cellular CD81, which serves a critical co-receptor function. CD81 soluble LEL binds to the virus E2 protein, thus preventing virus interaction with endogenous CD81 LEL¹¹². However, recent work shows that CD81 soluble LEL is minimally effective as an inhibitor in HCV assays that more closely mimic *in vivo* HCV infection in humans. Although CD81 is still required at an early step in the infection of normal primary human hepatocytes, viral particles appear to be less accessible to inhibitory interaction with CD81 LEL¹¹⁴.

In summary, soluble LELs from multiple tetraspanins have been used in a number of settings to disrupt tetraspanin-dependent functions. Although the mechanism of action has not been precisely clarified in many of these assays, it is presumed that soluble LELs can disrupt lateral interactions essential for assembly and/or maintenance of TEMs. Hence, by exerting a combination of direct and indirect effects, soluble LELs may offer a broader range of inhibitory possibilities than mAbs. However, in the case of HCV, in which the LEL binds to virus instead of cells, anti-CD81 mAbs may be more potent¹¹⁴.

RNAi approaches

Another strategy for interfering with tetraspanins involves small interfering (siRNA) knockdown. Knockdown of CD9, CD151 and CD81 can be readily achieved using siRNA and/or small hairpin RNA (shRNA)^{14,146,147}. This approach shows that endothelial cell CD9 and CD151 support lymphocyte transendothelial migration¹⁴⁶, CD9 supports integrin-dependent adhesion and reduced dissemination in ovarian carcinoma cells¹⁴⁸, and CD151 can regulate integrin-dependent cell adhesion and migration¹⁴⁷. Additional RNAi studies show that osteoclastogenesis is inhibited by TSPAN13 but promoted by TSPAN5 (previously known as NET-4, TM4SF9)¹⁴⁹. In infectious disease studies, silencing of CD81 with siRNA markedly inhibits (>90%) infection of primary normal human hepatocytes by HCV, much more effectively than soluble LEL from CD81 (REF. 114). Also, knockdown of CD151 significantly reduces susceptibility to porcine reproductive and respiratory syndrome virus infection in a model cell line¹⁵⁰. The RNAi approach has already been used to inhibit tetraspanins in at least one *in vivo* study. Using an inducible shRNA vector, CD81 was targeted in rat brain, resulting in suppression of cocaine-induced locomotor activity¹⁵¹.

The area of RNAi therapeutics is now sufficiently advanced, such that siRNAs can be made potent, specific (avoiding off-target effects) and chemically stabilized, although delivery to specific sites still needs to be optimized¹⁵². Regarding delivery, the recent use of specific mAbs to target siRNA-containing nanoparticles opens up a wide realm of new possibilities in which effects of relatively lethal siRNAs can be restricted to specific cell or tissue types¹⁵³. With a number of different RNAi therapeutics already in clinical trials¹⁵², this technology should be available for application to tetraspanin targets.

Other possibilities

Tetraspanin transmembrane domains are involved in crucial intramolecular and intermolecular interactions that are essential for correct intramolecular folding and for intermolecular associations with other transmembrane proteins in TEMs^{10,89,154,155}. A study of G-protein-coupled receptors shows that structural analogues of individual transmembrane domains can serve as potent and specific receptor antagonists¹⁵⁶. Hence, such an approach might also be applicable to tetraspanins. For example, transmembrane domain analogues that mimic single transmembrane domains in CD81 or CD151 conceivably could interfere with HCV infection or carcinoma progression, respectively.

The C-terminal cytoplasmic tail of tetraspanin CD63 interacts with a PDZ domain in Syntenin-1 to regulate CD63 endocytosis¹⁵⁷. Several other tetraspanins also contain C-terminal three amino-acid sequences corresponding to potential PDZ domain binding motifs. PDZ domains, with their well-defined binding sites, are promising targets for drug discovery¹⁵⁸. As additional tetraspanin–PDZ domain interactions are uncovered, prospects for PDZ intervention as a tetraspanin targeting strategy will be enhanced.

Other specific protein–protein interactions could also be targeted. The LELs of tetraspanins are being structurally characterized⁷⁻⁹, and key interaction sites are being mapped, such as between CD151 and integrins¹³⁷. So, it should become more feasible to disrupt these interactions with small peptides or with other small molecules. In this regard, the interaction between CD81–soluble LEL and the HCV–E2 protein has been used to screen for small-molecule inhibitors, with the intention of blocking HCV infection^{159,160}.

Palmitoylation of tetraspanins and partner proteins has a vital role during the assembly and/or maintenance of TEMs^{10,32-34,43}. Palmitoylation of tetraspanin CD82 is particularly important for its association with HTLV-1 Gag protein, which may support virus assembly and release¹²⁰. It is only recently that progress has been made in the identification of the protein acyl transferases that are responsible for protein palmitoylation¹⁶¹. Targeting relevant protein acyl transferases could provide a novel means of physically disrupting TEMs, thus leading to altered cellular functions. In this regard, a protein acyl transferase, called DHHC2, promotes tetraspanin palmitoylation while supporting TEM assembly¹⁶².

It is not yet clear how to take advantage of tetraspanin tumour suppressor functions therapeutically. However, in the case of the metastasis suppressor CD82, an interesting new possibility has emerged. Because CD82 is degraded by gp78 ubiquitin ligase, targeting of gp78 increases abundance of CD82 while reducing metastasis¹⁶³. Whether other tetraspanin suppressors (for example, CD9, CD63 and TSPAN13) undergo post-translational down-regulation that could be similarly targeted remains to be determined. Another possibility for amplifying tetraspanin suppression is through adenoviral transduction methods. Adenoviruses encoding CD9 or CD82 were administered, via the trachea, into mice orthotopically preimplanted with Lewis lung carcinoma cells, resulting in a dramatic reduction in metastasis⁶⁵. Curiously, host-cell expression of CD9 itself may facilitate adeno-associated virus serotype 2 infection¹⁶⁴.

Despite their abundance in humans (33 tetraspanins), flies (36 tetraspanins), fish (66 tetraspanins), worms (21 tetraspanins) and elsewhere^{1,2}, and despite the large numbers of unbiased genetic screens that have been carried out, relatively few tetraspanins have been identified as having essential functions. Furthermore, knockout of individual tetraspanins in mice has resulted in relatively mild phenotypes. One reason for the lack of more dramatic phenotypes could be that there is substantial compensation by the remaining tetraspanins. Consequently, it is predicted that engineering dominant negative tetraspanins will yield phenotypic effects that exceed those of individual knockouts, and will more closely resemble the effects seen using mAbs and RNAi strategies in developed organisms.

Summary and future prospects

So far, there have been few examples of tetraspanin targeting *in vivo*. Nonetheless, potential target opportunities abound as tetraspanins contribute to tumour progression, angiogenesis, fertilization, immune-cell functions, platelet clotting, infectious diseases and other processes. As more of the 33 mammalian tetraspanins are studied, and their functions and molecular interactions identified, the list of potential targets will grow as will possibilities for intervention.

Strategies for targeting tetraspanins include the use of mAbs and recombinant soluble LELs. The mAb and soluble LEL approaches can be complementary, as some processes are inhibited by one type of reagent but not the other. However, for both mAbs and soluble LELs, interpretation of results can be handicapped by not knowing the precise mechanism of action. It is often not clear which specific molecular interactions are being perturbed. Also, results can be a little unpredictable, as the same antibodies can stimulate or inhibit different types of cell fusion, and serve as both antagonists and agonists. The RNAi approach is clear in terms of mechanism and promising examples of both *in vitro* and *in vivo* applications have emerged.

The widespread distribution of many tetraspanins on numerous cell and tissue types is a potentially important obstacle to tetraspanin targeting. For example, agents that target CD9 on oocytes or CD151 on tumour cells might also affect the normal functions of platelets and endothelial cells, which also express abundant CD9 and CD151. Similarly, targeting of CD37 on B leukemic cells could also affect CD37-dependent functions of T cells. However, the development of cell-specific delivery systems may overcome this problem. Another issue is that of compensation and/or redundancy provided by non-targeted tetraspanins. To circumvent this, it may be necessary to focus on tetraspanins that have highly specific functions or to target multiple tetraspanins at once.

Finally, as tetraspanins function in the context of TEMs, a novel type of membrane microdomain, new strategies may need to be developed. For example, strategies aimed at non-protein elements in TEMs (such as gangliosides, cholesterol or palmitoylation), at specific transmembrane domain interactions, at specific cytoplasmic tail interactions, or at specific tetraspanin-partner combinations might be worth consideration.

Acknowledgements

The author gratefully acknowledges support from the National Institutes of Health grants GM38903 and CA42368.

Glossary

Integrins	A family of cell-surface transmembrane proteins (24 mammalian members), with $\alpha\beta$ -heterodimeric structures, which function as cell adhesion molecules.
EWI proteins	A family of four cell-surface immunoglobulin superfamily proteins, sharing a conserved glutamine-tryptophan-isoleucine (EWI) motif.
Protein palmitoylation	Post-translational acylation of a protein, typically on an intracellular cysteine residue.
Exosomes	Vesicles of 50–100 nm, enriched 10-fold to 100-fold for tetraspanins, and shed from the multivesicular bodies of cells.
Monoclonal antibody	A specific antibody produced in large quantity by a single hybrid cell clone formed in the laboratory by the fusion of a B cell with a tumour cell.
Angiogenesis	The process by which new blood vessels grow from pre-existing blood vessels.
RNA interference (RNAi)	A form of post-transcriptional gene silencing in which expression or transfection of dsRNA induces degradation, by nucleases, of the homologous endogenous transcripts, resulting in reduction or loss of gene activity.
DHHC2	A member of a family of enzymes (24 members in mammals) containing a conserved aspartate-histidine-histidine-cysteine (DHHC) motif, responsible for the <i>S</i> -palmitoylation of proteins.

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Box 1**Tetraspanin primary complexes**

Tetraspanins are involved in direct protein–protein interactions with both transmembrane and intracellular proteins.

For example, tetraspanins CD9 and CD81 can directly associate with EWI-2 and EWI-F, a pair of related cell-surface proteins in the immunoglobulin superfamily, named for a conserved Glu-Trp-Ile motif. EWI-2 negatively regulates cell motility, morphology and spreading in several cell lines^{81,139,165}. EWI-2 also impairs orthotopic tumour growth when expressed in a glioblastoma cell line (T. V. Kolesnikova *et al.*, personal communication). It is doubtful that EWI-2 functions without being associated with CD9 and/or CD81 because CD81 (and possibly also CD9) facilitate the cell-surface expression of EWI-2 (REF. 81). Also, EWI-2 redirects CD81 and CD9 into filopodia⁸¹ and causes CD9 to switch from homo oligomerization to EWI-2 hetero association¹⁴¹. A truncated form of EWI-2, called EWI-2wint, associates with CD81 and blocks hepatitis C virus entry¹⁶⁶.

In addition, CD9 directly associates with Claudin-1 when it is not present in tight junctions, leading to prolonged Claudin-1 half-life¹⁴. Other tetraspanins (CD81, CD151) may also associate physically and functionally with Claudin-1 (REFS 14,115), but it is not yet clear whether these associations are direct. Also, CD9 can associate with heparin-binding epidermal growth factor-like growth factor (HB-EGF), and other membrane-bound ligands for the epidermal growth factor receptor (EGFR), to promote juxtacrine signalling¹⁶⁷⁻¹⁶⁹. Furthermore, CD9 can modulate proteolytic release of soluble EGFR agonists, leading to either suppression¹⁶⁹ or stimulation¹⁷⁰ of paracrine signalling. Tetraspanin CD81 associates with and facilitates the biosynthesis of CD19 (REF. 89), a molecule that plays a pivotal role during signalling through the B-cell receptor¹⁷¹. CD81 also associates closely with the integrin $\alpha_4\beta_1$ (REF. 138), and regulates $\alpha_4\beta_1$ adhesion strengthening under shear flow conditions.⁹⁰ Tetraspanin CD151 associates directly with laminin-binding integrins ($\alpha_3\beta_1$, $\alpha_6\beta_1$, $\alpha_6\beta_4$, $\alpha_7\beta_1$)^{137,172}, with association occurring early in biosynthesis. Indeed, CD151 associates with the integrin α_3 subunit in the endoplasmic reticulum, even before $\alpha_3\beta_1$ association^{11,12}. CD151 contributes to laminin-binding integrin-dependent adhesion strengthening, cell spreading, motility and morphology^{11,23,32,173-176}.

Tetraspanins not only associate directly with other transmembrane proteins, but also with intracellular proteins. For example, the C-terminal cytoplasmic tail of tetraspanin CD63 associates directly with a PDZ motif in Syntenin-1, leading to a negative regulation of CD63 endocytosis¹⁵⁷. It remains to be determined whether PDZ-binding motifs in the C termini of several other tetraspanins will also bind to proteins containing PDZ domains.

Figure 1. Tetraspanin structural features

a | Shown in this 'unfolded' tetraspanin are membrane-proximal palmitoylations (red), hydrophilic residues within transmembrane domains (grey balls), and extracellular loops (EC1 and EC2). EC2, also called large extracellular loop (LEL) is divided into a constant region, containing A, B and E helices, and a variable region, containing the signature tetraspanin CCG motif, two conserved disulphide bonds (red) and a third loop and disulphide bond (dashed) that appears in some tetraspanins. **b** | This more realistic scheme emphasizes the close packing of the four transmembrane domains, the proximity of EC1 and EC2, and the overall rod shaped structure of tetraspanins. Disulphide bonds are not shown. This scheme is based on structural results seen for uroplakin tetraspanins UPK1A and UPK1B⁸, and modelled for other tetraspanins^{9,10}.

Figure 2. Strategies for targeting tetraspanins

a | Monoclonal antibodies (mAbs) might interfere with tetraspanins by blocking lateral interactions, although this has not yet been well documented. Also, they may sequester tetraspanins, leading to disruption of tetra spanin-enriched domains (TEMs), down-modulation or triggering of apoptosis. In addition, mAbs can be used to deliver a lethal hit to cells expressing particular tetraspanins. For example, the antibody might trigger apoptosis, complement-dependent cytotoxicity or antibody-dependent cellular cytotoxicity, or deliver a conjugated toxin or lethal radioisotope or nanoparticle. **b** | Recombinant soluble large extracellular loops (sLELs) most probably insert into TEMs, to disrupt lateral interactions among tetraspanins, or between tetraspanins and their partner molecules. In a special case, hepatitis C virus (HCV) infection, the CD81 sLEL acts in *trans* by binding to the virus and preventing interaction with cellular CD81. **c** | RNAi strategies are well documented to

demonstrate the functional importance of tetraspanins. **d** | Other approaches might be useful for tetraspanin targeting. For example, a small molecule could be designed to inhibit the interaction of a C-terminal tail with a PDZ-domain protein. A peptide specifically mimicking a tetraspanin transmembrane domain might be effective in disrupting lateral interactions. Interference with the appropriate protein acyl transferase in the Golgi would prevent tetraspanin palmitoylation, leading to an inability to assemble TEMs and resulting in impaired functions.

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Table 1
Directly associated tetraspanin partner proteins*

Tetraspanin	Partner protein	Function	Refs
CD9	CD9	CD9–CD9 dimer may be a basic structural unit	37
	EWI-2 (IGSF8, CD316)	Modulates integrin-dependent cell motility and/or spreading	13,81,139, 177
	EWI-F (C9P-1, FPRP)	Functions unknown	178,179
	Claudin-1	CD9 stabilizes expression of non-junctional Claudin-1	14
	HB-EGF	CD9 upregulates both diphtheria toxin binding and mitogenic functions of HB-EGF	128,167
CD81	CD81	CD81–CD81 dimer may be a basic structural unit	7,37,177
	EWI-2	Modulates integrin-dependent cell motility and/or spreading; also, CD81 supports maturation and surface expression of EWI-2	13,81,139, 177,180
	EWI-2wint	Inhibits hepatitis C virus interaction with CD81	166
	EWI-F	Functions unknown	178,179
	CD19	CD81 supports maturation and surface expression of CD19	89,181
	$\alpha_4\beta_1$ Integrin	CD81 supports $\alpha_4\beta_1$ integrin adhesion strengthening	90,138
CD151	CD151	CD151–CD151 dimer may be a basic structural unit	37
	$\alpha_3\beta_1$ Integrin	CD151 affects integrin-dependent adhesion and motility	53,137,138, 147,182
	$\alpha_6\beta_1$ Integrin	CD151 affects integrin-dependent adhesion strengthening	176
	$\alpha_6\beta_4$ Integrin	CD151 affects integrin-dependent adhesion and motility	45,53,147, 172,173
	$\alpha_7\beta_1$ Integrin	Functions not yet demonstrated	172
CD63	Syntenin-1	Endocytosis regulation	157

EWI-2wint, EWI-2 without its N-terminal immunoglobulin domain¹⁶⁶; HB-EGF, heparin-binding epidermal growth factor-like growth factor.

* This table does not include tetraspanins specialized to function in the eye (Peripherin, ROM1) or urothelial membrane (urolakin 1a, urolakin 1b).

Table 2
Tetraspanin functions amenable to targeting*

Tetraspanin	Activity	Evidence [‡]	Refs
<i>Normal physiology and non-infectious pathologies</i>			
CD9	Oocyte fertilization	KO, mAb, RNAi, sLEL	145, 183–185
CD37	B-cell leukemia and lymphoma growth	h-mAb, RIT-mAb	131,186
CD81	Oocyte fertilization	KO	79,187
	Spinal-cord injury	mAb	101
	Cocaine-induced locomotion	RNAi	151
	B-lymphocyte adhesion strengthening	KO	90
CD151	Pathological angiogenesis	KO	44
	Wound healing	KO	100
	Tumour metastasis	mAb, OE	51–53
	Primary tumour growth	RNAi	45
	Platelet aggregation, spreading, clot retraction	KO	95
TSPAN8 (CO-029)	Tumour metastasis, consumption coagulopathy	OE	61
TSPAN32 (TSSC6)	Thrombus stabilization	KO	96
<i>Infectious-disease pathologies</i>			
CD9	Canine distemper virus-induced cell–cell fusion	mAb	121,188
	Diphtheria toxin co-receptor	mAb, OE	128,189
	HIV-1 infection of macrophages	sLEL	106
	Feline immunodeficiency virus infection	mAb	126
CD63	HIV-1 infection of macrophages	mAb, sLEL	105,106
	Intracellular chlamydial development	mAb	190
CD81	Support of hepatitis C virus infectivity	KO, mAb, RNAi, sLEL	112,114
	Malaria sporozoite infectivity	KO, mAb	127
	HIV-1 infection of macrophages	sLEL	106
	HTLV-induced cell–cell fusion	mAb	118
CD82	HTLV transmission, cell–cell fusion	OE, mAb	118,119
CD151	Porcine RRS virus infection	mAb, RNAi	150
	HIV-1 infection of macrophages	sLEL	106

h-mAb, humanized mAb; RIT-mAb, mAb conjugated with radioisotope; RRS, reproductive and respiratory syndrome.

* This table is not intended to be an exhaustive list of tetraspanin functions, but focuses on those that might be worth targeting.

[‡]Functional evidence has been obtained either by knockout (KO) mouse experiments, monoclonal antibody (mAb) inhibition, RNAi intervention (either with siRNA or shRNA), soluble large extracellular loop (sLEL) incubation, or overexpression (OE) studies.