Tetraspanins in Cell Migration

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Abbreviations: ECM, extracellular matrix; TEM, tetraspanin-enriched microdomain; MMP, matrix metalloproteinase; JNK, c-Jun N-terminal kinase; RhoA, Ras homolog gene family, member A; PI4K, phosphatidylinositol 4-kinase; FAK, focal adhesion kinase; lck, lymphocyte-specific protein tyrosine kinase; p38, p38 mitogen-activated protein kinase; HCC, hepatocellular carcinoma; cdc42, cell division control protein 42; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; Sprouty2, Sprouty homolog 2; ROCK, Rho-associated protein kinase; PI3K, phosphatidylinositol-3, 4, 5-trisphosphate 3-kinase; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; ERK, extracellular signal-regulated kinase; PI4K, phosphatidylinositol 4-kinase; FAK, focal adhesion kinase; eNOS, endothelial nitric oxide synthase; HUVECs, human umbilical vein endothelial cells.

Tetraspanins are a superfamily of small transmembrane proteins that are expressed in almost all eukaryotic cells. Through interacting with one another and with other membrane and intracellular proteins, tetraspanins regulate a wide range of proteins such as integrins, cell surface receptors, and signaling molecules, and thereby engage in diverse cellular processes ranging from cell adhesion and migration to proliferation and differentiation. In particular, tetraspanins modulate the function of proteins involved in all determining factors of cell migration including cell–cell adhesion, cell–ECM adhesion, cytoskeletal protrusion/contraction, and proteolytic ECM remodeling. We herein provide a brief overview of collective in vitro and in vivo studies of tetraspanins to illustrate their regulatory functions in the migration and trafficking of cancer cells, vascular endothelial cells, skin cells (keratinocytes and fibroblasts), and leukocytes. We also discuss the involvement of tetraspanins in various pathologic and remedial processes that rely on cell migration and their potential value as targets for therapeutic intervention.

Introduction

Tetraspanins, also called the transmembrane 4 superfamily, are a family of small transmembrane proteins expressed in all multicellular eukaryotes. Thirty-four distinct tetraspanin family members have been found in mammals, of which 33 exist in humans. Tetraspanin proteins are structurally characterized by 4 transmembrane domains, 2 extracellular loops, and short intracellular N- and C-termini. One of the 2 extracellular loops is short (EC1), and the other is longer (EC2). Some tetraspanin proteins also have post-translational modifications including N-linked glycosylation on the EC2 loop and palmitoylation at a CXXC motif in their transmembrane region.

A schematic drawing of the general structure of tetraspanins is shown in Figure 1.

Although their actions and mechanisms are not fully understood, tetraspanins are known to function as scaffolding proteins in the plasma membrane of eukaryotic cells. Tetraspanins bind to one another and to numerous partner proteins, forming a “tetraspanin web” or tetraspanin-enriched microdomains (TEMs), which serve as structural and functional units in plasma membranes. Through direct protein–protein interactions and the specific organization of TEMs, tetraspanins modify the function of a wide variety of proteins including various integrins, immunoglobulin superfamily proteins, proteases, growth factor receptors, and intracellular signaling molecules. Consequently, they are engaged in a variety of cellular processes such as cell adhesion, migration, differentiation, and proliferation and are implicated in numerous pathological conditions including metastasis, inflammation, and viral infection. The four transmembrane domains of tetraspanins are involved in both intramolecular and intermolecular interactions that are crucial for the biosynthesis and assembly of TEMs. The EC2 loop is required for interactions between tetraspanins and other proteins. Despite conserved cysteine motifs, the EC2 loop is the most variable region among tetraspanin family members and likely plays a significant role in member-specific molecular recognition and function.

Tetraspanins are found in nearly all tissues and cell types. Each member exhibits a distinct expression profile. For example, the tetraspanins CD9, CD63, CD82, and CD151 have a wide distribution among various cell types, whereas CD37 and CD53 are mainly found in leukocytes. The functions of a given tetraspanin are likely defined by its protein sequence, post-translational modifications, and tissue and cellular distribution. Through regulation of integrins and other adhesion- and motility-related proteins, a number of tetraspanins have emerged as key regulators of cell adhesion and migration in both normal and pathological processes. The present review focuses on research advances made in this field.

Tetraspanins in cell migration

Cell migration is a fundamental process in both normal development and pathological conditions such as cancer metastasis.
Tetraspanins in cancer cell migration and metastasis

Aberrant expression of tetraspanins, especially CD151, CD9, CD82, CO-029, and CD63, is frequently detected in metastatic tumors and has been linked to cancer progression. In addition to their potential value as prognostic markers in patients with cancer, many studies have suggested that these tetraspanins also play active roles in cancer metastasis by promoting or inhibiting cancer cell migration and invasion. CD151 is the first member of the tetraspanin family to be identified as a promoter of metastasis. The promigratory effects of CD151 on cancer cells are mainly mediated by its association with laminin-binding integrins including α3β1, α6β4, and α6β1. In particular, CD151 forms a highly stoichiometric and stable association with integrin α3β1, which is linked to PI4K activation in many different cell lines. A considerable number of studies have shown that CD151 plays a role in metastasis of specific types of cancer; epidermoid carcinoma and breast cancer are the 2 most thoroughly investigated types of such cancers. CD151 promotes the in vitro migration and in vivo metastasis of epidermoid carcinoma cells by regulating α3β1 and α6β4 integrin-dependent cell adhesion and migration as well as the formation of Rho A-dependent cell–cell junctions. CD151 also promotes cancer cell migration and metastasis in colon cancer, fibrosarcoma, and several other cancer types (Table 1). Interestingly, CD151-null mice exhibit reduced lung metastasis of injected cancer cells and diminished cancer cell transendothelial migration and adhesion to CD151-null lung endothelial cells, suggesting that endothelial CD151 plays a role in fostering a tumor microenvironment that facilitates cancer cell invasion.

CD82, also known as KAI1, is a tetraspanin family member that functions as a metastasis suppressor. In addition to its association with various integrins, CD82 directly interacts with the epidermal growth factor (EGF) receptor (EGFR) and attenuates EGF-induced signaling by promoting EGFR desensitization. CD82 was first identified as a metastasis suppressor in prostate cancer. Subsequent studies have suggested that the antimetastatic effects of CD82 are mediated by inhibition of integrin-dependent activation of c-Met and Src kinases as well as suppression of fibronectin expression and β1 integrin activation. Other studies have shown that CD82 inhibits pathway. Taken together, these findings indicate that tetraspanins regulate the function of key proteins involved in all aspects of cell migration.

Increasing evidence also shows that tetraspanins play important roles in the migration of many different cell types, including but not limited to cancer cells, endothelial cells, keratinocytes, fibroblasts, and leukocytes, and are implicated in various normal and pathological conditions that rely on cell migration. These roles are discussed in more detail in the following sections.

**Figure 1.** Schematic drawing of the general structure of tetraspanins. Tetraspanins are composed of 4 transmembrane domains (pink), a small (EC1) and a large extracellular domain (EC2), a very small intracellular domain, and short cytoplasmic N- and C-terminal tails. The EC2 contains a variable region presenting a conserved Cys-Cys-Gly (CCG) motif and 2–6 additional cysteine residues, which form intramolecular disulfide bonds (red dotted lines).
| Tetraspanin       | Cancer type                  | Cell line; animal model                                                                 | Promoter (↑) or suppressor (↓) | References |
|-------------------|------------------------------|----------------------------------------------------------------------------------------|-------------------------------|------------|
| CD151 (TSPAN24)   | Epidermoid carcinoma         | HEp-3; in vitro migration/in vivo metastasis                                           | ↑                             | 29, 33     |
|                   |                              | HEp3; in vitro migration/in vivo metastasis                                             |                              |            |
|                   |                              | A431; in vitro migration                                                                | ↑                             | 32         |
|                   |                              | A431; in vitro migration                                                                | ↑                             | 33         |
|                   | Breast cancer                | MDA-MB-231; in vitro migration                                                         | ↑                             | 36         |
|                   |                              | MDA-MB-231; in vitro migration/in vivo progression                                      | ↑                             | 35         |
|                   |                              | MDA-MB-231; in vitro migration/in vivo metastasis                                       | ↑                             | 39         |
|                   |                              | MDA-MB-231; in vitro migration                                                          | ↑                             | 37         |
|                   | Prostate cancer              | PC3; in vitro migration                                                                 | ↑                             | 69         |
|                   |                              | PC3; in vitro migration/in vivo metastasis                                              | ↑                             | 70         |
|                   | Hepatocellular carcinoma     | HCCLM3, HepG2; in vitro migration/in vivo metastasis                                    | ↑                             | 40         |
|                   |                              | HepG2; in vitro migration                                                                | ↑                             | 41         |
|                   | Colon cancer                 | RPM/4788; in vitro migration/in vivo metastasis                                         | ↑                             | 71         |
|                   | Tongue squamous carcinoma    | Tca8113; in vitro migration                                                            | ↑                             | 72         |
|                   | Lung adenocarcinoma          | A549; in vitro migration                                                                | ↑                             | 73         |
|                   | Fibrosarcoma                 | HT1080; in vitro migration/in vivo metastasis                                          | ↑                             | 71         |
|                   | Glioblastoma                 | A172; in vitro migration                                                                | ↑                             | 71         |
|                   | Gastric cancer               | SGC7901; in vitro migration                                                            | ↑                             | 74         |
|                   | Cervical cancer              | HeLa; in vitro migration                                                                | ↑                             | 29         |
|                   | Epithelial ovarian cancer    | SKOV3, OVCARS; in vitro migration                                                      | ↑                             | 75         |
| CD82 (KAI1, TSPAN27) | Prostate cancer              | AT6.1; in vivo metastasis                                                              | ↓                             | 45         |
|                   |                              | PC3; in vitro migration                                                                 | ↓                             | 46         |
|                   |                              | DU145; in vitro migration                                                                | ↓                             | 47         |
|                   | Melanoma                     | B16-BL6; in vitro migration/in vivo metastasis                                         | ↓                             | 49         |
|                   | Fibrosarcoma                 | MMRU, MMLU; in vitro migration                                                         | ↓                             | 50         |
|                   | Glioblastoma                 | UACC903M, A375M; in vitro migration/in vivo metastasis                                  | ↓                             | 22         |
|                   | Non-small cell lung cancer   | H1299; in vitro migration                                                                | ↓                             | 25         |
|                   | Pancreatic cancer            | Panc1, MiaPaca-2; in vitro migration                                                    | ↓                             | 77         |
|                   | Hepatocellular carcinoma     | SMMC-7721; in vitro migration                                                           | ↓                             | 52         |
|                   | Ovarian cancer               | HCC-LM3; in vitro migration/in vivo metastasis                                         | ↓                             | 53         |
|                   | Fibrosarcoma                 | U266; in vitro migration                                                                | ↓                             | 78         |
| CD9 (TSPAN29)     | Small-cell lung cancer       | OS3-R5; in vitro migration                                                              | ↓                             | 47         |
|                   | Melanoma                     | OS3-R5; in vitro migration/in vivo metastasis                                          | ↓                             | 50         |
|                   | Breast cancer                | A375; in vitro transendothelial invasion                                               | ↑                             | 61         |
|                   | Ovarian cancer               | Early-stage VGP WM793; in vitro migration                                              | ↓                             | 59         |
|                   | Breast cancer                | MDA-MB-231; in vitro migration                                                          | ↓                             | 62         |
|                   | Multiple myeloma             | MDA-MB-231; in vitro migration                                                          | ↓                             | 63         |
|                   | Prostate cancer              | B02; in vitro metastasis                                                                | ↓                             | 56         |
| TSPAN1            | Colon cancer                 | HT-8; in vitro migration                                                                 | ↑                             | 79         |
|                   | Cervical cancer              | SiHa, HeLa; in vitro migration                                                         | ↑                             | 80         |
|                   | Non-small cell lung cancer   | A549, SK-MES-1; in vitro migration                                                     | ↑                             | 81         |
|                   | Hepatocellular carcinoma     | SMMC-7721; in vitro migration                                                           | ↑                             | 82         |
|                   | Squamous cell skin carcinoma | A431; in vitro migration                                                                | ↑                             | 83         |
| TSPAN8 (CO-029)   | Pancreatic adenocarcinoma     | BSF73AS; in vivo metastasis                                                            | ↑                             | 84         |
|                   | Colon cancer                 | Isrec0; in vitro migration                                                              | ↑                             | 24         |
|                   | Esophageal cancer            | KYSE150, EC9706; in vitro migration/in vivo metastasis                                  | ↑                             | 85         |
| CD63 (TSPAN30)    | Melanoma                     | KM3; in vitro migration                                                                 | ↓                             | 86         |
|                   | Colon cancer                 | MeluSo; in vitro migration                                                              | ↓                             | 88         |
| CD81 (TSPAN28)    | Hepatocellular carcinoma     | HepG2, 5W480, HuH7; in vitro migration/in vivo metastasis                              | ↓                             | 89         |
|                   | Melanoma                     | HepG2, HuH-7.5; in vitro migration                                                      | ↓                             | 90         |
|                   |                                | MeluSo; in vitro migration/in vivo metastasis                                         | ↓                             | 91         |
melanoma cell migration and metastasis in vitro and in vivo through suppression of Rho-associated kinase-mediated formation of stress fibers, inhibition of MMP-2, and regulation of inhibitor of growth 4. Further, CD82 suppresses HCC cell migration in vitro via upregulation of Sprouty2 with subsequent downregulation of sphingosine kinase 1, as well as via inhibition of EGFR and c-Met signaling. Intriguingly, CD82 is a direct target of miR-197, a metastasis promoter of HCC, and mediates the effects of miR-197 on HCC migration via regulation of Rac1 and ROCK activity. CD82 also suppresses migration and metastasis in several other cancer types including non-small cell lung carcinoma, pancreatic cancer, ovarian cancer, and fibroblastoma (Table 1).

CD9 is a tetraspanin family member that exhibits both promigratory and antimigratory properties. CD9 is associated with α1β1, α2β1, α3β1, α4β1, α5β1, α6β1, α7β1, α1bb3, and α6β4 integrins. The regulatory effects of CD9 on cell migration are mediated by integrin-dependent signaling such as phosphorylation of FAK and activation of PI3K, Akt, and p38 kinases. CD9 also directly interacts with EGFR in gastric cancer cells, and further expression of CD9 in EGFR/CD9-transfected HepG2 cells attenuates EGFR signaling, likely by downregulation of EGFR surface expression. CD9 can either promote or suppress cancer cell migration and metastasis depending on the type of cancer, the type of cells involved, and the migratory signal. CD9 inhibits both in vitro migration and in vivo metastasis of OS3-R5 cells, a small-cell lung cancer cell line. However, the effects of CD9 on melanoma migration and invasion are somewhat controversial. CD9 antagonizes osteopontin-induced migration and invasion of early-stage VGP WM793 melanoma cells, but supports transendothelial migration of A375 melanoma cells by strengthening interactions between tumor cells and the endothelial cell monolayer. Similar controversy surrounds the effects of CD9 on the migration of breast cancer cells. CD9 supports native type IV collagen-induced migration of MDA-MB-231 breast cancer cells in vitro, but suppresses the migration of these cells in response to fibronectin. An in vitro study showed that CD9 overexpression promotes bone metastasis of BO2 breast cancer cells, an osteotropic cell line derived from aggressive MDA-MB-231. Other studies have demonstrated that CD9 suppresses the migration of fibrosarcoma cells and multiple myeloma cells, but enhances the migration of prostate cancer cells (Table 1). Interestingly, the promigratory effects of CD9 on prostate cancer cells in vitro do not translate into prometastatic effects in vivo.

Other tetranspanin family members that have roles in cancer cell migration or invasion include TSPAN1 and TSPAN8 (promigratory), CD63 (antimigratory), and CD81 (promigratory or antimigratory) (Table 1). Collectively, these data indicate that select tetranspanin family members are key regulators of cancer cell migration, invasion, and metastasis and that modulation of their activity may have promising results in the treatment of specific types of cancer. The description of specific strategies to target tetranspanins for cancer therapy is beyond the scope of the present paper and has been discussed elsewhere.

Tetraspanins in endothelial cell migration and angiogenesis

Angiogenesis, the formation of new blood vessels from pre-existing ones, is an integral part of many developmental and pathological conditions including embryonic development, wound healing, tissue regeneration, and cancer progression. Migration of capillary endothelial cells is an essential component of angiogenesis and is typically driven by growth factors such as vascular endothelial growth factor or activated by integrins that bind to ECM components. Human endothelial cells express at least 23 tetranspanins including CD151, CD9, CD81, CD82, CD63, and TSPAN8. Many of these tetranspanins, especially CD151 and CD9, have been shown to regulate endothelial cell migration and angiogenesis in vitro and in vivo. In human umbilical vein endothelial cells (HUVECs), TEMs form endothelial adhesive platforms that recruit cell adhesion proteins such as ICAM-1 and VCAM-1 at cell–cell contact sites. Specifically, CD151 is associated with α1, α3, α4, and α6 integrins at lateral junctions, and antibodies to CD151 inhibit HUVEC migration and in vitro angiogenesis. Subsequent studies have shown that these effects of CD151 on HUVECs are mediated by integrin-dependent activation of the PI3K/Akt and ERK signaling pathways. Additionally, CD151 promotes migration, proliferation, and tube formation of ECV304 endothelial cells by activating endothelial NO synthase (eNOS). CD151-null mice exhibit normal vascular development but impaired angiogenesis of pathologic conditions such as tumor growth, and CD151-null mouse lung endothelial cells display aberrant migration and tube formation in vitro, along with reduced adhesion-dependent activation of PKB/Akt, eNOS, Rac, and Cdc42. CD151 gene delivery in rats and pigs following myocardial infarction enhances both myocardial angiogenesis and cardiac function, and these effects are correlated with the activation of FAK, PI3K, and MAPK signaling. One study showed that in a rat model of hind limb ischemia, CD151 gene transfer promoted angiogenesis and improved the skin temperature of the lateral ischemic limb, and activated the FAK, ERK, and PI3K/Akt/eNOS pathways. Importantly, these effects of CD151 are abrogated by transfer of a CD151 mutant with impaired integrin association, indicating that CD151-integrin complex formation is required for CD151-induced angiogenesis.

CD9 is another tetranspanin that plays a key role in endothelial cell migration and angiogenesis. Like CD151, tetranspan CD9 is localized at endothelial cell–cell junctions and associates with α3β1 integrin. Anti-CD9 antibody inhibits the migration of human saphenous vein and mammary artery endothelial cells toward fibronectin and vitronectin via modulation of β1 or β3 integrin-dependent signaling. In HUVECs, CD9 forms a ternary complex with αβ3 integrin and junctional adhesion molecule A and positively regulates basic fibroblast growth factor–induced cell migration and tube formation following release of junctional adhesion molecule A and activation of MAPK. GS-168AT2, a truncated form of CD9-partner 1 protein, which depletes cell surface CD151 and CD9, inhibits vascular endothelial growth factor–induced HUVEC migration and tube formation in vitro and attenuates tumor-associated angiogenesis in...
Moreover, anti-CD9 antibody was shown to inhibit tumor progression in a human gastric cancer xenograft model via inhibition of both tumor growth and tumor-associated angiogenesis. Interestingly, a recent study showed that CD9 deletion attenuates lymphatic endothelial cell migration and tube formation \textit{in vivo} and diminishes both tumor metastasis to lymph nodes and tumor-associated lymphangiogenesis \textit{in vivo}. These data indicate that targeting CD9 may subdue cancer progression via inhibition of both angiogenesis and lymphangiogenesis. Intriguingly, anti-CD9 antibody also inhibits the migration of microvascular endothelial cells of the bovine retina toward fibronectin, and intravitreous injection of siRNA-CD9 or anti-CD9 antibodies reduces laser-induced retinal and choroidal neovascularization in mice. These findings suggest that CD9 may be a therapeutic target for macular degeneration. Furthermore, tumor cells overexpressing rat TSPAN8 promote endothelial cell branching \textit{in vitro} and induce systemic angiogenesis \textit{in vivo}; these effects are driven by selective uptake of tumor cell-derived, TSPAN8-containing exosomes by endothelial cells, a process directed by exosomal TSPAN8. Other tetraspanins implicated in endothelial cell migration and possibly angiogenesis include CD81 and CD63, which have been identified as positive regulators, and CD82, which has been reported as a negative regulator. Therefore, accumulating evidence indicates that targeting specific tetraspanins may hold promise as a novel treatment for cancer and other conditions involving angiogenesis, such as macular degeneration and post-ischemic revascularization.

### Tetraspanins in keratinocyte migration during wound healing

The wound healing process is divided into 4 sequential, yet overlapping phases: (1) hemostasis, (2) inflammation, (3) proliferation, and (4) remodeling. The entire process involves coordinated action of different cell types, including immune cells, endothelial cells, keratinocytes, and fibroblasts. Re-epithelialization of the epidermis, which involves proliferation and migration of keratinocytes from the wound edges across the wound bed to cover the injured area, is an integral part of the proliferation phase of wound healing. Several tetraspanins are expressed on the keratinocyte surface; of these, CD151, CD9, and CD81 are colocalsed with \(\alpha_3\) and \(\beta_1\) integrins at intercellular junctions. One study showed that antibodies to CD151, CD9, CD81, \(\alpha_3\), and \(\beta_1\) inhibit the migration of human keratinocytes in an \textit{in vitro} wound-healing assay. Consistent with these results, CD151 expression has been found to be upregulated during wound healing in C57BL/6 mice, especially within the migrating epidermal tongue at the wound edge. CD151-null mice show impaired wound healing that is primarily attributed to a re-epithelialization deficit, and CD151-null keratinocytes migrate poorly on Matrigel (a basement membrane equivalent) and laminin-332 (a key player in re-epithelialization) and in skin explant cultures. Collectively, these data indicate that CD151 positively regulates wound healing by promoting keratinocyte migration during re-epithelialization. In the proliferation phase of wound healing, fibroblasts grow, migrate, and from a new ECM by secreting collagen and fibronectin. This process is an essential prerequisite to epidermal re-epithelialization. CD151 is also expressed in normal skin fibroblasts, and CD151-null fibroblasts migrate much faster on collagen I while showing no significant changes in adhesion, proliferation, or the ability to cause contraction in response to transforming growth factor \(\beta-1\) or platelet-derived growth factor. These results show that CD151 has a potential role in fibroblast migration during wound healing and may thus warrant further investigation.

Similar to CD151, CD9 is colocalsed with \(\alpha_3\) and \(\beta_1\) integrins at intercellular junctions of keratinocytes. Previous studies have shown that anti-CD9 antibody attenuates the migration of primary human keratinocytes; however, CD9 silencing enhances the migration of HaCaT cells, an immortal human keratinocyte cell line, through activation of the JNK pathway and subsequent MMP-9 expression. One possible explanation for these seemingly inconsistent results is that the binding of anti-CD9 antibody to CD9 does not inhibit CD9 function, but rather enhances it. The finding that CD9 is downregulated in migrating keratinocytes during wound healing both \textit{in vitro} and \textit{in vivo} supports the antiangiogenic effect of CD9 on keratinocytes under these conditions. Similar to CD151-null mice, CD9-null mice show delayed wound healing that is attributed to impaired epidermal migration. Because abnormal elevations of MMP-9 are detected in CD9-null wounds, this delayed epidermal migration may be attributed to excessive degradation of type IV collagen in the basement membrane at the wound site rather than to changes in the migrating keratinocytes themselves. Moreover, because CD9 promotes endothelial cell migration and angiogenesis, loss of CD9 might negatively affect angiogenesis at the wound site, additionally contributing to impaired epidermal migration and re-epithelialization. In summary, these data implicate tetraspanins CD151 and CD9 as important regulators of the wound healing process, indicating their role as potential therapeutic targets for pathological wound repair. Tetraspanins CD63 and CD81 are also found in keratinocytes, and their roles in wound healing may warrant future investigation.

### Tetraspanins in immune cell migration

Tetraspanins were first identified as cell surface antigens in lymphocytes. Later studies showed that immune cells express at least 20 tetraspanins on their surface. In immune cells, tetraspanins interact with many key leukocyte proteins, including immunoreceptors, integrins, and signaling molecules, allowing them to regulate a range of fundamental immune cellular processes such as antigen presentation, antibody production, degranulation, proliferation, and migration/ extravasation. In the present review, we focus on the role of tetraspanins in the migration and extravasation of leukocytes, a critical process in the immune response.
Dendritic cells (DCs) are antigen-presenting cells that stimulate both naive B and T cells during immune responses, and their effectiveness depends on their ability to capture, process, and present antigens and migrate to secondary lymphoid tissues. Tetraspanins CD63, CD9, CD81, CD82, and CD151 are expressed in immature DCs, and antibodies to CD63, CD9, CD81, and CD82 (but not CD151) enhance chemokine-induced migration of these cells. CD81-null DCs display drastically impaired motility because of their inability to form actin protrusions. CD81 silencing in human and mouse DCs produces a similar phenotype along with a selective loss of Rac1 activity. Although CD37-null DCs potently stimulate T cells in vitro, these cells induce poor T-cell responses when injected into wild-type mice. This is attributed to impaired migration from skin to draining lymph nodes. In Jurkat T lymphocytes, tetraspanin CD9 enhances cell migration, activation, and proliferation by regulating the expression and clustering of ALCAM, a member of the immunoglobulin superfamily of cell adhesion molecules. In mast cells, CD9 colocalizes with high-affinity IgE receptor and the transmembrane adaptor protein non-T-cell activation linker (NTAL), promoting antigen-driven chemotaxis via cross talking with these partner proteins. Natural killer cells show substantial expression of CD81, CD63, and CD151 on their cell surface, and stimulation of CD81 with an immobilized antibody induces phosphorylation of ezrin/radixin/moesin proteins, facilitating natural killer cell migration toward various chemokines. In endothelial cells, tetraspanins associate with cell adhesion proteins such as ICAM-1 and VCAM-1 at cell–cell contact sites with transmigrating leukocytes, and endothelial CD9/CD151 silencing prevents lymphocyte transendothelial migration. Additionally, CD63-null HUVECs fail to recruit leukocytes, and CD63-null mice show reduced leukocyte rolling, recruitment, and extravasation, a phenotype similar to that associated with loss of P-selectin. Interestingly, antibodies to CD81 and CD9 block monocyte migration across brain endothelial monolayers by acting on both leukocyte and endothelial tetraspanins. Taken together, these data indicate that both leukocyte and endothelial tetraspanins play crucial roles in leukocyte migration and extravasation during immune responses.

Leukocyte infiltration into the central nervous system is a key process in the development of demyelinating lesions in multiple sclerosis. In mice, administration of an anti-CD81 antibody reduces inflammation in the spinal cord and ameliorates the development of neurological symptoms of experimental autoimmune encephalomyelitis. These results suggest that targeting specific tetraspanins may be a novel therapeutic approach for inflammatory disorders such as multiple sclerosis.

**Conclusions**

The regulatory function of tetraspanin proteins in cell migration has been integrated in the present review (Fig. 2). Tetraspanins interact with a wide range of membrane proteins such as integrins, cell surface receptors, and signaling molecules. They also modulate all 4 determining factors of cell migration: cell–cell adhesion, cell–ECM adhesion, cytoskeletal protrusion/contraction, and proteolytic ECM remodeling. Numerous in vitro and in vivo studies have highlighted the important regulatory function of tetraspanins in the migration of cancer cells, vascular endothelial cells, skin cells (keratinocytes and fibroblasts), and leukocytes. Consequently, tetraspanins are implicated in many pathologic or remedial processes that rely on cell migration, such as cancer, macular degeneration, ischemic injury repair, wound healing, and inflammation. Targeting tetraspanins via
small molecule agents, RNAi, or antibodies may allow the development of novel therapy for these diseases.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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