Tetraspanin protein contributions to cancer

Hong-Xing Wang1, Qinglin Li1, Chandan Sharma1, Konstantin Knoblich1 and Martin E. Hemler1,2

Department of Cancer Biology and AIDS, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, U.S.A.

Abstract

Among the 33 human tetraspanin proteins, CD151, CD9 and Tspan12 play particularly important roles in cancer. Tetraspanin CD151, in partnership with integrins α6β1 and α6β4, modulates tumour cell growth, invasion, migration, metastasis, signalling and drug sensitivity. Tetraspanin CD9 has suppressor functions in multiple tumour cell types. Major CD9 partner proteins, such as EWI-2 and EWI-F, may modulate these tumour-suppressor functions. Tetraspanin Tspan12 mutations are linked to a human disease called familial exudative vitreoretinopathy. In addition, as a regulator of the metalloprotease ADAM10 (a disintegrin and metalloprotease 10) maturation and function, Tspan12 probably contributes to the pro-tumorigenic functions of ADAM10.

Introduction

Tetraspanin proteins make multiple contributions to tumorigenesis, for example by supporting or inhibiting tumour growth, invasion, metastasis and angiogenesis. The present review focuses on a few key tetraspanin proteins (CD151, CD9 and Tspan12) and their effects on tumour behaviour. In addition, the roles of major tetraspanin partner proteins [integrin α6β4, EWI-2 and ADAM (a disintegrin and metalloprotease 10)] are considered.

Tetraspanin CD151

CD151 genetics and partner proteins

Tetraspanin protein CD151 [PETA3 (platelet–endothelial cell tetraspanin antigen 3), SFA-1 (SF-ITI-activated gene 1), Tspan24] is broadly expressed in endothelial cells, epithelial cells, Schwann cells and dendritic cells, as well as in skeletal, smooth and cardiac muscle [1]. CD151-knockout mice in C57BL/6 and 129Lv genetic backgrounds were viable and fertile, with only a few minor defects [2,3]. However, CD151-knockout FVB/N mice showed major renal pathology, including proteinuria, focal glomerulosclerosis and disorganization of the glomerular basement membrane [4,5]. Although CD151-null mice showed no obvious abnormalities in normal skin in either C57Bl/6 or FVB strains, skin wound healing in CD151-null mice was significantly impaired, due to dysregulation of basement membrane laminin 5, impaired keratinocyte proliferation and diminished up-regulation of α6β4 in the wound [6]. In humans, a CD151 mutation was associated with end-stage kidney failure [7,8], apparently due to disruption of the glomerular basement membrane [7]. Pretribial bullous skin lesions were also observed [7,8], as were sensorineural deafness, bilateral lacrimal duct stenosis, nail dystrophy and severe anaemia probably arising from defective erythropoiesis [7,8]. It is speculated that all of these defects arise due to dysregulated integrin–laminin organization and function [7].

CD151 interacts with many other transmembrane proteins either directly or indirectly. CD151 partners include laminin-binding integrins (α3β1, α6β1, α7β1 and α6β4) [9], PI4K (phosphoinositide 4-kinase) [10] and PKC (protein kinase C) [11], and other tetraspanins (CD9, CD81 and CD63), which assemble together to form TEMs (tetraspanin-enriched microdomains) [12]. As a major partner for laminin-binding integrins, CD151 regulates integrin-dependent adhesion strengthening, cell morphology and cell migration [12]. At least part of the effect of CD151 on integrins may be due to CD151 regulating the trafficking/endocytosis of integrin complexes. CD151 contains a potential tyrosine-based sorting motif in the C-terminal domain. The YXXΦ (where Φ is a hydrophobic residue) motif is required for endocytosis at the plasma membrane, and it sorts proteins from the TGN (trans-Golgi network) to lysosomes for degradation. Mutations in the CD151 YXXΦ motif diminished CD151 internalization, thus affecting integrin-dependent cell migration [13].

CD151 and cancer

Integrin-mediated cell adhesion is plays a central role in the development of cancer, as integrins control tumour cell migration, invasion, proliferation, metastasis, angiogenesis and survival. Consequently, integrins have emerged as important targets for therapeutic intervention in cancer [14]. Because CD151 is a major partner and regulator of laminin-binding integrins, it is perhaps not surprising that CD151.
together with α3β1 and α6β4, plays a vital role in cancer cell motility, invasion and metastasis [15]. CD151 regulates TGF (transforming growth factor) β1-induced activation of p38 as well as HGF (hepatocyte growth factor)/c-Met signalling in breast cancer cells, and is associated with reduced survival in breast cancer patients [16–18]. In liver cancer, the expression of CD151 was positively correlated with metastatic potential of HCC (hepatocellular carcinoma) cell lines [19], and also overexpressed in HCC samples compared with normal liver tissues [19]. CD151 may promote HCC progression by increasing expression MMP9 (matrix metalloproteinase 9), a zinc-dependent type IV collagenase, through the PI3K (phosphoinositide 3-kinase)/Akt/GSK3β (glycogen synthase kinase 3β)/Snail pathway [20]. Also, increased CD151 expression correlates with an increased risk of metastasis and reduced survival in various other cancers, including colon, pancreatic, lung and prostate cancer. Evidence obtained using an anti-CD151 monoclonal antibody in a chick embryo xenograft model suggests that CD151 is contributing to spontaneous metastasis [21].

Studies from our laboratory show that CD151 expression is especially elevated in high-grade and oestrogen receptor-negative human breast cancer subtypes [22]. Silencing of CD151 in basal-like breast cancer cells resulted in reduced in vivo tumour growth in both ectopic and orthotopic xenograft models [22]. In addition, CD151 ablation reduced basal-like mammary cell migration, invasion, spreading and signalling, while disrupting collaboration between the EGFR (epidermal growth factor) and α6β4 integrin [22]. In human ErbB2+ breast cancer cells, CD151 worked together with laminin, integrin and FAK (focal adhesion kinase) to regulate anti-ErbB2 drug-sensitivity [23]. Furthermore, CD151-null mice were impaired in pathological angiogenesis, as seen in multiple in vivo assays, including tumour implantation [3]. The combination of in vitro cell studies, in vivo mouse studies and human clinical studies all point to CD151 playing a key role in the development and metastasis of several important tumour types. Hence CD151 could be used as a target for future anticancer therapy.

α6β4 and α6β1 integrins

Properties of α6 and β4 integrins

The α6 integrins (α6β1 and α6β4) together with α3β1 and α7β1 form a distinct integrin subgroup with respect to ligand specificity and protein sequence similarity. Among integrin β subunits, the β4 subunit is unique. Its distinctively long cytoplasmic domain (~1000 amino acids) contains two FN (fibronectin) type III repeats, which flank a CS (connecting segment). In the CS region, β4 can be phosphorylated at Ser536, Ser630, Ser736 and Ser1024 partly by PKC, in connection with HD (hemidesmosome) disassembly [24–26]. The α6β4 integrin also can undergo Src-family kinase tyrosine phosphorylation in the FN–CS region [27], leading to association with signalling molecules, such as Shc [28], IRS (insulin receptor substrate)-1 and IRS-2 [29]. A distinctive property of LB (laminin-binding) integrins is their post-translational palmitoylation. Palmitate is added to single membrane-proximal cysteine residues in the α6, α3 and α7 subunits, and to seven membrane-proximal cysteine residues in the β4 subunit. Palmitoylation of the β4 subunit affects integrin-dependent spreading and signalling [30,31]. A physical consequence of integrin β4 palmitoylation is the recruitment of the α6β4 integrin into cell-surface membrane complexes called TEMs [30]. Integrin α-chain palmitoylation is also likely to contribute to integrin–TEM association, but this is not yet definitive.

The α6β4 integrin, present on nearly all types of epithelial cells, is a key component of HDs. The HD molecular complex links extracellular matrix laminin with cytoskeletal intermediate filaments [32]. Hence deletion or mutation of genes for α6 or β4 in mice or humans results in epithelial blistering [33]. The α6β4 integrin also supports wound healing [34], mammary epithelial branching morphogenesis [35] and mammary cell survival within the three-dimensional polarized architecture [36].

α6 and β4 on tumour cells

Expression of the α6 subunit [37] or α6β4 [38] correlates with poor prognosis of breast cancer, whereas α6β1 expression is linked to metastatic potential and survival of human breast carcinoma cells [39,40]. In prostate cancer, α6β1 replaces α6β4, to support progression to invasive carcinoma [41]. The α6β1 integrin may facilitate activation of HIF-1 (hypoxia-inducible factor-1), leading to production of VEGF (vascular endothelial growth factor), which supports tumour survival by increasing vascular permeability and promoting angiogenesis [42].

As epithelial cells progress to tumours, the α6β4 integrin disconnects from intermediate filaments in normal HDs, translocates to lamellipodia, associates with the actin-rich cytoskeleton, and then plays a pro-invasive pro-survival role, partly through the PI3K/Akt pathway [24]. During this transition, the β4 cytoplasmic domain undergoes EGFR (epidermal growth factor)-dependent PKC-dependent phosphorylation at Ser362 and Ser1424. This results in HD disassembly and integrin relocation to the leading edges of lamellar protrusions, thus contributing to tumour cell migration and invasion [26,43]. However, α6β4 contributions vary in different cell environments [44]. For example, α6β4 supports survival in p53-deficient carcinoma cells, but causes apoptosis when p53 is normal [45]. Integrin α6β4 also supports keratinocyte transformation that is induced by oncogenic Ras and blockade of NF-κB (nuclear factor κB) [46], and can collaborate with ErbB2 to promote mammary tumorigenesis and progression to metastasis [47]. Additionally, α6β4 contributes to tumour progression by promoting VEGF- and bFGF (basic fibroblast growth factor)-initiated angiogenesis [48].

Our laboratory has recently focused on two aspects of α6β1 and α6β4 integrin physiology, involving (i) collaborations with CD151, and (ii) the role of palmitoylation. In
basal-type breast cancer cells, removal of CD151 affects α6 integrin-dependent invasion, migration, spreading, signalling and EGFR collaboration [22]. In another study, CD151 and α6 integrins worked together to support the resistance of ErbB2+ tumour cells to the anti-ErbB2 drugs trastuzumab and lapatinib [23]. At present, we are investigating the consequences of CD151 ablation, with respect to changes in β4 phosphorylation, in parallel with HD disruption (Q. Li, unpublished work). As we demonstrated previously, removal of β4 integrin palmitoylation impairs cell spreading and signalling through p130Cas on laminin substrates, and impairs association with tetraspanin-enriched microdomains [30]. At present, we are studying the specific protein acyl transferase, within the ‘DHHC’ (Asp-His-His-Cys) family of protein acyl transferases [49,50], which is primarily responsible for integrin α6 and β4 palmitoylation (C. Sharma, unpublished work).

**Tetraspanin CD9**

**CD9 domains and key partner proteins**

CD9, a member of tetraspanin family, is widely expressed on the surface of several cell types, including haemopoietic cells, endothelial cells, epithelial cells and many malignant tumour cells [51,52]. CD9 affects major cell signalling pathways and regulates multiple cellular functions such as sperm–egg fusion [53–55], cell adhesion, migration, cell apoptosis, and tumour cell motility and metastasis. Like other tetraspanins, CD9 has four putative TMs (transmembrane domains), short N- and C-terminal cytoplasmic domains, a small intracellular loop and two extracellular loops [51,52]. CD9 interacts with a number of transmembrane proteins including integrins, EWI proteins (EWI-2 and EWI-F) and other tetraspanins (e.g. CD81 and CD151) to form TEMs [12,56]. Within CD9, multiple distinct domains contribute to its functions. The large extracellular loop of CD9 contains Ser173-Phe-Gln175, residues which are required for gamete fusion [57]. TM1 and TM2 of CD9 are required for the inhibition of platelet aggregation [58]. A chimaeric CD81/CD9 containing CD81 TM1–TM3 and EC (extracellular loop) 1 domains combined with CD9 EC2 and TM4 regions could mimic intact CD9 function by increasing the sensitivity of LHBEGF cells to diphtheria toxin [59].

**CD9 and tumour suppression**

An inverse correlation between CD9 expression in primary tumours and patient survival rate has been established for melanomas, and colon, lung and breast carcinomas. Overexpressed CD9 in melanoma and breast, lung, pancreas and colon carcinoma cells appears to suppress the motility and metastatic potential of these cells. Similarly to CD82, CD9 can hamper metastasis formation by prohibiting integrin-mediated motility. In ovarian carcinoma cells, expression levels of CD9 and integrin subunits β1, α2, α3, α5 and α6 are closely correlated, and down-regulation of CD9 is associated with reduction of matrix adhesion and diffuse growth in vitro [60]. In addition, anti-CD9 monoclonal antibodies can inhibit the migration of different carcinoma tumour cell types and the transendothelial migration of melanoma cells [61,62].

**CD9 and cell signalling**

For both CD9 and the closely related tetraspanin CD81, regulation of proliferation is positively associated with activation of the ERK (extracellular-signal-regulated kinase) 1/2/MAPK (mitogen-activated protein kinase) pathway. While suppressing ERK1/2 signalling, anti-CD9 antibodies concurrently activated the JNK (c-Jun N-terminal kinase)/SAPK (stress-activated protein kinase) and p38 MAPK pathway [63,64]. CD9 can associate with conventional PKC isozymes including PKCa and PKCβ [11], as well as type II PI4K [65], which could contribute to signalling and tumour-suppressor functions. In addition, CD9’s effects on cell migration may involve CD9 associating with the β1 integrin chain and EGFR to alter EGFR–EGFR signalling [63], and CD9 may affect the Wnt signalling pathway by down-regulating Wnt genes [66]. Finally, CD9 associates with TGFα and protects TGFα from cleavage, thereby regulating cell proliferation and cell migration [67].

**CD9 association with EWI-2 and protein organization**

Our laboratory, together with others, have demonstrated that the major protein partners for CD9 are cell-surface Ig superfamily proteins called EWI-1F (CD9P1 (CD9 partner 1), FPRP (prostaglandin F2 receptor-associated protein)) and EWI-2 [68–70]. On T-leukaemic cells and on glioblastoma cells, EWI-2 appeared to have tumour-suppressor functions, affecting both cell spreading/invasion and in vivo tumour growth [71,72]. However, our more recent results suggest that EWI-2 in other tumour cell environments does not have suppressor activity (H.-X. Wang, unpublished work). At present, it is not yet clear the extent to which CD9 tumour suppressor activity involves associated proteins EWI-2 and EWI-1. In another study, we have focused on the C-terminal tail of CD9, which is short (only eight residues), while terminating in a potential PDZ domain-binding motif. Extensive conservation of the CD9 C-terminal sequence across many vertebrate species is suggestive of its functional importance. We find that a conservative mutation within the CD9 C-terminal tail markedly affects cell adhesion, spreading and homotypic cell–cell aggregation. Furthermore, this mutation appears to alter markedly the size and oligomerization characteristics of CD9 complexes with its partner proteins (H.-X. Wang, T.V. Kolesnikova, C. Denison, S.P. Gygi and M.E. Hemler, unpublished work).

**Tspan12**

**Tspan12 functions in mouse and humans**

Tspan12 is a tetraspanin protein with an uncharacteristically long C-terminal tail (~60 amino acids). It is widely expressed in most human tissues and organs. Tspan12-knockout mice...
appear normal, except for retinal vascular defects [73] which closely resemble the retinal defects in a human disease called FEVR (familial exudative vitreoretinopathy) [74]. Tspan12 strongly enhances the signalling which occurs upon binding of the Norrin protein to its receptor Frizzled-4, with co-receptor assistance from Lrp5 (low-density-lipoprotein receptor-related protein-5). This signalling goes through β-catenin, which interacts with TCF (T-cell factor)/LEF (lymphoid enhancer factor) transcription factors to turn on target genes [75]. Consequently, Tspan12-null mice show severely impaired Norrin/β-catenin signalling [73]. In humans, nine different TSPAN12 mutations have been observed, resulting in a spectrum of FEVR disease phenotypes [74,76], and demonstrating conclusively that TSPAN12 mutation can cause human FEVR.

**Tspan12 as a regulator of ADAM10**

Other studies have shown that Tspan12 associates with the membrane metalloproteinase ADAM10 under relatively stringent detergent conditions, thus making it the most robust tetraspanin partner for ADAM10 yet identified [77]. ADAM10 is a metalloproteinase that acts as a sheddase in its mature form, to cleave cell-surface molecules such as CD44, amphiregulin, CD23, L1-CAM (L1 cell adhesion molecule), N-cadherin, and E-cadherin [78]. ADAM10-dependent shedding of membrane-bound growth factors enhances EGFR stimulation, whereas shedding of ErbB2 provides oncogenic activation, leading in both cases to accelerated tumour cell growth. ADAM10 itself can be up-regulated on cancer cells [79]. There is a growing effort to devise strategies for inhibiting ADAM10, which may have therapeutic anticancer effects [78].

Tspan12 promotes ADAM10 maturation, and enhances ADAM10-dependent proteolysis of multiple substrates, including APP (amyloid precursor protein) [77]. Mutations of critical Tspan12 palmitoylation sites confer dominant-negative-like properties on Tspan12, leading to poor translocation of endogenous Tspan12 to the plasma membrane, resulting in inhibition of ADAM10 maturation [77]. Importantly, Tspan12 neither associates with nor modifies the functions of ADAM17, a protease somewhat similar to ADAM10. The shedding of several cell-surface proteins targeted by ADAM10 is likely to contribute to pro-tumorigenic functions for ADAM10 [78]. To the extent that TSPAN12 promotes the maturation and function of ADAM10, it also should be pro-tumorigenic.

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