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REVIEW

Tetraspanins as regulators of the tumour microenvironment: implications for metastasis and therapeutic strategies

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One of the hallmarks of cancer is the ability to activate invasion and metastasis. Cancer morbidity and mortality are largely related to the spread of the primary, localized tumour to adjacent and distant sites. Appropriate management and treatment decisions based on predicting metastatic disease at the time of diagnosis is thus crucial, which supports better understanding of the metastatic process. There are components of metastasis that are common to all primary tumours: dissociation from the primary tumour mass, reorganization/remodelling of extracellular matrix, cell migration, recognition and movement through endothelial cells and the vascular circulation and lodgement and proliferation within ectopic stroma. One of the key and initial events is the increased ability of cancer cells to move, escaping the regulation of normal physiological control. The cellular cytoskeleton plays an important role in cancer cell motility and active cytoskeletal rearrangement can result in metastatic disease. This active change in cytoskeletal dynamics results in manipulation of plasma membrane and cellular balance between cellular adhesion and motility which in turn determines cancer cell movement. Members of the tetraspanin family of proteins play important roles in regulation of cancer cell migration and cancer-endothelial cell interactions, which are critical for cancer invasion and metastasis. Their involvements in active cytoskeletal dynamics, cancer metastasis and potential clinical application will be discussed in this review. In particular, the tetraspanin member, CD151, is highlighted for its major role in cancer invasion and metastasis.

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Abbreviations
EC2, large extracellular loop; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; MT1-MMP, membrane-type 1 MMP; PI3-K, phosphoinositide 3-kinase; PI4-K, PI-4-kinase; RNAi, interference RNA; TME, tumour microenvironment

Introduction

Cell motility and invasion is an important biological process for the generation and development of the organism in normal and pathological conditions. Cancer cell motility and invasion is driven by similar cellular mechanisms to normal cell migration but is lacking in the inhibitory responsiveness that controls normal migration (Palmer *et al.*, 2011).

Therefore, the concept of targeting cancer cell migration as a therapeutic approach is unclear. However, the potential advantages of specifically targeting cancer cell migration are worth exploring. The cellular cytoskeleton provides cell structure and shape, and is essential for intrinsic cellular vesicle and organelle movements and also cellular external movement (Pardee, 2009).

The cytoskeleton and cancer metastasis

Single cell and collective cell movement are important features of cancer that enable cancer cells to invade and form metastases (Friedl and Gilmour, 2009). As stated by Friedl and Wolf (2010), the determining factors of cell migration include cell-cell adhesion (cadherins), cell-extracellular matrix (ECM) adhesion (integrins), cytoskeletal protrusion/contraction (Rac/Rho), traction force/propulsion and proteolysis. The subject of discussion in this review is the tetraspanin superfamily which has a role in various key determinants of cell migration. They interact directly with various integrins (Berditchevski and Odintsova, 1999; Berditchevski, 2001), matrix metalloproteinases (MMPs) (Takino *et al.*, 2003; Yanez-Mo *et al.*, 2008; Lafleur *et al.*, 2009; Yáñez-Mó *et al.*, 2011; Schroder *et al.*, 2013) and E-cadherin (Greco *et al.*, 2010). Specifically, the tetraspanin CD151 is important in maintaining the balance between RhoA and Rac-1 signalling in endothelial cells (Zhang *et al.*, 2011a) and epidermal carcinoma cells (Johnson *et al.*, 2009). In contrast, CD82 was found to modulate membrane composition leading to down regulation of cytoskeleton rearrangement via Src, p130CAS/Crk, and Rho/Rac pathway (Sridhar and Miranti, 2006; Liu *et al.*, 2012). In addition, CD81 also interacts directly with Rac proteins which delays Rac inactivation, leading to enhanced cell migration (Tejera *et al.*, 2013). Integrins are major partners of tetraspanins and they have been implicated in the control of cell adhesion and migration (Huttenlocher and Horwitz, 2011). They are best known to form focal adhesions, which are a complex of integrins, signalling proteins and actin cytoskeleton. This will be discussed in more detail in the following sections.

Tetraspanins

Tetraspanins are 4-transmembrane spanning proteins with short cytoplasmic N- and C- termini (Maecker *et al.*, 1997; Berditchevski, 2001). They are expressed on the cell surface and/or intracellular vesicles (Wright *et al.*, 2004b). This family contains 33 members in mammals (Berditchevski, 2001; Hemler, 2005; Levy and Shoham, 2005), each of which has a distinctive pattern of expression (Table 1). For example, CD9, CD81, TSPAN7, CD63, CD82, and CD151 are found in virtually all tissues whereas CD37 and CD53 are found in haemopoietic cells (Maecker *et al.*, 1997; Bienstock and Barrett, 2001; Wright *et al.*, 2004b). Tetraspanins are involved in fertilization, immune interaction and brain development (Yáñez-Mó *et al.*, 2001) and have been linked to various processes including signal transduction pathways, cellular activation, proliferation, motility, adhesion, tissue differentiation, angiogenesis, tumour progression and metastasis (Hasegawa *et al.*, 1998; Berditchevski, 2001; Yáñez-Mó *et al.*, 2001; Ang *et al.*, 2010).

An overall rod-shaped structure of tetraspanins was revealed by a 6 Å resolution cryo-electron microscopy structure of uroplakin (Min *et al.*, 2006). The rod-shaped structure consists of four close packed transmembrane helices that extend into the extracellular loops, capped by a disulfide-stabilized head domain (Figure 1). Of the 200–350 amino acids that are found in tetraspanins, 12–31 of them reside in the short extracellular loop for which structural information is not yet available.

The large extracellular loop (EC2) of tetraspanins is of highly variable structure, despite conserved cysteine motifs, which may indicate tetraspanin-specific recognition processes (Kitadokoro *et al.*, 2001). Conserved motifs include four invariant cysteine residues in the EC2 domain: CCG (Cys-Cys-Gly), PXSC (Phe-X-Ser-Cys) and EGC (Glu-Gly-Cys) (Hemler, 2001; Clark *et al.*, 2004; Kovalenko *et al.*, 2005) (Figure 1). The transmembrane domains stabilize heteromultimerization among different tetraspanins forming 'tetraspanin webs' (Fitter *et al.*, 1998; Stipp *et al.*, 2003b; Kovalenko *et al.*, 2005). Besides conserved EC2 homology across species for any particular tetraspanin (Bienstock and Barrett, 2001), Seigneuret *et al.* suggested that the EC2 domain was organized into two subdomains: one conserved subdomain with a highly conserved fold despite significant residue differences and a second variable subdomain with extreme variability in size, amino acid sequence and protein fold governed by key disulfide bridges (Seigneuret *et al.*, 2001). The EC2 regions of tetraspanins are required for interactions between tetraspanins and other transmembrane proteins such as integrins, and other signalling molecules (Maecker *et al.*, 1997; Yáñez-Mó *et al.*, 2001). In addition, mutations within transmembrane domains 1, 2 and 4 of peripherin/rds tetraspanin are linked to various types of retinal dystrophies (Stipp *et al.*, 2003b). The short cytoplasmic tails show no obvious functional significance in signalling processes, suggesting association with other signalling molecules (Fitter *et al.*, 1998). Tetraspanins are thought to act as molecular facilitators, recruiting groups of specific cell-surface proteins which stabilize functional signalling complexes (Maecker *et al.*, 1997).

The tetraspanin 'web' or tetraspanin-enriched microdomain is an important biological feature of tetraspanin members and involves interactions with various leucocyte receptors, signalling molecules such as integrins, PKC, PI-4-kinase (PI4-K) and with each other (Wright *et al.*, 2004b) (Figure 2). These interactions are important in determining fundamental biological activities such as cell adhesion, proliferation and cell motility. Interactions between tetraspanin members are important in maintaining the integrity of the tetraspanin web and providing binding sites for different ligands. However, these interactions are weaker than tetraspanin-partner interactions, such as CD151-α3β1 integrin (Wright *et al.*, 2004b). Palmitoylation, post-translational acylation in most cases with cysteine residues, is found to be critical for organization of tetraspanin-enriched microdomains (Zhou *et al.*, 2004) and the loss of palmitoylation affects tetraspanin-partner interactions, subcellular distribution, stability during biosynthesis and cell morphology (Yang *et al.*, 2002; Stipp *et al.*, 2003b).

Integrins are major partners of the tetraspanins and interact with a wide range of ECM proteins (Berditchevski, 2001; Hood and Cheresh, 2002) (see below for more discussion).

Table 1

Known human tetraspanins: distribution and function

Tetraspanins	Other names	Tissue and organ distribution	Functions
TSPAN1	TSP-1 NET-1	Endometrium, colon, kidney, heart, lung, pancreas, prostate, thyroid gland and trachea (Todd *et al.*, 1998; Puls *et al.*, 1999).	• Possible role in thiamine (vitamin B1) transportation via direct interaction with thiamine transporter, hTHTR-1 (Nabokina *et al.*, 2011).
TSPAN2	TSP-2 NET-3	Adrenal gland, brain, duodenum, intestine, liver, lung, ovary and testis. Weak expression in heart, pancreas, skin, stomach and uterus (Todd *et al.*, 1998) Myeloid cells and T lymphocytes (Serru *et al.*, 2000).	• Possible role together with CD9 and CD81 in oligodendrocyte signalling (Terada *et al.*, 2002)
TSPAN3	TSP-3	Brain, endometrium, colon, kidney, heart, lung, melanocytes, pancreatic islets, pancreas, prostate, retina, thyroid gland, trachea and dendritic cells (Todd *et al.*, 1998). Osteoclast precursor cells (Iwai *et al.*, 2007)	• Regulates oligodendrocyte cell proliferation and migration (Terada *et al.*, 2002) via direct interaction with claudin-11 and β1 integrins (Tiwari-Woodruff *et al.*, 2004).
TSPAN4	TSP-4 NAG-2 TM4SF7	Brain, heart, melanocytes (Todd *et al.*, 1998), osteoclast precursor cells (Iwai *et al.*, 2007), spleen, colon, lymphocytes, pancreas, prostate and salivary gland (Tachibana *et al.*, 1997),	• Possible role in autoimmune response causing endothelial apoptosis via complex with integrin (Traggiai *et al.*, 2010)
TSPAN5	TSP-5 NET-4	Brain, colon, liver/spleen, pancreas, retina, T-lymphoid cells (Todd *et al.*, 1998; Serru *et al.*, 2000) and osteoclast precursor cells (Iwai *et al.*, 2007)	• Possible role in osteoclastogenesis (Iwai *et al.*, 2007) • Regulation of ADAM10 trafficking and activity (Dornier *et al.*, 2012)
TSPAN6	TSP-6	Brain, colon, liver/spleen, heart, lung, melanocytes, ovary, pancreas, prostate and retina (Maeda *et al.*, 1998; Todd *et al.*, 1998)	Not known
TSPAN7	CD231 TALLA-1 A15	Brain, lung, kidney, skeletal muscle, spleen. Expression is also found in T-cell acute lymphoblastic leukaemia and neuroblastoma (Takagi *et al.*, 1995)	• Possible role in cell proliferation and cell motility. • Mutation causes X-linked mental retardation (Zemni *et al.*, 2000) • Interact with Herpes simplex virus type 1 (HSV-1) and play a role in HSV-1 replication (Wang *et al.*, 2010).
TSPAN8	CO-029	Gastric, oesophageal, hepatic, colorectal, and pancreatic carcinomas (Richardson *et al.*, 2011)	• Possible role in cancer cell motility and metastasis (Richardson *et al.*, 2011)
TSPAN9	NET-5	Megakaryocytes, platelets and hematopoietic cells (Serru *et al.*, 2000)	• Have a role in regulation of collagen-induced platelet adhesion and activation via complex with collagen receptor, glycoprotein VI, integrin α6β1 and other tetraspanin members (Protty *et al.*, 2009)
TSPAN10	OCULOSPANIN	Retinal pigment epithelium and choroid (Wistow *et al.*, 2002)	Not known
TSPAN11		Not known	Not known
TSPAN12	NET-2 TM4SF12	Lymphoid cells (Serru *et al.*, 2000)	• Retinal vascularization (Junge *et al.*, 2009) • Mutation causes vitreoretinopathy exudative type 5 (Nikopoulos *et al.*, 2010) • Regulatory role in proteolytic function of membrane proteinases such as ADAM10 (Xu *et al.*, 2009) and MMP14/MT1-MMP (Lafleur *et al.*, 2009)
TSPAN13	NET-6	Osteoclast precursor cells and haematopoietic cells (Serru *et al.*, 2000; Iwai *et al.*, 2007)	• Possible role in osteoclastogenesis (Iwai *et al.*, 2007)

Table 1

Continued

Tetraspanins	Other names	Tissue and organ distribution	Functions
TSPAN14	DC-TM4F2	Not known	• Regulation of ADAM10 trafficking and activity (Dornier *et al.*, 2012; Haining *et al.*, 2012)
TSPAN15	NET-7	Myeloid cells, B-lymphoid cells and T-lymphoid cells (Serru *et al.*, 2000)	• Regulation of ADAM10 trafficking and activity (Prox *et al.*, 2012)
TSPAN16	TM4-B TM4SF16	Most tissues, strong expression in spinal cord, prostate, and salivary gland (Puls *et al.*, 1999)	Not known
TSPAN17	FBXO23 TM4SF17	Not known	• A subunit of ubiquitin protein ligases (Cenciarelli *et al.*, 1999).
TSPAN18	N/A	Not known	Not known
TSPAN19	N/A	Not known	Not known
UPK1B	UP1b TSPAN20	Bladder epithelium (Yu, 1994)	• Regulation of UPII and UPIII maturation, stabilization and cell surface expression (Hu *et al.*, 2005)
TSPAN21	UP1a UPK1A, TSPAN21	Bladder epithelium (Yu, 1994)	• Regulation of UPII and UPIII maturation, stabilization and cell surface expression (Hu *et al.*, 2005)
PRPH2	RDS ROCA-1 TSPAN22	The nervous system (Kaprielian and Patterson, 1993) and retina (Travis *et al.*, 1991).	• Rod-photoreceptor disk morphogenesis (Molday *et al.*, 1999). • Mutation causes retinitis pigmentosa (Dryja *et al.*, 1997)
TSPAN23	ROM1	Retina (Bascom *et al.*, 1992)	• Rod-photoreceptor disk morphogenesis (Molday *et al.*, 1999). • Mutations in ROM1 and PRPH2 cause retinitis pigmentosa (Bascom *et al.*, 1995; Dryja *et al.*, 1997)
CD151	PETA-3 SFA-1 MER2 TSPAN24	Most tissues including vascular endothelium, epidermis platelets and erythroid cells, except brain, red blood cells and lymphocytes (Sincock *et al.*, 1997)	• Hemidesmosome assembly • Tumour metastasis promoter • Wound healing
CD53	TSPAN25 MOX44	Lymphocytes, monocytes, granulocytes and osteoclast precursor cells (Olweus *et al.*, 1993; Iwai *et al.*, 2007)	• Direct association with palmitoylated transmembrane adaptor protein, SCIMP, CD37 and CD81 to form membrane platform important in antigen presentation in immune response (Draber *et al.*, 2011). • Regulatory role on the cell-surface expression and function of MT1-MMP (Schroder *et al.*, 2013)
CD37	TSPAN26	Mature B cells and osteoclast precursor cells (Iwai *et al.*, 2007)	• Regulation of T-lymphocyte proliferation (van Spriel *et al.*, 2004; Gartlan *et al.*, 2010) • Regulatory role on the cell-surface expression and function of MT1-MMP(Schroder *et al.*, 2013)
CD82	KAI1 ST6 SAR2 4F9 TSPAN27 C33 antigen	Most tissues, except smooth muscle, adrenal cortex, urothelium, myelin of peripheral nerves, epithelium of amnion (Dong *et al.*, 1995; Huang *et al.*, 1997). Lymphocytes and monocytes (Imai *et al.*, 1992).	• Associates with CD4 or CD8 and delivers co-stimulatory signals for the TCR/CD3 pathway (Nojima *et al.*, 1993) via association with β1 integrin (Iwata *et al.*, 2002). • Metastasis suppressor in prostate cancer (Dong *et al.*, 1995). • Indirect regulation of proteolytic function of urokinase type plasminogen uPA/uPAR system that possibly control cell motility and adhesion (Bass *et al.*, 2005) • Regulatory role on the cell-surface expression and function of MT1-MMP (Schroder *et al.*, 2013)

Table 1

Continued

Tetraspanins	Other names	Tissue and organ distribution	Functions
CD81	TAPA-1 TSPAN28	Most cells, including lymphoid cells (Oren *et al.*, 1990; Berditchevski *et al.*, 1996; Levy *et al.*, 1998; Berditchevski, 2001; Hemler, 2005)	• Role in the regulation of lymphoma cell growth (Boismenu *et al.*, 1996; Sagi *et al.*, 2012). • HCV viral receptor (Pileri *et al.*, 1998). • Sperm-egg fusion (Rubinstein *et al.*, 2006) • Regulatory role on the cell-surface expression and function of MT1-MMP (Lafleur *et al.*, 2009) • Support EWI-2 maturation and localization and facilitate interaction of EWI-2 to integrin $\alpha3\beta1$ (Stipp *et al.*, 2003a)
CD9	MIC-3 MRP-1 TSPAN29	Most tissues including haematopoietic, osteoclast precursor cells and epithelial cells, except red blood cells and pancreas (Huang *et al.*, 1997; Sincock *et al.*, 1997; Nakamura *et al.*, 2001)	• Regulation of cell adhesion and motility in normal and cancerous cells (Ikeyama *et al.*, 1993; Kotha *et al.*, 2008; Powner *et al.*, 2011). • Platelet activation and aggregation (Boucheix *et al.*, 1983; Hato *et al.*, 1988; Slupsky *et al.*, 1989) • Regulates paranodal junction formation (Ishibashi *et al.*, 2004). • Sperm-egg fusion (Higginbottom *et al.*, 2003; Rubinstein *et al.*, 2006) • Regulatory role on the cell-surface expression and function of MT1-MMP (Lafleur *et al.*, 2009) and CD9P-1 (Chambrion and Le Naour, 2010; Schroder *et al.*, 2013) • Expresses on CD4+ T-cells and involved in T-cell activation (Tai *et al.*, 1996; Kobayashi *et al.*, 2004)
CD63	MLA1 TSPAN30 Ocular melanoma-associated antigen	Haematopoietic cells, lymphoid tissues, osteoclast precursor cells and tissue macrophages (Metzelaar *et al.*, 1991; Radford *et al.*, 1996; Iwai *et al.*, 2007). Bladder, gut, kidney, lung, ocular tissues, pancreas, prostate, salivary gland, spleen and uterus, except brain, red blood cells and lymphocytes (Donoso *et al.*, 1985; Sincock *et al.*, 1997)	• Association with melanoma progression (Donoso *et al.*, 1986). • Possible role in MHC II-dependent T-cell stimulation (Petersen *et al.*, 2011) • Possible role in HIV replication (Chen *et al.*, 2008a) • Regulatory role on the cell-surface expression and function of MT1-MMP (Takino *et al.*, 2003; Schroder *et al.*, 2013) • Trafficking of the heterodimeric ion pump H, K-ATPase in gastric parietal cells (Duffield *et al.*, 2003)
TSPAN31	SAS	Osteoclast precursor cells (Iwai *et al.*, 2007) and sarcoma	Not known
TSPAN32	TSSC6 PHEMX	High level of expression in haematopoietic tissues including peripheral blood leukocytes, thymus and spleen (Nicholson *et al.*, 2000; Robb *et al.*, 2001)	• May have a role in T-lymphocyte proliferative response (Tarrant *et al.*, 2002; Gartlan *et al.*, 2010)
TSPAN33	PEN	Predominantly in erythroblasts (Heikens *et al.*, 2007)	• Role in differentiation of erythroid progenitors (Heikens *et al.*, 2007)

Data sourced from cited references, the human protein atlas (http://www.proteinatlas.org) and the protein database (http://www.uniprot.org). N/A denotes data not available. ADAM10, A disintegrin and metalloprotease 10.

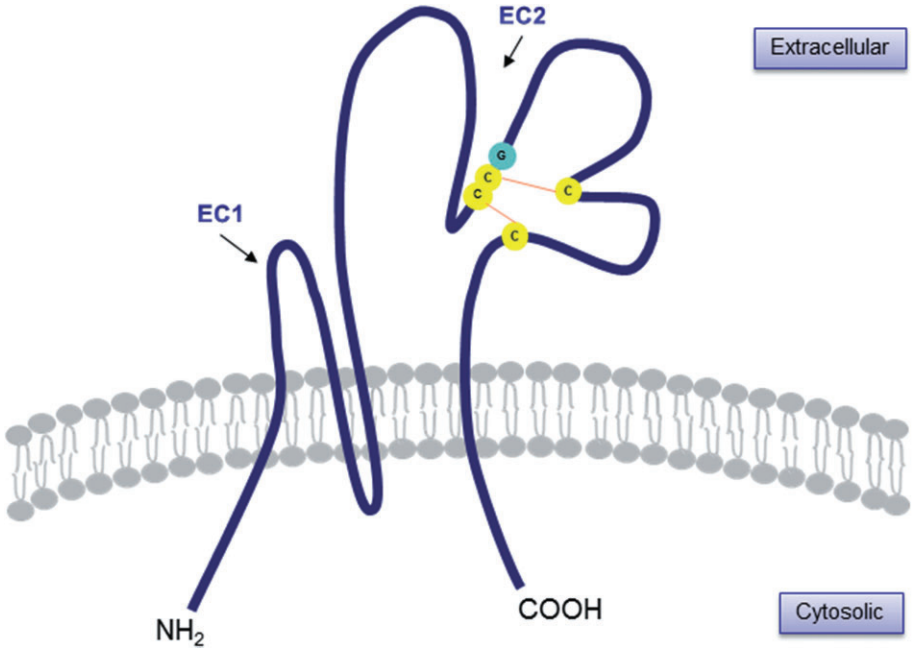

Figure 1

Representation of structural feature of tetraspanins. A variable domain (EC2) is stabilized by two disulfide bonds (orange lines) and consists of four invariant Cys residues (in yellow), two of which are in a Cys-Cys-Gly (CCG) motif (in yellow and green).

The interaction of tetraspanins with laminin-binding integrins may be the pathway by which tetraspanins have an effect on migration and metastasis (Serru *et al.*, 1999). Berditchevski emphasized that the tetraspanins CD81 and CD151 interact with integrins directly and as a consequence, may bring other family members into integrin proximity (Berditchevski, 2001). For example, the PKC family of phospholipid-dependent serine and threonine kinases is among the intracellular proteins which bind indirectly to tetraspanins and participate in various biological activities (Berditchevski, 2001). PKCs associate with several different tetraspanins such as CD9, CD81, CD82 and CD151, in which specificity resides within the intracellular domain of tetraspanins and tetraspanins act as linker molecules recruiting PKC into close proximity with β1, β2 and β3 integrins (Zhang *et al.*, 2001b). PKCα and PKCε isoforms are found to interact with β1 integrins, promoting integrin-dependent cell motility and control of internalization of integrins (Ng *et al.*, 1999; Ivaska *et al.*, 2002).

Integrin involvement in cell invasion and metastasis is well established. Brakebusch *et al.*, in particular, discussed the role of integrins in invasive growth *in vivo* and demonstrated the importance of integrin-mediated binding events in cell proliferation and invasion. For example, up-regulation of integrin α5β1 expression inhibits programmed cell death and β1 integrins promote metastasis (Brakebusch *et al.*, 2002). Integrin αvβ4, when constitutively activated, promotes breast cancer metastatic activity in a human breast cancer cell line and mouse model (Felding-Habermann *et al.*, 2001). β1 integrin is required for cancer cell adhesion and invasion by promoting formation of focal adhesion complexes to the extracellular matrix and mediating anti-apoptotic signal transduction pathways activating Akt/phosphoinositide 3-kinase (PI3-K), which may or may not involve focal adhesion kinase activation (Velling *et al.*, 2004; Brockbank *et al.*, 2005). Integrin expression profiles have been studied intensively and various integrin heterodimers, such as α3β1 and α6β1, are correlated with aggressiveness of prostate cancer, while expression of β4 integrin is often lost (Knox *et al.*, 1994; Cress *et al.*, 1995). A study of MDA-MB-231 cells, a breast cancer cell line, has shown that the α3β1-tetraspanin protein complex may be linked to an invasive phenotype of tumour cells via modulation of various signalling pathways, including degradation and activation of MMP-2 (a MMP family protein associated with supporting protrusive activity and invasive migration of the cells) and affecting PI3-K signalling pathways, which control actin cytoskeleton dynamics (Sugiura and Berditchevski, 1999). Mutagenesis of the α3 domain of integrin reveals a phosphorylation site within the conserved motif at the α3A cytoplasmic tail corresponding to integrin-related signalling, motility and morphology (Zhang *et al.*, 2001a). In addition, the integrin α6β4 has novel functions in migration of epithelial and epithelial-derived carcinoma cells via the formation of adhesive structures, hemidesmosomes. These in turn link to the intermediate filament cytoskeleton and activate PI3-K which then stimulates other integrins, especially α3β1 (Mercurio *et al.*, 2001). Co-localization of integrin β4 with CD151 also leads to activation of PKC which in turn promotes integrin internalization and increases cell motility (Gesierich *et al.*, 2005). Binding of CD151 to α3β1 is highly stoichiometric and CD151 association with PI4-K brings PI4-K in closer proximity to α3β1 (Yauch *et al.*, 1998), which may be one of the mechanisms by which CD151 promotes cell motility.

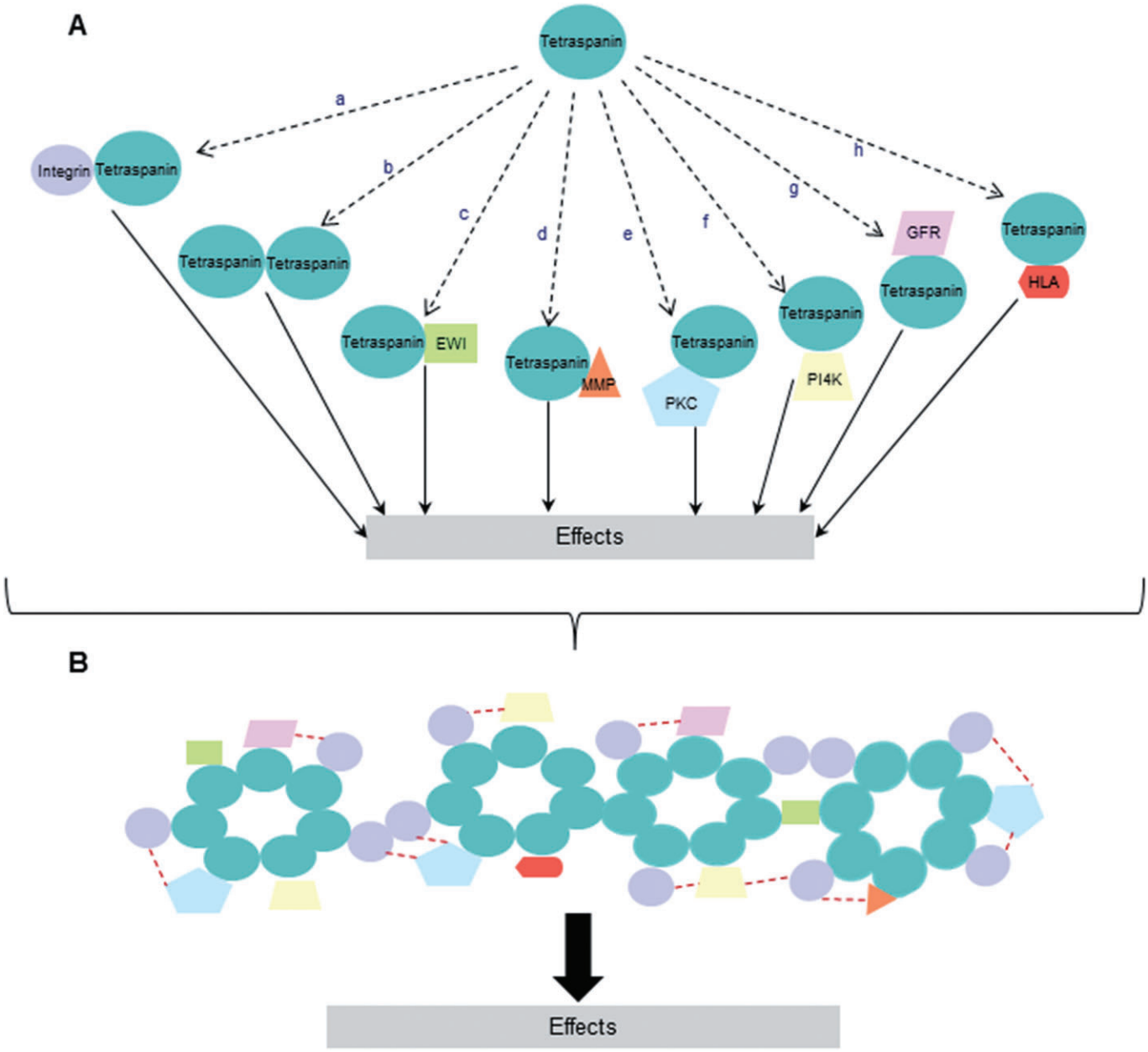

Figure 2

Schematic representation of formation and complexity of the tetraspanin web. (A) Tetraspanins interact with various molecules including other members of tetraspanin superfamily (a–h) and these interactions can result in downstream signalling and biological function. (B) Representative formation of tetraspanin web forming specific signalling network of cell membrane and cytosolic proteins are shown. The network depending on tetraspanins and their partners are determinants of signal transduction and tetraspanins can bring together different signalling proteins into close proximity (red dotted lines). (EWI, a cell surface immunoglobulin SF protein; GFR, growth factor receptor; HLA, human leukocyte antigen).

Expression and prognostic value of tetraspanins in various cancers

Many studies have found correlations between tetraspanins and progression of cancer. Most tetraspanins become down-regulated in metastatic tumours but the CD151 glycoprotein was the first tetraspanin member to be identified as a promoter of metastasis (Testa *et al.*, 1999). Similarly, TSPAN8 is up-regulated in advanced stages in pancreatic, hepatic, oesophageal, gastric and colorectal carcinomas (Richardson *et al.*, 2011). Although most studies have found CD9 to be down-regulated during cancer progression (see Tables 2 and 3), the opposite has been found in osteosarcoma (Kubista *et al.*, 2004), prostate cancer (Zvierev *et al.*, 2005) and breast cancer (Kischel *et al.*, 2012). These findings are supported by the finding that CD9 promotes expression of the matrix proteolytic enzyme, MMP-2 (Hong *et al.*, 2005). Various tetraspanins have been investigated for their potential

Table 2

Tetraspanins are prognostic indicators in many cancers (↑ denotes increased levels = more progression and ↓ denotes decreased levels = more progression)

Cancer type	Tetraspanins and correlation with prognosis	References
Astrocytoma	CD63↑	(Rorive *et al.*, 2010)
Breast cancer	CD9↓, CD82↓	(Huang *et al.*, 1998)
	CD151↑	(Sadej *et al.*, 2009; Kwon *et al.*, 2012)
Clear cell renal cell carcinoma	CD151↑	(Yoo *et al.*, 2011)
	TSPAN7	(Wuttig *et al.*, 2012)
Colon cancer	CD9↓, CD82↓, CD151↑	(Hashida *et al.*, 2003)
	TSPAN8↑	(Greco *et al.*, 2010)
Colorectal cancer	TSPAN1↑	(Chen *et al.*, 2009)
Endometrial carcinoma	CD9↓	(Miyamoto *et al.*, 2001)
	CD151↑	(Voss *et al.*, 2011)
Gallbladder adenocarcinoma	CD9↓	(Qiong *et al.*, 2012)
Gastric cancer	CD9↓	(Hori *et al.*, 2004; Soyuer *et al.*, 2010; Chen *et al.*, 2011b)
	TSPAN1↑	(Chen *et al.*, 2008b)
	CD151↑	(Yang *et al.*, 2013)
Gastric gastrointestinal stromal tumour	CD9↓	(Setoguchi *et al.*, 2011)
Gingival squamous cell carcinoma	CD151↑	(Hirano *et al.*, 2009)
Glioblastoma	CD63↑	(Rorive *et al.*, 2010)
Head and neck squamous cell carcinoma	CD9↓	(Mhawech *et al.*, 2003)
Hepatocellular carcinoma	CD81↓	(Inoue *et al.*, 2001)
	CD151↑	(Ke *et al.*, 2009; Shi *et al.*, 2010; Devbhandari *et al.*, 2011)
	TSPAN1↑	(Chen *et al.*, 2010a)
	TSPAN8↑	(Kanetaka *et al.*, 2001)
Intrahepatic cholangiocarcinoma	CD151↑	(Huang *et al.*, 2010)
Lung adenocarcinoma	CD9↓	(Higashiyama *et al.*, 1997)
	CD63↓	(Kwon *et al.*, 2007)
Melanoma	CD9↓	(Si and Hersey, 1993)
	CD63↓	(Radford *et al.*, 1997)
Merkel cell carcinoma	CD9↓, CD151↑	(Woegerbauer *et al.*, 2010)
Multiple myeloma	CD81↑	(Paiva *et al.*, 2012)
Non-small cell lung cancer	CD82↓	(Adachi *et al.*, 1996)
	CD63↓	(Kwon *et al.*, 2007)
	CD151↑	(Tokuhara *et al.*, 2001)
Oral squamous cell carcinoma	CD9↓	(Kusukawa *et al.*, 2001; Buim *et al.*, 2010)
Oesophageal squamous cell carcinoma	CD9↓	(Uchida *et al.*, 1999)
	CD82↓	(Uchida *et al.*, 1999)
	CD151↑	(Suzuki *et al.*, 2011)
Ovarian carcinoma	CD9↓	(Houle *et al.*, 2002)
	CD82↓	(Schindl *et al.*, 2001; Houle *et al.*, 2002)
	TSPAN1↑	(Scholz *et al.*, 2009)
	CD63↓	(Zhijun *et al.*, 2007)
Pancreatic cancer	CD9↓, CD82↓	(Sho *et al.*, 1998)
	CD151↑	(Zhu *et al.*, 2011)
Prostate cancer	CD82↓	(Lijovic *et al.*, 2002)
	CD151↑	(Ang *et al.*, 2004)
	TSPAN13↓	(Arencibia *et al.*, 2009)
Thyroid cancer	CD82↓	(Chen *et al.*, 2004)

Table 3

Tetraspanin members have a role in cancer cell motility and invasion

Tetraspanin	Cancer type	Promoter/ Suppressor of motility/ invasion	Proposed mechanism	Reference
CD9	Small cell lung cancer	Suppressor	Not determined	(Zheng *et al.*, 2005)
	Fibrosarcoma	Suppressor	Inhibition of cell motility and colony formation via formation of complexes of CD9 and its partners TGFα, EGFR, EWI-2, EWIF and β1 and activation of Akt, p38, and EGFR pathways	(Chen *et al.*, 2011a)
	Ovarian carcinoma	Suppressor	CD9 has a role in cell adhesion on ECM and down regulation of CD9 resulted in altered integrins β1, α2, α3β1, α 5, and α 6 expression and localizations	(Furuya *et al.*, 2005)
	Prostate cancer	Promoter	Not determined	(Zvierev *et al.*, 2005)
	Lung cancer	Suppressor	Association with β1 integrin	(Funakoshi *et al.*, 2003)
	Breast cancer	Promoter	Not determined	(Kischel *et al.*, 2012)
		Promoter	CD9/CD81 support MT1-MMP, a proteolytic enzyme, expression and CD9/CD81/MT1-MMP association enhances invasion in *in vitro* 3D collagen and fibrin gel	(Lafleur *et al.*, 2009)
		Promoter	CD9 and CD81 complex may independently promote α3β1 integrin association with PKCα	(Gustafson-Wagner and Stipp, 2013)
CD63	Melanoma	Suppressor	Association with β1 integrin and may regulate β1 integrin expression	(Radford *et al.*, 1997; Jang and Lee, 2003)
	Colon cancer	Suppressor	CD63/α3 integrin complex regulates adhesion and migration on substrate laminin-5	(Sordat *et al.*, 2002)
CD81	Hepatocellular carcinoma	Suppressor	Interacting with PI4KIIβ and together affect actin cytoskeleton rearrangement	(Mazzocca *et al.*, 2008)
	Histiocytic lymphoma	Promoter	CD81 promotes cell membrane protrusive structures	(Bari *et al.*, 2011)
	Breast cancer	Promoter	CD9/CD81 support MT1-MMP, a proteolytic enzyme, expression and CD9/CD81/MT1-MMP association enhances invasion in *in vitro* 3D collagen and fibrin gel	(Lafleur *et al.*, 2009)
		Promoter	CD9 and CD81 complex may independently promote α3β1 integrin association with PKCα	(Gustafson-Wagner and Stipp, 2013)
CD82	Ovarian cancer	Suppressor	Inhibiting αvβ3 integrin/vitronectin-mediated cell motility and proliferation	(Zlatna *et al.*, 2009)
	Non-small cell lung cancer	Suppressor	Regulating β1 integrin maturation and its cell surface expression	(Jee *et al.*, 2007)
		Suppressor	Stabilizing E-cadherin–b-catenin complex (promoting cellular adhesion)	(Abe *et al.*, 2008)
	Oral squamous cell carcinoma line and non-small cell lung carcinoma	Suppressor	Direct association with c-Met inhibiting HGF-promoted motility	(Takahashi *et al.*, 2007)
	Prostate cancer	Suppressor	Not determined	(Dong *et al.*, 1995; Lijovic *et al.*, 2002; Bari *et al.*, 2009)
		Suppressor	CD82 modulates integrin-mediated activations of c-Met and Src signallings	(Sridhar and Miranti, 2006)
		Suppressor	Attenuates cell membrane protrusive structures	(Bari *et al.*, 2011)
		Suppressor	Direct association with EWI2/PGRL immunoglobulin member to mediate tumour cell migration	(Zhang *et al.*, 2003)
		Suppressor	Regulates β1 integrin activation at the cell surface affecting focal adhesion complex formation	(Lee *et al.*, 2011)

Table 3

Continued

Tetraspanin	Cancer type	Promoter/ Suppressor of motility/ invasion	Proposed mechanism	Reference
CD151	Melanoma	Promoter	Association with α3β1 and α6β1 integrins. Linking β1 integrins to Ras, Rac1 and Cdc42	(Hong *et al.*, 2006; 2012)
	Skin squamous cell carcinoma	Promoter	CD151 supports PKCα-α6β4 integrin association and α6β4 integrin distribution	(Li *et al.*, 2012)
	Gastric cancer	Promoter	Association with integrins α3	(Yang *et al.*, 2013)
	Prostate cancer	Promoter	Not determined	(Ang *et al.*, 2004; 2010)
	Pancreatic and colorectal carcinoma	Promoter	Association with α6β4 integrin and tetraspanin TSPAN8	(Gesierich *et al.*, 2005)
	Salivary gland cancer	Promoter	Association with c-Met and integrins α3/α6 and promotes HGF/c-Met signalling pathway	(Klosek *et al.*, 2005)
	Breast cancer	Promoter	Association with c-Met and integrins α3/α6 and promotes HGF/c-Met signalling pathway	(Klosek *et al.*, 2009)
		Promoter	Assisting ErbB2-integrin pathway through focal adhesion kinase (FAK) signalling	(Yang *et al.*, 2010; Deng *et al.*, 2012)
	Intrahepatic cholangiocarcinoma	Promoter	Not determined	(Huang *et al.*, 2010)
	Hepatocellular carcinoma	Promoter	Association with α6 integrin Increase Rac/Cdc42 activity	(Ke *et al.*, 2011; Fei *et al.*, 2012)
	Colon cancer, glioblastoma and fibrosarcoma	Promoter	Possibly via FAK association	(Kohno *et al.*, 2002)
	Human epidermoid carcinoma and fibrosarcoma	Promoter	Supports tumour cell detachment and tumour intravasation	(Zijlstra *et al.*, 2008)
	Ovarian cancer	Promoter	Not determined	(Mosig *et al.*, 2012)
TSPAN1	Colon cancer	Promoter	Not determined	(Chen *et al.*, 2010b)
	Hepatocellular carcinoma	Promoter	Not determined	(Wang *et al.*, 2012)
	Skin squamous cell carcinoma	Promoter	Not determined	(Chen *et al.*, 2010c)
TSPAN8	Oesophageal carcinoma	Promoter	ADAM12, a type of matrix metalloprotease enzyme is involved in TSPAN8's promotion of motility and invasion	(Zhou *et al.*, 2008)
	Colon cancer	Promoter	Modulating regulation of E-Cadherin/p120ctn complex on cell motility	(Greco *et al.*, 2010)
	Colorectal cancer	Suppressor	Promotes cell motility through regulation of tumour cell-matrix and cell-cell adhesion	(Guo *et al.*, 2012)
TSPAN13	Breast cancer	Suppressor	May promotes cell-matrix adhesion via down-regulation of MMPs	(Huang *et al.*, 2007)

MET, mesenchymal-epithelial transition.

as prognostic factors in many cancers, as outlined in Table 2.

Association with integrins

Integrins are important in cell attachment and control cell migration, cell cycle progression and programmed cell death. They regulate these functions in synergy with other signalling pathways (Brakebusch *et al.*, 2002), including tetraspanins such as CD81, CD9, CD53, CD63, CD82 and CD151, in various types of human cells (Maecker *et al.*, 1997). They do not have any intrinsic activities but are present on the cell surface and respond to various ECM components and microenvironmental signals to form integrin-dependent signalling pathways to regulate proliferation, migration, invasion, apoptosis and angiogenesis (Brakebusch *et al.*, 2002; Stupack and Cheresh, 2004). Major laminin-binding integrins (laminins are cell-adhesive proteins in basement membranes) are α3β1, α6β1, α6β4 and α7β1 (Nishiuchi *et al.*, 2005). There is evi-

dence that CD81 associates with the α4β1 integrin, and CD151 with α3β1, α6β1, α6β4 and α7β1 (Serru *et al.*, 1999; Sterk *et al.*, 2002; Wright *et al.*, 2004b).

Evidence of a relationship between angiogenesis, lymphangiogenesis and cancer progression and involvement of integrins has become apparent in recent years. In endothelial cells, integrins have been found to be involved in the induction of angiogenesis (Dominguez-Jimenez *et al.*, 2001; Wang *et al.*, 2005; Mitchell *et al.*, 2009; 2010). Integrins α1β1, α2β1, α4β1, α5β1, α9β1, α6β4, αvβ3 and αvβ5 play a role in angiogenesis, while integrins α2β1, α4β1, α5β1 and α9β1 are involved in lymphangiogenesis (Hong *et al.*, 2004; Jin and Varner, 2004; Dietrich *et al.*, 2007; Avraamides *et al.*, 2008; Okazaki *et al.*, 2009; Garmy-Susini *et al.*, 2010). Integrins α5β1, αvβ5 and αvβ3 bind provisional ECM components (the permissive basal ECM required for angiogenesis) such as fibronectin and vitronectin which are up-regulated during angiogenesis, while integrins that bind to basal ECM components collagen and laminin such as α1β1, α2β1, α3β1, α5β1 and α6β4 tend to be down-regulated (Stupack and Cheresh, 2004). Integrin α9β1 directly associates with VEGFs -A, -C and -D, which are major modulators of blood (VEGF-A) and lymph (VEGF-C and VEGF-D) vessel formation (Timoshenko *et al.*, 2007; Vlahakis *et al.*, 2007; Oommen, 2011; Majumder *et al.*, 2012). Therefore, integrin protein expression is an important determinant to balance signal transduction pathways that occur within the cell.

Many studies have found various complexes of tetraspanins and integrins that are co-localized and together influence cell motility. In rat pancreatic adenocarcinoma, for example, the D6.1A tetraspanin (rat homologue of human CO-029 tetraspanin) and α6β4 integrin are co-expressed and together contribute to hematogenous spread of tumour cells (Gesierich *et al.*, 2005). Interestingly, most of CD151's role is through association with integrins. Complexes of CD151 and integrin α3β1 are the focus of most studies as their interaction is strong, direct and stoichiometric (Yauch *et al.*, 1998; Berditchevski *et al.*, 2001). CD151 and the integrin α3β1 were directly associated and were important in recruiting various signalling molecules (including other tetraspanin members) into close proximity to each other to form a signalling complex (Serru *et al.*, 1999). Loss of CD151 diminished the association of laminin-binding integrins (i.e. α3β1 and α6β1) with signalling proteins (Yauch *et al.*, 1998; Takeda *et al.*, 2007). CD151 was also associated with activation of various signalling molecules, PI3-K, PI4-K, PKB/Akt, endothelial nitric oxide synthase (eNOS), Rac and Cdc42 that are involved in cell migration, invasion and angiogenesis (Yauch *et al.*, 1998; Takeda *et al.*, 2007; Zheng and Liu, 2007a). The CD151-α3β1 complexes play a particular role in formation of focal contacts and intracellular signalling events, especially changes in actin-cytoskeleton dynamics leading to cell invasive migration (Berditchevski and Odintsova, 1999; Shigeta *et al.*, 2003). CD151 is an essential molecular linker in integrin-dependent cell motility signalling (Kazarov *et al.*, 2002) and important in determining integrin localization within cells (Sincock *et al.*, 1997; Chometon *et al.*, 2006; Hasegawa *et al.*, 2007). Changes in integrin localization are believed to be a determinant of cancer invasion and metastasis.

Tetraspanin influence in forming complexes with integrins may regulate cellular integrin distribution and integrin trafficking, which in turn regulates cellular motility and invasion (Berditchevski and Odintsova, 1999; Winterwood *et al.*, 2006; Berditchevski and Odintsova, 2007). Some of tetraspanin/integrin associations that are important in cancer cell motility/invasion and angiogenesis are shown in Tables 3 and 4. Significance of integrin-tetraspanin association is well reviewed in Boucheix and Rubinstein (2001), Hemler (2008), Stipp (2010) and Bassani and Cingolani (2012).

Role of tetraspanins in cancer metastasis

Cellular changes of carcinogenesis involve various mechanisms, including regulatory gene mutations, gene over- and under-expression, endocrine activation and epigenetic alterations. This accumulation of changes results in a loss of balanced gene expression, allowing cells to undergo transformation (Golias *et al.*, 2007). The cellular transformation together with changes in tumour microenvironment (TME) triggers invasive and/or metastatic phenotypes (Wall *et al.*, 2003; Hugo *et al.*, 2007). In order for tumour cells to create a progressive disease, they need to communicate with their surrounding cells. The TME plays an important part in the promotion of cancer cell growth, invasion, angiogenesis and survival (Fidler, 2002). Angiogenesis is one of the major characteristics of cancer metastasis and anti-angiogenic agents targeting various molecular targets are currently in clinical trials (Carmeliet and Jain, 2000; Detchokul and Frauman, 2011; Goel *et al.*, 2011).

Members of the tetraspanin superfamily are implicated in regulation of cell proliferation, motility, adhesion, angiogenesis and tumour metastasis (Hemler *et al.*, 1996; Hasegawa *et al.*, 1998; Berditchevski, 2001; Boucheix *et al.*, 2001; Longo *et al.*, 2001; Tokuhara *et al.*, 2001; Sadej *et al.*, 2009). Tetraspanins are also associated with the regulation of the net proteolytic activity at the plasma membrane providing additional dimension of regulatory mechanisms for cell adhesion, migration and growth factor signalling (Yáñez-Mó *et al.*, 2011; Dornier *et al.*, 2012; Haining *et al.*, 2012; Schroder *et al.*, 2013). CD82/KAI1 was found to negatively correlate with the progression of prostate cancer, suggesting this tetraspanin has a tumour suppressor role (Lijovic *et al.*, 2002). Since the first finding of the role of CD151 in the promotion of cancer migration and metastasis by Testa *et al.* (1999), numerous studies have been looking at this tetraspanin in various cancers: epidermoid, pancreatic, glioblastoma, breast, colorectal, amelanotic melanoma, osteosarcoma and hepatic fibrosarcoma (Testa *et al.*, 1999; Kohno *et al.*, 2002; Gesierich *et al.*, 2005; Hong *et al.*, 2006; Hasegawa *et al.*, 2007; Yang *et al.*, 2008; Shi *et al.*, 2010; Zhang *et al.*, 2010). CD151 affects cell motility and malignancy in non-small cell lung cancer (Sugiura and Berditchevski, 1999) and pancreatic and colorectal tumours (Gesierich *et al.*, 2005) and also has a role in migration of neutrophils, fibroblasts and endothelial cells (Yáñez-Mó *et al.*, 1998; Yauch *et al.*, 1998; Kohno *et al.*, 2002; Liu *et al.*, 2007; Takeda *et al.*, 2007; Zheng and Liu, 2007b; Zuo *et al.*, 2010). A link between CD151 expression and prostate cancer prognosis has been demonstrated (Ang *et al.*, 2004) and CD151 correlates with the prognosis and survival time of non-small cell lung cancer (Tokuhara *et al.*, 2001), colon cancer (Hashida *et al.*, 2003), renal cell carcinoma (Yoo *et al.*, 2011), pancreatic adenocarcinoma (Zhu *et al.*, 2011), oesophageal squamous cell carcinoma (Suzuki *et al.*, 2011),

Table 4

Tetraspanins and their role in cancer angiogenesis

Tetraspanin	Investigated cell types/animal models	Proposed mechanism	Reference
CD9	Multiple myeloma	Involved in transendothelial invasion and CD9 expression was up-regulated upon contact with bone marrow endothelial cells	(De Bruyne *et al.*, 2006)
	Human cervical carcinoma	Not determined. Preferentially expressed near vascular and lymphatic tumour invasions which may suggest role in transendothelial migration	(Sauer *et al.*, 2003b)
	Human umbilical vein endothelial cells (HUVECs) and melanoma cells	Promotes transendothelial migration of tumour cells and tumour-endothelial cells interactions	(Longo *et al.*, 2001)
	Human dermal microvascular endothelial cells	Promotes endothelial cell VEGF- and HGF- induced motility and invasion but not proliferation	(Kamisasanuki *et al.*, 2011)
	Human saphenous vein or mammary artery endothelial cells	Promotes endothelial cell motility via association with β1 and β3 integrins	(Klein-Soyer *et al.*, 2000; Soyuer *et al.*, 2010)
	Human gastric cancer cell xenografts in SCID mice	CD9 antibody treated mice had decreased angiogenesis. Mechanism was not determined	(Nakamoto *et al.*, 2009)
	HUVECs and erythroleukemic cells	Associates with adhesion receptor ICAM-1 at the apical membrane as part of endothelial adhesion platforms (EAPs) that regulate cellular adhesion	(Barreiro *et al.*, 2008)
CD81	HUVECs	Localised with CD151, CD9 and integrin α3β1 and promotes endothelial cellular migration	(Yáñez-Mó *et al.*, 1998)
CD82	HUVECs	Up-regulated CD82 gene expression under hypoxic condition (via HIF-2) and CD82 suppresses endothelial cell migration	(Nagao and Oka, 2011)
CD151	HUVECs and erythroleukemic cells	Associates with adhesion receptor VCAM-1 at the apical membrane as part of endothelial adhesion platforms (EAPs) that regulate cellular adhesion	(Barreiro *et al.*, 2008)
	HUVECs	Complex of tetraspanins/α3β1 mediates Ang II promotion of tubulogenesis	(Dominguez-Jimenez *et al.*, 2001)
	Hepatocellular carcinoma cells	Modulates MMP9 expression via the PI3-K/Akt/GSK-3b/Snail signal to promote angiogenesis	(Shi *et al.*, 2010)
	Breast cancer cells	Responds to endothelial factors, maybe via association with α3β1and α6β4 integrins	(Sadej *et al.*, 2010)
	HUVECs	Localized at cell-cell junction together with CD81, CD9 and α6β4 and promotes endothelial cell motility and ECM remodelling	(Yáñez-Mó *et al.*, 1998)
	Mouse lung endothelial cells derived from CD151-null mice	Activates along with FAK, ERK, PI3K/Akt/eNOS, and Rac1/Cdc42 pathways to promote angiogenesis	(Takeda *et al.*, 2007)
	Mouse lung endothelial cells derived from CD151-null mice and murine melanoma cells	Required for melanoma cell-endothelial cell adhesion and transendothelial migration	(Takeda *et al.*, 2011)
	Human dermal microvascular endothelial cells (HMECs) and HUVECs	Promotes cell-matrix adhesion via stabilizing focal adhesion maturation and promotion of cadherin-independent cell-cell adhesion	(Zhang *et al.*, 2011a)
	HUVECs	Localises at cell-cell junctions, promotes endothelial cell motility and promotes *in vitro* capillary tube formation	(Sincock *et al.*, 1999)
	Rat/pig models of myocardial ischemia	CD151 gene delivery improved microvessel densities in animal models of myocardial ischemia	(Zheng and Liu, 2006; Zuo *et al.*, 2009a,b)
	HUVECs	CD151-integrin complex may be needed for the promotions of *in vitro* endothelial proliferation, migration and tube formation acting via ERK-dependent signalling pathway	(Zuo *et al.*, 2010)
Tspan8	BDX-derived rat pancreatic adenocarcinoma	Induces angiogenic switch and promotes angiogenic factor expression	(Gesierich, 2006)
	BDX-derived rat pancreatic adenocarcinoma and rat aortic ring endothelial cells	Exosomes expressing Tspan8 secreted by tumour cells activates endothelial cell activation, maturation and motility	(Nazarenko *et al.*, 2010)

intrahepatic cholangiocarcinoma (Huang *et al.*, 2010), breast cancer (Sadej *et al.*, 2010), hepatocellular carcinoma (Ke *et al.*, 2009; Shi *et al.*, 2010) and Merkel cell carcinoma (Woegerbauer *et al.*, 2010) (see Table 5 below). CD151 has attracted much interest in cancer research and this will be discussed in the following sections. We have also summarized the significance of tetraspanins in various cancers (Tables 3 and 4), according to their role in cancer invasion/metastasis and the angiogenesis process, respectively.

Role as a promoter/suppressor of motility and invasion machinery. Changes in oncogene mutations, growth factor signalling, adhesion receptor profiles, actin cytoskeletal architecture, E-cadherin expression at cell-cell contacts and basement membrane composition are important determinants of tumour invasion and metastasis (Wells, 2006). Many tumours originate from epithelial cells, therefore it is important to look at changes in the development of intrinsic cellular transition (Hugo *et al.*, 2007). Epithelial-mesenchymal transition (EMT) is a cellular transition of morphogenetic and organogenetic processes (Boyer *et al.*, 2000), which results in induction of increased cell motility and dissociation from intercellular complexes in transformed cells. These changes include cell-cell dissociation, actin cytoskeleton reorganization and cell-substratum interactions (Savagner, 2001). The transformation was first recognised during gastrulation at an embryonic developmental stage where epithelial cells transform into embryonic mesoderm. However, this process also occurs during organogenesis and somitogenesis, somite formation in the embryo that differentiate into skeletal muscle, vertebrae and dermis of all vertebrates, and is involved in pathophysiological conditions such as wound healing, kidney fibrosis and cataract formation (Lee *et al.*, 2006; Chaffer *et al.*, 2007). Epithelial and mesenchymal cells are different in their appearance, composition adhesiveness and mechanism of migration (Lee *et al.*, 2006). These differences allow detection of the occurrence of EMT and mesenchymal-epithelial transitions within the cells. In cancer, such

Table 5

Prognostic value of CD151 in various cancers

Cancer type	Associated proteins	Clinical correlation	Reference
Breast cancer	Integrins α3β1, α6β1 and α6β4	Increased expression correlates with lower survival	(Sadej *et al.*, 2010)
	Not determined	Increased expression correlates with lower survival	(Kwon *et al.*, 2012)
Colon cancer	Not determined	Increased expression correlates with metastasis and lower survival	(Hashida *et al.*, 2003)
Clear cell renal cell carcinoma	Not determined	Increased expression correlates with metastasis and lower survival	(Yoo *et al.*, 2011)
Endometrial carcinoma	Expression correlates with E-cadherin expression	Increased expression correlates with aggressive forms and lower survival	(Voss *et al.*, 2011)
Gastric cancer	Integrins α3	Increased expression correlates with lower survival	(Yang *et al.*, 2013)
Gingival squamous cell carcinoma	Not determined	Increased expression correlates with lower survival	(Hirano *et al.*, 2009)
Hepatocellular carcinoma	Expression correlates with proto-oncogene c-Met expression	Increased expression correlates with metastasis, lower survival	(Ke *et al.*, 2009)
	Not determined	Increased expression concomitant with MMP9 and MVD correlates with lower survival	(Shi *et al.*, 2010)
	Integrin β1	Increased expression correlates with lower survival and high recurrence rate and expression of CD151/ integrin β1 complex greatly indicated poor prognosis	(Devbhandari *et al.*, 2011)
Intrahepatic cholangiocarcinoma	Expression correlates with proto-oncogene c-Met expression	Increased expression correlates with metastasis and lower survival	(Huang *et al.*, 2010)
Non-small cell lung cancer	Not determined	Increased expression correlates with lower survival	(Tokuhara *et al.*, 2001)
Oesophageal squamous cell carcinoma	Not determined	Increased expression correlates with metastasis and lower survival	(Suzuki *et al.*, 2011)
Pancreatic ductal adenocarcinoma	Expression correlates with proto-oncogene c-Met and integrins α3/α6 expression	Increased expression correlates with metastasis and lower survival	(Zhu *et al.*, 2011)
Prostate cancer	Not determined	Increased expression correlates with metastasis and lower survival	(Ang *et al.*, 2004)

transformation is recognized due to its similar changes in protein/gene expression as that observed during embryonic morphogenesis, although the process is typically not complete and often forms a metastable phenotype. However, in cancers this concept is still controversial due to limited knowledge of modulatory mechanisms (Lawrence *et al.*, 2007) and difficulties in studying the process in the clinical setting. Delocalization of the adhesion molecule cadherin leads to a detachment of cells from cell-cell contacts and these cadherins are relocalized with the help of integrins (Borghi, 2010). Integrins play important parts in cellular interactions with the ECM and signalling pathways, supporting the promotion of tumour cell adhesion profiles and tumour cell motility (Jin and Varner, 2004). This in turn promotes tumour dissemination and metastasis.

Cell motility is one of the critical steps for cancer invasion and metastasis (Wells, 2006). Cell motility is a complex coordinated process in which cells acquire a motile phenotype involving changes in their protein expression profiles; these involve changes in oncogene mutations, growth factor signalling (including EGF and TGFα), adhesion proteins (e.g. integrins and E-cadherins), proteinases (e.g. MMPs and uPA), actin cytoskeletal proteins and structures (e.g. vimentin, microtubules and actin microfilaments), cell membrane proteins (e.g. tetraspanins) and basement membrane composition, which can lead to loss of cell-cell adhesion and promotion of cell-matrix interaction (Wells, 2006).

Tetraspanins are involved in cytoskeletal dynamics

Targeting actin rearrangement or dynamics is one of the initial approaches to target tumour cell motility and invasion (Fenteany and Zhu, 2003), as investigated in ovarian cancer using actin-targeting agent cytochalasin D (Bijman *et al.*, 2008) and in prostate cancer looking at ZNF185, an actin-associated protein (Zhang *et al.*, 2007), and SWAP70, a F-actin binding protein (Chiyomaru *et al.*, 2011). However, severe cytotoxicity remains an issue and because actins exist in many isoforms, careful selection of an actin population or design of drugs that target actin will need to be elucidated (Fojo, 2006; Stehn *et al.*, 2006; Terracciano *et al.*, 2008; Blain *et al.*, 2010). This has placed emphasis on actin rearrangement as a modulator of tumour cell motility. Tetraspanins are modulators of pathways that control actin remodelling and reorganization,

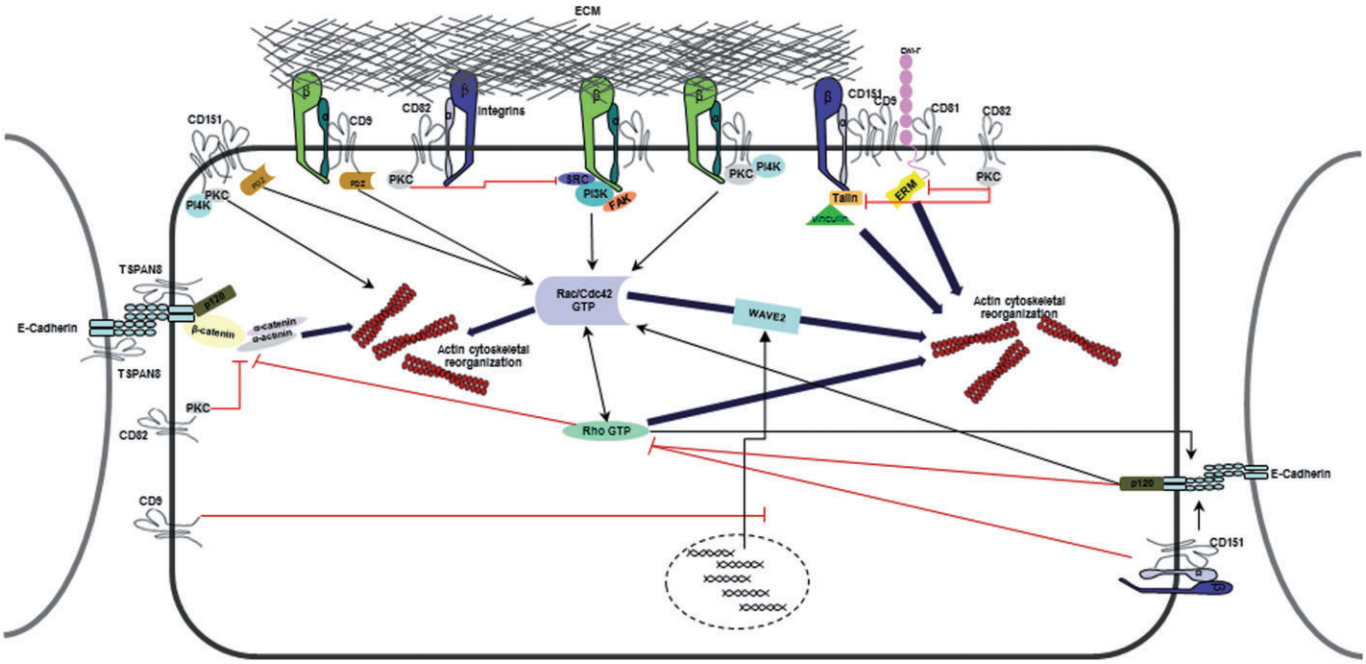

Figure 3

Tetraspanins play many roles in the regulation of the dynamics of cytoskeletal actin and thus in regulation of cell motility. Through binding to integrins, tetraspanins control the cytoskeletal rearrangements. Members of Rho family of GTPases (Rac/Rho/Cdc42 GTPases) mediate many aspects of actin dynamics. They also regulate cell-cell adhesion through cadherin-catenin complexes. E-cadherin-mediated cell-cell adherence involves reorganization of the actin cytoskeleton. p120ctn (represented as p120 in the diagram) associates directly with E-cadherins and plays a role in cell-cell adhesion and it also regulates Rho GTPase activity. The CD151-α3β1 integrin complex also affects the stability of E-cadherin-based junctions and Rho activation. CD151 also mediates activation of Rac/Cdc42 and CD151-PKC association mediates actin reorganization. CD151, CD9 and CD81 contain PDZ-domain-binding motifs and binding to PDZ-domain-containing proteins connects tetraspanins to the cytoskeleton. CD9 strengthens integrin adhesion to the ECM. CD9 also inhibits WAVE2 transcription affecting Rac-WAVE2-Arp2/3 complex associated activation of actin reorganization. There is evidence that TSPAN8 binds directly to E-cadherins. CD82 can regulate β-catenin/α-catenin via PKC. PKC also controls integrin-binding Talin and the EWI-F-binding protein ERM. PKC has been shown to inhibit, leading to inhibition of actin polymerisation. EWI-F, members of the Ig superfamily of proteins; FAK, focal adhesion kinase; PDZ, common structural domain of 80–90 amino acids; SRC, proto-oncogene encoding a tyrosine kinase.

which is an early step in cell migration machinery. These are demonstrated in Figure 3 showing the involvement of tetraspanins in regulation of the dynamics of cytoskeletal actin.

Role in angiogenesis. Improved understanding of new blood vessel formation from existing vessels has changed the paradigm for cancer therapeutics with anti-angiogenesis drugs being used to complement traditional chemotherapy (Jones and Fujiyama, 1999). After carcinogenic transformation and growth, basement membrane degradation, invasion of the surrounding stroma and migration of endothelial cells in response to angiogenic stimuli, angiogenesis is a necessary step in order for a tumour mass to grow bigger than 1 mm (Fidler, 2002). Pathological angiogenesis is thought to be less tightly regulated than neovascularization occurring during development and wound healing (Stupack and Cheresh, 2004). In an adult, normal blood vessels remain dormant and the endothelial cells forming the lining of blood vessels enter a cell cycle at a rate of 1 in 10^3 cells. However, under pathological conditions, levels of pro-angiogenic factors are highly up-regulated allowing new vessel outgrowth (Stupack and Cheresh, 2004). There are many anti-angiogenic agents that are now available for cancer treatments including bevacizumab (for glioblastoma, renal cell carcinoma, non-squamous non-small cell lung cancer and colorectal cancer) (FDA, 2004), everolimus (for advanced renal cell carcinoma, pancreatic neuroendocrine tumours, subependymal giant cell astrocytoma) (FDA, 2009b), imatinib mesylate (for chronic myelogenous leukaemia, acute lymphoblastic leukaemia, dermatofibrosarcoma protuberans and metastatic gastrointestinal stromal cancer) (FDA, 2003), pazopanib (for advanced renal cell carcinoma) (FDA, 2009a), sunitinib mesylate (for advanced renal cell carcinoma, gastrointestinal stromal cancer and pancreatic neuroendocrine tumours) (FDA, 2006) and sorafenib (for hepatocellular carcinoma and advanced renal cell carcinoma) (FDA, 2005). These drugs target VEGF, VEGF receptors or PDGF receptors, which are known biomarkers of the angiogenic process.

Tumour-induced angiogenesis requires interaction and communication between endothelial cells, tumour cells and ECM (Jones and Fujiyama, 1999). Like tumour cells, endothelial cells express various tetraspanins including CD9, CD63, CD81, CD82, CD151, Tspan4 and Tspan8 and these tetraspanins have been shown to play a role in angiogenesis, leucocyte recognition, tumour-endothelial binding and vascular development (Bailey *et al.*, 2011). These tetraspanins could potentially be a direct target in controlling the communication between endothelial cell, tumour cells and ECM. Longo *et al.* demonstrated involvement of CD9, CD151 and CD81 in angiogenesis (Longo *et al.*, 2001) showing that CD9 facilitated tumour-endothelial transcellular migration. CD9 also regulated angiogenic activity, activated either by VEGF or hepatocyte growth factor (HGF) in endothelial cell migration and invasion assays *in vitro* and *in vivo* rat cornea micropocket angiogenesis assays (Kamisasanuki *et al.*, 2011).

A study using CD151-null mice has indicated a role for CD151 in tumour angiogenesis (Takeda *et al.*, 2007) and *in vitro* studies using endothelial cells have shown that CD151-integrin complexes have a role in endothelial cell proliferation, morphogenesis and migration, all of which are important in the angiogenesis process (Yáñez-Mó *et al.*, 1998; Sincock *et al.*, 1999; Zhang *et al.*, 2002). Angiogenesis has been considered as a prognostic indicator in prostate cancer (Borre *et al.*, 1998; Mehta *et al.*, 2001; Bono *et al.*, 2002) and correlates with the aggressiveness of the disease (Weidner *et al.*, 1993; Ferrer *et al.*, 1998). In animal models of myocardial ischaemia, introduction of CD151 via gene delivery improved capillary densities (Lan *et al.*, 2005; Zuo *et al.*, 2009a; Wei *et al.*, 2011). Studies using CD151-null mice found that these mice were viable and no phenotypic change was reported. However, pathological angiogenesis in these mice was greatly affected, supporting a pro-angiogenic role for CD151 specifically in pathological conditions (Wright *et al.*, 2004a; Takeda *et al.*, 2007). The role of CD151 in angiogenesis is perhaps through assisting communication between tumour cells and endothelial cells (Sadej *et al.*, 2009). Many studies found that CD151-integrin (especially α3β1 and α6β4) complexes are localized at the tumour cell and endothelial cell contact area (Yáñez-Mó *et al.*, 1998; Longo *et al.*, 2001; Sadej *et al.*, 2009). CD151 is expressed in vascular endothelial cells and associates with integrin β1, β3, β4, α2, α3, α5 and α6 chains (Sincock *et al.*, 1999). As discussed earlier, integrin expression is an important determinant to balance signal transduction pathways that occur within the cell. Expression of CD151 is required for integrin distribution within the endothelial intercellular contacts, which promotes angiogenesis (Takeda *et al.*, 2007). While cross-talk between tumour cells and endothelial cells assisting in transendothelial migration of tumour cells is important in angiogenesis, CD151 has been found to be a membrane linker through which other signalling proteins stimulate the important regulator of endothelial cell function eNOS (Zheng and Liu, 2007b).

An important pathway that has been intensively studied and implicated in clinical trials for inhibition of angiogenesis and motility is extracellular-signal-regulated kinases 1/2 (ERK1/2), one of the MAPKs. Integrins can directly regulate ERK 1/2 stimulation or via integrin-mediated-growth-factor activation, especially αvβ3 integrin mediating VEGF and basic fibroblast growth factor signalling (Hood and Cheresh, 2002; Stupack and Cheresh, 2004). Peptide inhibitors, such as cilengitide, and humanized monoclonal antibodies against integrins αvβ3 and α5β1 have been tested in human clinical trials for various cancers (Hood and Cheresh, 2002; Stupack and Cheresh, 2004). The role of tetraspanins in angiogenesis has been investigated in various endothelial cells and tumour models, which are summarized in Table 4.

CD151 has a major role in cancer metastasis

The involvement of CD151 in the progression of cancer metastasis is well established. Besides its role in cancer invasion and metastasis, CD151 has also been associated with other important physiological and pathological conditions; primary glomerular disease (Baleato *et al.*, 2008), hereditary nephrotic syndrome (Karamatic Crew *et al.*, 2004) and wound healing (Penas *et al.*, 2000). Loss of CD151 in mice results in defects in renal function but mice were viable and fertile, similar to humans with nonsense mutations in CD151 who develop end-stage hereditary nephropathy associated with pretibial epidermolysis bullosa and sensorineural deafness (Karamatic Crew *et al.*, 2004; Sachs *et al.*, 2006). Increasing interest in tetraspanins has marked their importance in the

area of cancer biomarker research. It is increasingly appreciated that tetraspanins are potential candidates for therapeutic targeting to develop immunotherapies, biological agents, small molecule drugs and aptamers, which will be discussed in the following sections.

CD151 is a prognostic indicator in many cancers. So far, studies of solid tumours in humans suggest that CD151 expression increases as the disease progresses, with metastatic stages having the highest CD151 levels compared to primary tumours; this is true for non-small cell lung cancer (Tokuhara *et al.*, 2001), colon cancer (Hashida *et al.*, 2003), Merkel cell carcinoma (Woegerbauer *et al.*, 2010), hepatocellular carcinoma (Ke *et al.*, 2009) and prostate cancer (Ang *et al.*, 2004) (see Table 5). The majority of studies have found a correlation between CD151 expression and cancer invasion and have thus contributed to the understanding that CD151 is a promotor of cell motility and tumour invasion processes (Sugiura and Berditchevski, 1999; Gesierich *et al.*, 2005). Interestingly, there have been some contradictory findings that challenge this conclusion. A study in breast carcinoma found that decreased expression of CD151, CD9 and CD63 tetraspanins was associated with a more malignant phenotype (Sauer *et al.*, 2003a). Another finding by Lin *et al.*, also found an inverse relationship between CD151 expression and metastatic progression in colorectal cancer patients (Lin *et al.*, 2011). However, in these studies, the levels of CD151 expression were not indicative of the disease outcomes.

Clinical application and potential use of tetraspanins in cancer drug development

Tetraspanins are potential targets for drug development in the area of infectious disease given that many tetraspanins are known to facilitate infection processes of various pathogens, for example, viral, bacterial and protozoan infections (Hassuna *et al.*, 2009; Green *et al.*, 2011). Hassuna *et al.* summarized how pathogens exploit tetraspanins and ways in which tetraspanins could be used to prevent infections via disruption of the tetraspanin web with antibodies to tetraspanin members, knock-down of tetraspanin expression via siRNA and use of tetraspanin to traffic target drugs (Hassuna *et al.*, 2009). It may be possible to apply the same strategies for the treatments of cancer, which is regarded as a chronic disease. The variety of technologies that can be used to molecularly target cancer cells, including immunotherapy, aptamers and RNAi (Imai and Takaoka, 2006; Chen *et al.*, 2010b; Kohmo *et al.*, 2010; Seigneuric *et al.*, 2011), in combination with novel delivery systems, provides exciting possibilities for targeting specific tetraspanins in a highly selective manner (Table 6).

Current interest in cancer immunotherapy is growing, as evidence for cancer immunosurveillance is becoming stronger. Cancer immunosurveillance suggests that the immune system can detect and destroy precursors of cancer cells to stop progression to cancer (Zitvogel *et al.*, 2006). Moreover, immunodeficient individuals are more susceptible to cancer incidence and development (Veenbergen and van Spriel, 2011). Immunotherapy involves biological treatments that stimulates or restores the patients' immune system in order to fight disease or infection (Shih *et al.*, 2010; von Hofe, 2011). A number of novel drugs also offer more specific treatments targeting either cancer cell surface proteins or introduce irradiated tumour cells which may lead to activation of the host immune system. In the treatment of cancer, successful responses to immunotherapy require overcoming various factors including the tumour architecture, reduced antigen presentation, the immunosuppressive tumour microenvironment, resistance to cytotoxic T lymphocyte killing and active suppression of the immune system (Davis and Cebon, 2011). Hege *et al.* have summarized clinical trials that combined immunotherapy with other treatments to enhance the effectiveness of anti-tumour activity and this includes the use of CD40 and Toll-like receptors for DC activation, anti-CTLA4 and anti-CD25 antibodies to inhibit down-modulation of T-cell responses, VEGF blockade to prevent the inhibitory effects of the VEGF receptor and IFN-α for promotion of immunomodulatory responses (Hege *et al.*, 2006). Furthermore, chemotherapy (e.g. docetaxel, a cytotoxic anti-microtubule agent) and anti-androgen therapy has been combined with cancer vaccines in clinical trials of prostate cancer to test whether combination of traditional therapy with immunotherapy can enhance anti-tumour responses (Arlen *et al.*, 2005; 2006; Madan *et al.*, 2008). The results were encouraging and indicated beneficial outcome for patients receiving vaccine prior to conventional therapy. The administration routes for cancer immunotherapy can be via cancer vaccines using the patient's own cancer cells, allogeneic cancer cell lines or nucleic acid-based vaccines enabling expression of cancer-specific antigens or coupled with antibody-based immunotherapy. Recently, the US. Food and Drug Administration (FDA) approved Provenge® (sipuleucel-T), autologous CD54+ cells activated with PAP-GM-CSF [a stimulant made up of a unique prostate cancer antigen, prostate acid phosphatase (PAP) and an immune cell activator GM-CSF], in the treatment of asymptomatic or minimally symptomatic metastatic castrate resistant prostate cancer (FDA, 2010). This novel cancer vaccine comprises autologous cellular immunotherapy which involves manufacturing of the patient's own personalized cancer vaccine and it is the first therapeutic cancer vaccine to be approved by the FDA (Higano *et al.*, 2010). Development of biological agents, including mAbs are still advancing in the area of cancer therapeutics. Twelve mAbs have been approved in the treatment of cancers including non-Hodgkin's lymphoma, metastatic breast cancer, chronic lymphocytic leukaemia, acute myeloid leukaemia and metastatic colorectal cancer (Shih *et al.*, 2010).

Tetraspanins have various roles in immune responses and play different roles in infectious diseases facilitating microbial recognition, entry and stimulation of immune responses (van Spriel and Figdor, 2010; Veenbergen and van Spriel, 2011). CD81 is a co-receptor of the important human pathogen, hepatitis C virus (Pileri *et al.*, 1998) and CD9 binds to diphtheria toxin (Cha, 2000). These roles of tetraspanins in activation of the immune system and antigen recognition are favourable indications for their potential use in cancer treatments of cancer biomarkers that is. for uses in therapeutic delivery vehicles such as RNA interference (RNAi), aptamers and exosomes. Potential clinical applications of tetraspanins and drug development strategies are summarized in Table 6.

Table 6

Potential clinical application of tetraspanins for the development of cancer therapeutic agents

Therapeutic approach	Tetraspanin application	Potential use in cancer therapeutics and mechanism of action
Prognostic markers	Markers of prognosis and clinical outcome	• Expression is indicative of clinical course and outcome • Treatment decision may be based (in part) on tetraspanin expression
Immunotherapy	**Vaccine target** Potential candidates as cancer vaccine target markers	**Vaccine target** • The presence of markers that are tissue-specific and cancer type-specific would be potential candidates for cancer vaccines • The mechanism of action of this immunotherapy involves dendritic cells (DC) (adaptive) immune responses to tumour cell antigens, thus promoting differentiation of bone marrow-derived progenitors into DCs at the local injection site (Hege *et al.*, 2006). • Tetraspanins were used as vaccine target in the treatment of hepatic alveolar echinococcosis, a rare and life-threatening parasitic disease and was found that antibodies raised by a number of tetraspanins could successfully inhibit formation of cyst lesion in mice, which they speculated to be via inhibition of tetraspanins's role in *Echinococcus multilocularis* host immune survival and host-parasite interaction (Dang *et al.*, 2009). This finding strongly supports the potential use of tetraspanins as vaccine targets in the protection against formation of secondary tumours.
	Antibody-based immunotherapy Antibody against tetraspanins	**Antibody-based immunotherapy** • A study of radioimmunology using three different tumour antigens in ovarian cancer tried to overcome the issue of tumour antigen expression heterogeneity. TAG-72, MUC1, and CA125 antigens, which are overexpressed in ovarian cancers, in triple labelling were able to label tissue samples with high sensitivities and detected 98% of ovarian cancer samples compared with individual staining which indicated limited efficacy of single labelling antigen in antibody-guided therapy (Chauhan *et al.*, 2007). • The use of CD151 mAb has been studied to examine CD151 functions and found to inhibit cell invasion and metastasis in different pathological settings (Yáñez-Mó *et al.*, 1998; Berditchevski and Odintsova, 1999; Testa *et al.*, 1999; Zijlstra *et al.*, 2008; Haeuw *et al.*, 2011).
Exosome	Markers of exosomes	• Exosomes are small membrane vesicles, 40–100 nm vesicles exocytosed from the fusion of plasma membrane and multivesicular bodies (a cellular component, intraluminal membrane-bound vesicles), from different cell types (Denzer *et al.*, 2000; Simpson *et al.*, 2009). Functions include disposal of unwanted proteins, T-cell stimulation, transferring of antigen between cells i.e. tumour cells to dendritic cells and antigen-independent immunosuppression (Thery *et al.*, 2002). Exosomes secreted from tumours may assist in cell-cell communication (Denzer *et al.*, 2000; Camussi *et al.*, 2010) and tumour cell escape from the immune detection via exosome-assisted immune suppression (Taylor and Gercel-Taylor, 2011) • According to curated web-based exosome database, Exocarta (http://www.exocarta.org), CD9 and CD81 tetraspanins are listed as exosomal membrane molecule markers detected from exosomes secreted from various cancer cells; bladder cancer, melanoma, colorectal cancer, ovarian cancer and pancreatic adenocarcinoma (Mathivanan *et al.*, 2011). Tumour-rejection antigens that are present on tumour exosomes are pattern of antigens that are important in the initiation of immune reaction against tumours (Andre *et al.*, 2002) • Exosome represents a natural cell-free tumour immunization that derives from either dendritic or tumour cells (Hao *et al.*, 2007). Exosomes isolated from human B-lymphocytes and dendritic cells are enriched in tetraspanins CD9, CD37, CD53, CD63, CD81 and CD82 (Escola, 1998; Thery *et al.*, 2001; Lamparski *et al.*, 2002). • Exosomes extracted from BDX-derived pancreatic adenocarcinoma cells expressed TSPAN-8 stimulated endothelial cell activation and angiogenesis (Nazarenko *et al.*, 2010).

Table 6

Continued

Therapeutic approach	Tetraspanin application	Potential use in cancer therapeutics and mechanism of action
Aptamers	Potential use of tetraspanins in nucleic acid based aptamers for cancer cell recognition, which will offer better targeted treatment in a cell-type specific manner. Potential use as a cancer therapeutic target in inhibitory aptamer in the prevention of cancer metastasis.	• Aptamers are synthetic, modified oligonucleotides that are isolated from natural nucleic acids via Systematic Evolution of Ligands by EXponential enrichment (SELEX) process, a process involving selection and amplification of binding sequences from combinatorial library of random oligonucleotides (Tuerk and Gold, 1990; Hicke and Stephens, 2000). • Clinical and medical applications of aptamers in cancer therapeutics includes drug delivery tools (Farokhzad *et al.*, 2004; Soontornworajit *et al.*, 2011; Tan *et al.*, 2011; Zhang *et al.*, 2011b), targeting of disease-associated targeted sequence (Esposito *et al.*, 2011; Rockey *et al.*, 2011), cancer imaging (Hong *et al.*, 2011; Santra and Malhotra, 2011; MK Yu *et al.*, 2011), cancer cell recognition and detection (Li *et al.*, 2011). • The Aptamer targeted cancer cell biomarkers and was used as a drug carrier to the target cancer cells. A recent study using nanoparticles-conjugated aptamer targeting MUC1, a transmembrane glycoprotein often overexpressed in many cancers, found increase in an uptake of cytotoxic drug, Paciltaxel-loaded nanoparticles in MUC1-positive tumour cells and enhanced cytotoxicity of these cancer cells *in vitro* (CC Yu *et al.*, 2011). • In oncology therapeutics, several cancer biomarkers are subjected to the development of aptamers including VEGF, PDGF and PSMA, in which their nucleic acid sequences have now been registered and protected by US patents (Majumder *et al.*, 2009). Tetraspanins may also be used as biomarkers; however, this possibility has not yet been explored.
RNAi therapeutics	Targets of RNAi therapy	• RNAi and antibody treatment to CD9 sensitized chemoresistant non-small cell lung cancers cells to chemotherapy (Kohmo *et al.,* 2010) • siRNA targeting CD81 has been investigated in rheumatoid arthritis (Nakagawa *et al.*, 2009) • *In vitro* RNAi targeting TSPAN1 inhibits cell proliferation and invasion in colon cancer (Chen *et al.*, 2010b)

Potential therapeutic benefits and limitations

Given the clinical heterogeneity of cancers, it is currently difficult to establish uniform and optimal diagnostic screening and treatment regimens for individual patients (Fidler, 2002). With the limitations of current approaches, researchers are searching for biomarkers that can more accurately indicate the prognosis of individual cases and thus lead to better personalized treatment options. This aim is very challenging and it is unlikely that any single biomarker will, by itself, be adequate for treatment decisions (Shariat *et al.*, 2007).

We have recently reviewed new drugs targeting different aspects of prostate cancer development and potential biomarkers that have made it to clinical trials (Detchokul and Frauman, 2011). These drugs were administered in conjunction with traditional systemic therapies and targeted biomarkers were categorized into five therapeutic approaches; prostate cancer vaccines, epigenetic therapies, pro-apoptotic agents, prostate cancer antibodies and anti-angiogenesis approaches. These approaches have not exhibited therapeutic benefits over the mainstream cytotoxic treatments in prostate cancer due mainly to the participants recruited having developed castration-resistant prostate cancer. The beneficial outcome of these trials may become more obvious with patients with earlier stages of prostate cancer. The review emphasized the importance of biomarker targets and their potential use in the treatment of prostate cancer. A number of novel drugs also offer more specific treatment targeting either prostate cancer cell surface proteins or introduction of irradiated tumour cells which may lead to activation of the host immune system. The obvious benefits of targeted therapeutics would be the potential for minimization of adverse effects, hence better quality of life for patients. Although none of the drugs reviewed specifically targeted migration, it is undeniable that cell motility is at the heart of cancer invasion and dissemination, the most common cause of cancer morbidity and mortality. Notably, the successful story of cancer biomarkers is Her2/Neu in breast cancer. Trastuzumab, mAb to Her2/Neu, has been used in the treatment of Her2+ breast cancer patients in addition to first-line therapy and was found to prolong disease progression (Slamon *et al.*, 2001; Baselga *et al.*, 2012). This has encouraged more emphasis on individualization in cancer therapy.

Targeting cell migration in modifying or preventing metastasis is still in its infancy. Comprehensive reviews of tumour cell motility targets in metastasis treatment have been published recently (Palmer *et al.*, 2011; Thiolloy and Rinker-Schaeffer, 2011). They have drawn attention to the increasing interest in cell migration research in cancer metas-

tasis in the past two decades. Tetraspanins are known for their roles in tumour cell motility and invasion but their potential influence on tumour progression is still not well understood. Because cancer research has shifted from focusing mainly on the tumour itself to include the tumour's interaction with its microenvironment, cancer biomarkers not only are an indication of tumour origins but also of disease progression. Tetraspanins, having a role in two characteristics of cancer invasion/metastasis and angiogenesis, are possible biomarkers of tumour progression and therapeutic targets which may allow for more personalised therapy in the future.

Conclusions and future directions

The importance of tetraspanins in different aspects of cancer metastasis is integrated in this review. In particular, CD151 is a marker of disease progression clinically and encompasses diverse regulatory roles in the metastatic process. Inhibition of *in vitro* and *in vivo* motility and metastasis in various experimental settings highlight the major role of CD151 in the control of signalling complexes that drive cancer metastasis. CD151 also influences the localization and organization of its partner proteins, which are important determinants of metastatic behaviour in many cancers. The clinical applications of tetraspanin modulation are not limited to direct targeting via RNAi or antibodies, but will also be relevant to new classes of therapies, for example, biological agents, aptamers and exosomes. Tetraspanins are certainly a potential therapeutic target, which as cell surface molecules, can be targeted as a preventative and/or palliative strategy in cancer treatments.

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Conflict of interest

We certify that there is no conflict of interest with any financial organizations regarding the materials discussed in the manuscript.

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