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Tetraspanins and malignancy

Claude Boucheix, Guy Huynh Thien Duc, Claude Jasmin and Eric Rubinstein

Tetraspanins, also called tetraspans or the transmembrane 4 superfamily (TM4SF), are cell-surface proteins that span the membrane four times and are found on many different cell types in many organisms. They display numerous properties that indicate their physiological importance in cell adhesion, motility, activation and proliferation, as well as their contribution to pathological conditions such as metastasis or viral infection. A major characteristic of tetraspanins is their ability to form cell-surface complexes with other molecules participating in cell adhesion, either to the extracellular matrix (ECM) or to other cells, and with molecules required for signalling. It is not yet known how the structure of the complexes might affect the functions of other molecules or what basic biochemical mechanisms allow their formation and regulation. Nevertheless, an intriguing association between tetraspanin expression and metastatic potential indicates that these molecules may provide novel insights into tumour progression.

The existence of a superfamily of molecules that was later called tetraspans, tetraspanins or the transmembrane 4 superfamily (TM4SF) became evident in the early 1990s after gene cloning of several cell-surface molecules identified up to a decade earlier using monoclonal antibodies (mAbs). The tetraspanins are integral membrane proteins characterised by the presence of four hydrophobic (transmembrane) domains delimiting two extracellular regions of unequal sizes (Fig. 1). An important sequence homology with conserved amino acids distinguishes them from other proteins with four transmembrane domains: all tetraspanins have four, six or eight

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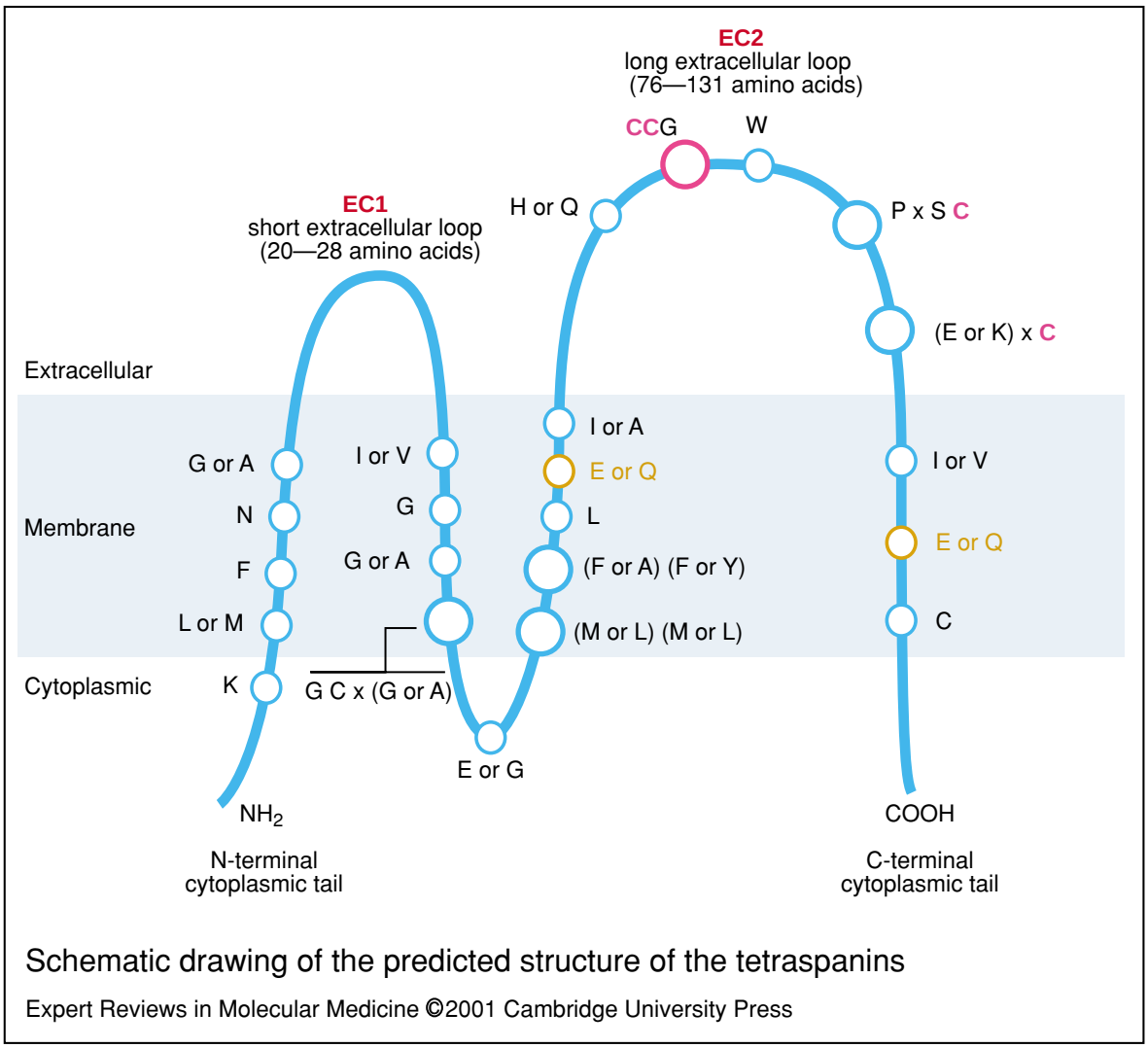

Schematic drawing of the predicted structure of the tetraspanins

Figure 1. Schematic drawing of the predicted structure of the tetraspanins. The structure proposed here is based on primary amino acid sequence and has yet to be confirmed by crystallographic studies. The conserved amino acids are indicated as circles (blue = conserved amino acids; magenta = conserved cysteine residues of the EC2 domain; orange = charged amino acids found in the third or fourth transmembrane domains). The size of the circles indicates whether there are one (small circle) or more (large circle) amino acids conserved at this position; x indicates a position where all amino acids are potentially accepted. The tetraspanins are composed of 210 (tetraspanin SAS) to 347 (tetraspanin RDS, encoded by the retinal dystrophy syndrome gene) amino acids. The highest level of homology is found within the hydrophobic (transmembrane) domains. The small extracellular loop (EC1) contains 20–28 amino acids, whereas the large extracellular loop (EC2) contains 76–131 amino acids. The cytoplasmic tails contain fewer than 15 amino acids, although the tetraspanins RDS and NET-2 have approximately 60 amino acids in their C-terminal cytoplasmic domains (Refs 7, 9). Most tetraspanins are potentially glycosylated in EC2, except for CD9, which is glycosylated in EC1 (Ref. 97), and CD81 (Ref. 13), which is not glycosylated. Although the tetraspanins are acylated, the cysteine residues involved have not yet been identified **(fig001cbv)**.

cysteine residues with a CCG motif in the large extracellular domain (Refs 1, 2, 3).

The tetraspanin superfamily has now grown to 25 members. Among these are the leukocyte differentiation antigens CD9, CD37, CD53, CD63, CD81/TAPA-1, CD82/Kai1 and CD151/PETA-3. Other tetraspanins include: CO-029 and SAS, which were discovered on nonhaematopoietic tumours (Refs 4, 5); the uroplakins UP1a and UP1b, which are constituents of the asymetric

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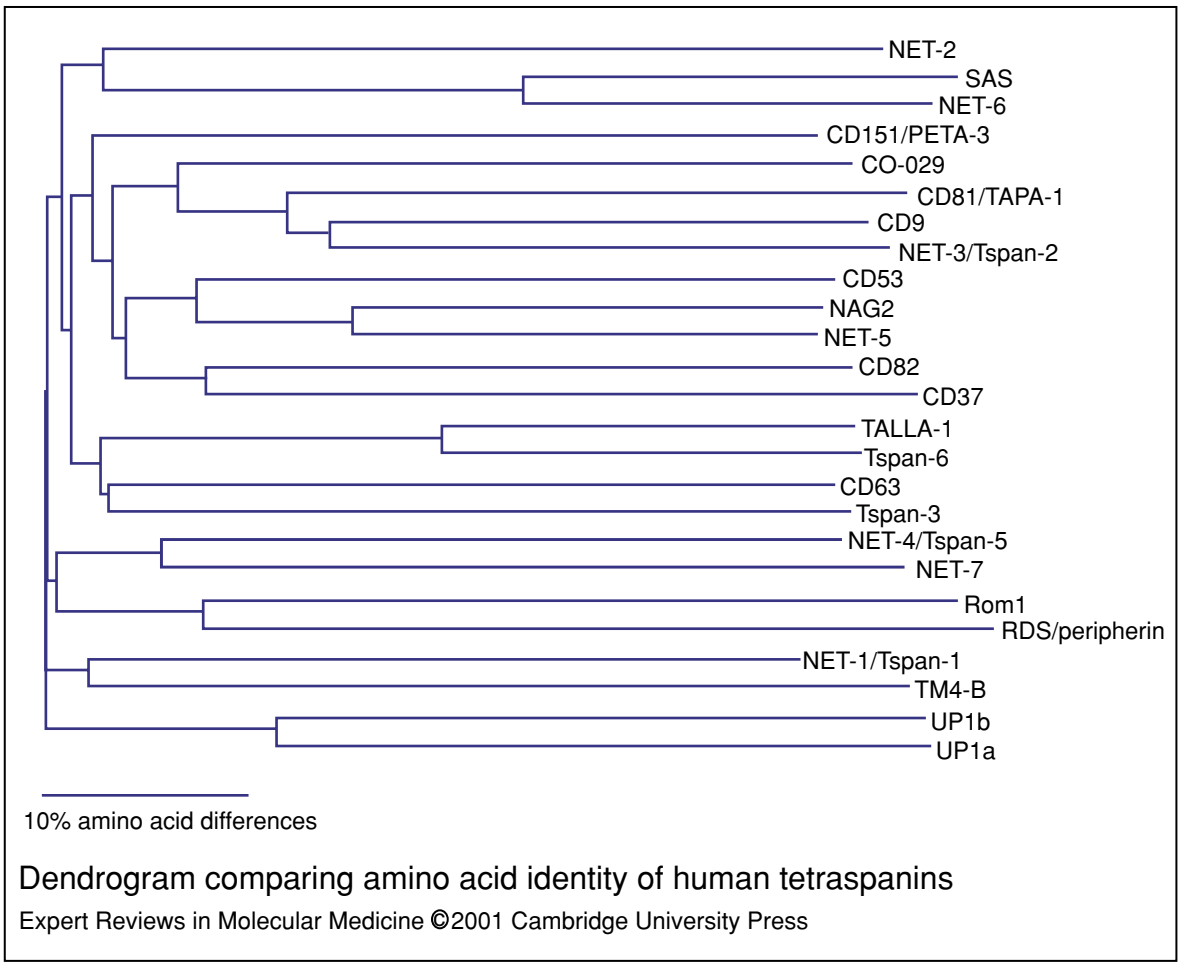

Figure 2. Dendrogram comparing amino acid identity of human tetraspanins. The relative branch length of this dendrogram (or distance tree) indicates the distance (% of amino acid differences) between the different tetraspanins. Comparisons were based on the whole amino acid length of each tetraspanin found by Blast2 search of cDNA sequences in Genbank. Abbreviations: NET, new EST tetraspanins; RDS, retinal dystrophy syndrome; UP, uroplakin **(fig002cbv)**.

unit membranes of the urothelium (Ref. 6); and the proteins encoded by the retinal dystrophy syndrome genes, RDS/peripherin and Rom1, which are found in the photoreceptor outer segment disc (Ref. 7). Analysis of human cDNA databases by several laboratories has also lead to the discovery of ten new members, variously called Tspan-1–6 (Ref. 8), NET-1–7 (for 'new EST tetraspanin'; Ref. 9) and TM4-B (Ref. 10). The molecules L6 and IL-TMP also have four transmembrane domains and were originally considered as tetraspanins, but the discovery and sequence analysis of two closely related molecules (L6D and TM4SF5) suggest that they constitute a separate superfamily (Ref. 11). KRAG/sarcospan, a protein of the dystrophin/dystroglycan complex, has also been suggested to belong to the tetraspanin superfamily on the basis of structure; however, this molecule has a very low level of homology with genuine tetraspanins (Ref. 12). Figure 2 shows a dendrogram of 25 human tetraspanins created from a Blast2 search of cDNA sequences in GenBank, indicating the molecular distance between them. The superfamily of tetraspanins is old in evolutionary terms since the invertebrates *Drosophila*, *Schistosoma* and *Caenorhabditis elegans* also express these molecules. Such conservation of tetraspanin gene structure strengthens the assumption that these molecules derive from a common ancestor.

Certain tetraspanins have a restricted pattern of expression (for example CD53 is highly restricted to leukocytes). Others, such as the leukocyte differentiation antigens CD81 and

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CD82, which were originally described on haematopoietic cells, can be found on most studied cultured cells. All studied mammalian cells express several members of the tetraspanin family, with the exception of red blood cells, which express none (Ref. 3).

Elucidating tetraspanin functional properties

What do tetraspanins do? Three different types of experiments have yielded information on their functional properties. The first approach has been the analysis of the cellular effects of anti-tetraspanin antibodies, the second evaluates the effects of overexpression by transfection and the third relates the phenotype of genetic defects caused either by gene knockout or by human genetic diseases. The molecular mechanims of the pleiotropic cellular effects are not known but may rely, as discussed in a later section, on the existence of a network of molecular interactions orchestrated by tetraspanins.

Anti-tetraspanin antibodies

The targeting of tetraspanins by specific mAbs has yielded a variety of functional effects. For instance, anti-CD81 mAbs inhibit cell proliferation (Ref. 13), anti-CD82 (Ref. 14), -CD81 and -CD53 (Ref. 15) mAbs costimulate lymphoid cells, anti-CD81 and -CD151 mAbs inhibite neurite outgrowth (Ref. 16), anti-CD9 mAbs induce homotypic cellular adhesion (Ref. 17), and a variety of anti-tetraspanin antibodies inhibit or stimulate cell migration (Ref. 10; Table 1). The induction of human platelet activation/aggregation by anti-CD9 mouse IgG1 is initiated by the crosslinking of the platelet FcγRII (Refs 18, 19). However, this mechanism does not seem to be involved in other major effects of tetraspanin antibodies.

Experiments aimed at identifying the surface molecules that control migration of cultured tumour cells yielded the first indication that a tetraspanin, CD9, is involved in cell migration. Among the 3000 hybridoma antibodies produced against the lung adenocarcinoma cell line MAC8, the strongest inhibition of cell motility was found with an antibody that was shown to recognise CD9 (Ref. 20). This was repeatedly confirmed for CD9 and other tetraspanins in various cellular models (Ref. 10). In addition, a correlation between the level of expression of the tetraspanin CD63 in transfected melanoma cells and the inhibition of migration by anti-CD63 mAbs has been reported (Ref. 21). Furthermore, particular experimental conditions may lead to a stimulation of cell migration, as for Schwann cells on axons by anti-CD9 antibodies (Ref. 22), MDA-MB231 breast carcinoma cells by several anti-tetraspanin antibodies (Ref. 23) and endometrial carcinoma cells by anti-CD9 antibodies (Ref. 24).

Overexpression of tetraspanins

The ectopic expression of tetraspanins in cultured cell lines induces apparently contradictory effects on cell migration. Tetraspanin expression seems to reduce migration when no extracellular matrix (ECM) component is added (Refs 6, 21, 25), whereas motility seems to increase in the presence of some β_1 integrin substrates (Refs 10, 17, 21, 26). Cell motility is a complex process that is influenced, on the one hand, by the nature of the ECM and the presence of growth factors, proteinases and other components and, on the other hand, by the pattern of expression and state of activation of cell-surface receptors (Refs 27, 28). These parameters were not controlled in the reported experiments; in addition, as discussed below, ECM substrates such as laminin, fibronectin and matrigel do not necessarily reproduce the composition and structure of ECM found in tissues. Thus, results of in vitro experiments must be interpreted cautiously regarding their in vivo relevance.

Genetic defects in tetraspanins

The consequence of the absence of CD81, CD37 and CD9 has been investigated using gene-knockout mice. CD81 knockout leads to reduced expression of the B-cell antigen CD19 and is associated with decreased calcium mobilisation following CD19 engagement (Refs 2, 29, 30). Two groups have observed a reduction of B-1 lymphocytes in the peritoneum (Refs 29, 30) and a third group reported an apparent reduction in T helper 2 (Th2)-dependent IgG1 production (Ref. 31). Mice deficient in CD37, which is a tetraspanin expressed on mature B cells, exhibited a reduced humoral response to T-cell-dependent antigens, suggesting a role for CD37 in mediating B- and T-cell interactions (Ref. 32). CD9 knockout led to severely reduced female fertility linked to a defect in sperm/egg fusion without other gross abnormalities (Refs 33, 34, 35). In wild-type mice, CD9 is strongly expressed on the surface of oocytes (Ref. 36). It was initially hypothesised that CD9 could play a role in the fusion process

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Table 1. Tetraspanins and cell migration (tab001cbv)

(a) Inhibition of migration by anti-tetraspanin antibodies

Tetraspanin	Cell type	Refs
CD9	Adenocarcinoma	20
CD9, CD81, CD82	Haematopoietic cells	17, 43, 98
CD9	Keratinocytes	99
CD9	Colon carcinoma	85
CD63	Melanoma	21
CD9, CD81, CD151	Endothelial cells	100, 101
CD151	Neutrophils	57
CD151	Carcinoma	26

(b) Stimulation of migration by anti-tetraspanin antibodies

Tetraspanin	Cell type	Substrate	Ref.
CD9	Schwann cells	Living axons	22
CD9, CD53, CD81, CD151	Carcinoma	Matrigel[a]	23
CD9	Endometrial carcinoma	Matrigel	24

(c) Effect of transfection on cell migration/motility

Tetraspanin	Cell type	Substrate/medium	Assay and effect on spontaneous cell motility[b]	Ref.
CD9	Adenocarcinoma and CHO cell line	BSA	Cell penetration and phagokinesis (–)	25
CD9	Raji cells	BSA	Cell penetration (=)	17
		Laminin/Fn	Cell penetration (+)	17
CD63	Melanoma	FCS	Cell penetration (–)	21
		Fn	Cell penetration (+)	21
CD82	Colon carcinoma	BSA	Phagokinesis (–)	102
		Matrigel	Cell penetration (–)	102
CD151	Hela	Matrigel	Cell penetration (+)	26

[a] Matrigel is a solubilised basement membrane matrix extracted from the EHS mouse tumour, which is rich in basement membrane proteins (laminin, collagen I, entactin, heparan sulfate proteoglycan) and also contains growth factors, matrix metalloproteinases and other proteases (Refs 103, 104).
[b] (–) Indicates a reduced motility; (+) indicates an increased motility; (=) indicates no change.
Abbreviations: BSA, bovine serum albumin; CHO, Chinese hamster ovary; FCS, fetal calf serum; Fn, fibronectin.

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by regulating the interaction between the sperm ADAM protein fertilin and the oocyte integrin $\alpha_6\beta_1$ [with which CD9 associates (Ref. 37)]; however, this has been challenged by the recent finding that integrin $\alpha_6\beta_1$ is not required for sperm/egg fusion (Ref. 38). Therefore, another mechanism involving CD9 appears to be required for fusion to occur.

In addition to gene-knockout data, there is evidence that a translocation [t(X;2)] disrupting the tetraspanin gene encoding TALLA-1/TM4SF2 is associated with a case of X-linked mental retardation (Ref. 39), which has prompted the study of other patients with this disease. Studies of two other families have found, respectively, a truncating mutation and a C to A mutation resulting in a non-conservative amino acid substitution (P172H) in a consensus motif present in several tetraspanins – this points to a critical functional site in the TALLA-1/TM4SF2 protein (Ref. 39). In addition, numerous mutations of RDS/peripherin are associated with retinal dystrophies that often result in a dominant phenotype (Ref. 7).

Therefore, knockout and genetic data have already confirmed the physiological importance of four tetraspanins: CD9, CD37, CD81 and TALLA-1. In particular, the CD9-associated fusion defect might prove to be an interesting model for the dissection of molecular interactions controlled by tetraspanins. The loss of function as a consequence of a single amino acid mutation observed with TALLA-1 raises the possibility of a specific and unidentified TALLA-1 partner that could lose its connection to a 'tetraspanin web' (as described in the next section). CD37 and TALLA-1 have a restricted pattern of expression – CD37 is found on mature B lymphoid cells in humans (there are no mAbs available for murine CD37) and TALLA-1 is found within the nervous system. It is therefore not surprising that genetic defects lead to pathological conditions limited to these organs. The situation is different for CD9 and CD81, which have a wide tissue distribution and for which gene knockout induces an abnormal phenotype limited to a single tissue. In such cases, compensation of their loss by substitution with molecules of the same family or by other mechanims have to be considered. Generation of mice that have double or triple tetraspanin gene knockouts might help to resolve this question.

Tetraspanin molecular complexes: 'the tetraspanin web'

One of the properties peculiar to the tetraspanins is their capacity to associate with a significant number of other cell-surface molecules. Among the molecules associated with the known tetraspanins are the lymphoid antigens CD4/CD8 (Ref. 40) and CD19 (Refs 41, 42), the β_1 integrins (Refs 43, 44), the membrane precursor of heparin-binding epidermal growth factor (HB-EGF) (Refs 45, 46), the HLA-DR major histocompatibility complex (MHC) antigens (Ref. 47) and the tetraspanins themselves (Refs 37, 48).

Although the association of the tetraspanins with β_1 integrins was first reported for CD9 and CD63 (Refs 43, 44, 49), this association has subsequently been confirmed for other tetraspanins (Refs 37, 50, 51). Analysis of the complexes after overexpression of the tetraspanin CD9 in the Burkitt cell line Raji showed that CD9 did not compete with the tetraspanin CD81 but was added to the preformed complexes (Ref. 37). This type of interaction suggests that the tetraspanins take part in a network of molecular interactions on the surface of the cells, termed the 'tetraspanin web' (Ref. 37). Other arguments in favour of this assumption include the large size of tetraspanin-containing immunoprecipitated complexes (Ref. 50), the presence of several copies of the same tetraspanin in these complexes (Ref. 37) and the presence of at least two types of tetraspanin-associated molecules (integrins and HLA-DR) in the same complexes (Ref. 37). The fact that the associated tetraspanins and molecules are recognised by antibodies having similar functional effects (Ref. 15) suggests that these complexes occur physiologically on the cell surface.

Analysis of the complexes isolated using different detergents has been extremely informative (Ref. 51; Fig. 3). Whereas large complexes could be isolated using mild detergents such as CHAPS or Brij96/97, cell lysis with digitonin showed a much more restricted pattern of interactions involving one tetraspanin and one or a few specific immunoprecipitated partner molecules (Ref. 52). Under these conditions, the following associations were reported: (1) CD151 with the integrins $\alpha_3\beta_1$ or $\alpha_6\beta_1$ (Ref. 52); (2) CD81 with the integrin $\alpha_4\beta_1$ (Ref. 52) or the B-cell lymphoid antigen CD19 (Ref. 53); (3) CD9 and CD81 with CD9P-1, the 135 kDa product of the KIAA1436 gene (the human orthologue of

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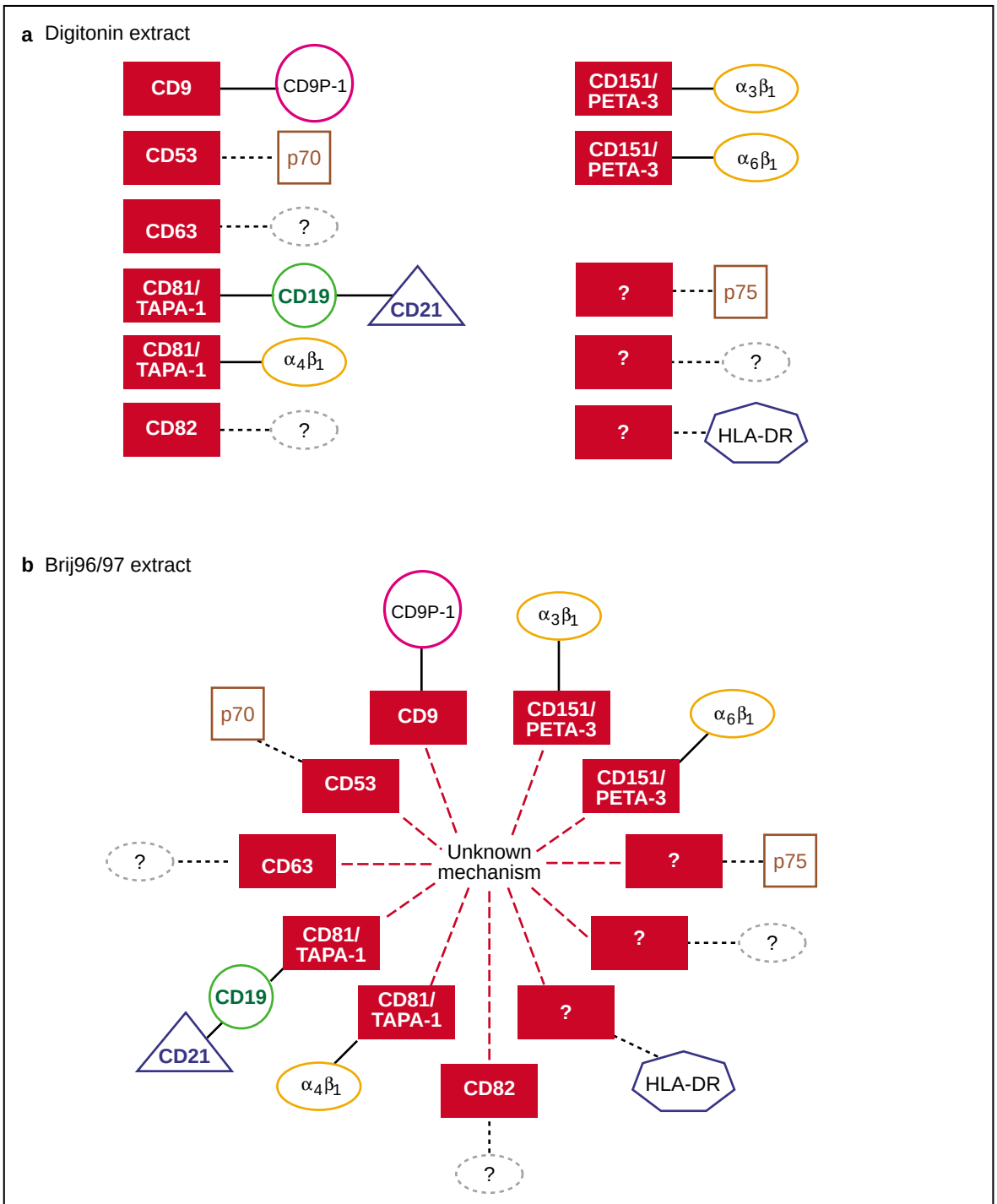

Detergent-dependent co-immunoprecipitation of two different types of tetraspanin complexes

Figure 3. Detergent-dependent co-immunoprecipitation of two different types of tetraspanin complexes *(see next page for legend)* **(fig003cbv).**

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Figure 3. Detergent-dependent co-immunoprecipitation of two different types of tetraspanin complexes. The pattern of co-immunoprecipitation observed after surface labelling with biotin and extraction with the mild detergents (a) digitonin and (b) Brij96/97 suggests that tetraspanins (in red-filled squares) may exist in two different types of molecular complexes (Ref. 52). First-order complexes, indicated by a solid or dotted black line, revealed by digitonin extraction contain only a single tetraspanin and its specific co-immunoprecipitation partner(s) (e.g. CD81 and its partner $\alpha_4\beta_1$ integrin). (b) By contrast, second-order large complexes, co-immunoprecipitated following Brij96/97 extraction, comprise first-order complexes associated through tetraspanin–tetraspanin interactions by an unknown mechanism indicated by red dashed lines. An arbitrary arrangement of the molecules within the complexes is shown in both (a) and (b). Immunoprecipitation partners are grouped as follows: orange ovals = integrins; brown squares = unidentified molecules; red-filled rectangles = tetraspanins; green circles = the B lymphoid antigen CD19; dark blue heptagons = the HLA-DR major histocompatibility complex; pink circle = CD9P-1. The CD21 antigen, which is linked indirectly through CD19 to the tetraspanin complexes, is shown as a blue triangle. In the digitonin extract, question marks indicate that no specific partners have yet been found for the corresponding tetraspanins (CD63 and CD82). In the Brij extract, question marks indicate that no tetraspanin has been found specifically associated with proteins linked to tetraspanin complexes (p75 or HLA-DR). In both (a) and (b), linked question marks (i.e. tetraspanin and partner) indicate that there are new tetraspanins, such as NET/Tspan proteins, that still require characterisation at this protein level **(fig003cbv)**.

prostaglandin F2a receptor regulatory protein (FPRP) (Refs 52, 110, 111); and (4) CD53 with an unidentified molecule p70 (Ref. 52). Because no tetraspanin–tetraspanin complexes were observed in digitonin-soluble fractions, it could be suggested that digitonin only extracts primary complexes (one tetraspanin with one specific partner), whereas larger (secondary) complexes are preserved by milder detergents.

Thus, it is possible to consider a model in which each tetraspanin would link its molecular partner(s) to the other tetraspanins and their own partners, thereby organising the positioning of cell-surface proteins so as to allow signal transduction, cell adhesion or motility. In this context, the tetraspanins would play the role of 'surface organisers' (Ref. 37), adaptors (Ref. 54) or facilitators (Ref. 2). As a first step towards defining this model further, the molecular requirements for the interaction of tetraspanins with their specific partners has been partially studied. This has highlighted the role of the large extracellular region (EC2) in interactions between CD19 and CD81 (Refs 41, 55), CD9 and proHB-EGF (Refs 15, 56), and CD151 and $\alpha_3\beta_1$ (Ref. 57).

Tetraspanins and viruses

There have been several examples of tetraspanins playing a role in the viral life cycle. Anti-tetraspanin antibodies inhibit syncytium formation and/or virus production. This was observed for the tetraspanins CD81 and CD82 with human T-lymphotropic virus 1 (HTLV-1) (Ref. 40), and for the tetraspanin CD9 with the feline immunodeficiency virus (Ref. 58) and the canine distemper virus (Refs 59, 60).

Importantly, the tetraspanin CD81 might play a role in the aetiopathogenesis of hepatitis C virus (HCV), which infects 170 million people worldwide. HCV is responsible for the disease hepatitis C, which can evolve to a hepatocellular cirrhosis and carcinoma, and also for immune diseases related to lymphoid B cells (e.g. cryoglobulinaemia, lymphoproliferative disorder, autoantibody production) (Ref. 61). Recently, it has been shown that HCV particles fix CD81, probably via binding of the viral envelope protein E2 to the tetraspanin EC2 loop (Ref. 62), and in this way could allow the virus entry into the cell. On the basis of sequence comparison between human and monkey CD81, combined with mutagenesis studies, it was shown that certain amino acids are essential for CD81 recognition of E2 (Ref. 63); however, it was also shown that recognition is not predictive of a productive infection (Ref. 64).

Tetraspanins and malignancy

Tetraspanins as differentiation markers in tumours

Some tetraspanins have been viewed as useful markers for the characterisation of tumoural cells. CD9 was initially described on the surface of cells of B-lineage acute lymphoblastic leukaemia (Ref. 65). It is expressed on 90% of B-lineage acute leukaemias, and on 50% of acute myeloid leukaemias and B-lineage chronic lymphoid leukaemias (Ref. 66). In particular, CD9 is a constant marker of acute promyelocytic

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leukaemia, in association with the CD13$^+$/CD33$^+$/HLA-DR$^-$ phenotype (Ref. 67).

The tetraspanin TALLA-1 is expressed in acute neuroblastomas and T-lymphoid leukaemias (Ref. 68). The expression of this tetraspanin is correlated in leukaemic cell lines with that of the Tal1 transcription factor, whose gene is rearranged and expressed in certain translocations observed in T-cell acute leukaemias. The Tal1 transcription factor acts in cooperation with the rhombotin gene products cofactors RBTN1 and RBTN2, and transfection of Tal1 and RBTN1 can induce the expression of tetraspanin TALLA-1 (Ref. 69). The antigen CO-029 was discovered in colorectal carcinomas (Ref. 4), while the antigen L6 is overexpressed in breast, lung, colon and ovary tumours (Ref. 70). Correlations such as these, together with others described in Table 2, indicate a possible role for tetraspanins in tumour growth, as described below.

Table 2. Tetraspanins and cancer: correlations (tab002cbv)

Tetraspanin	Cell type	Refs
(a) Examples of where the level of tetraspanin expression is inversely related to the metastatic potential		
CD9	Melanoma	105
	Breast carcinoma	79, 106
	Lung carcinoma	46
	Colon carcinoma	107
CD63	Melanoma	71
CD82	Prostate carcinoma	72, 73
	Pancreas carcinoma	74, 81
	Lung carcinoma	75
	Colon carcinoma	83
(b) Examples of where the level of tetraspanin expression is a good prognosis factor[a]		
CD9	Breast carcinoma	79, 106
	Lung carcinoma	46
	Colon carcinoma	107
	Pancreas carcinoma	108
CD82	Prostate carcinoma	72, 73
	Lung carcinoma	75
	Colon carcinoma	76
	Hepatoma	74, 82
	Lung non-small-cell carcinoma	109
	Breast carcinoma	84

[a] Simultaneous reduction of CD9 and CD82 has an additive effect on metastatic potential and is a bad prognosis factor (Refs 77, 84).

Tetraspanins, tumour progression and metastasis

CD63, the first tetraspanin to be cloned, is strongly expressed at early stages of melanoma formation and is downregulated at advanced stages (Ref. 71). The role of CD9 in the tumour process was investigated following the initial work on cell motility mentioned above, since motility is considered to be an essential component of the mechanism of metastasis (Ref. 20). Moreover, the search for tumour suppressor genes unexpectedly revealed the suppressor role of the tetraspanin CD82 in prostate cancer metastasis (Ref. 72). CD82 expression is reduced during the tumoural progression of prostate, lung, pancreas and colorectal cancers (Refs 73, 74, 75, 76). Accordingly, it has been shown in various tumours that the level of expression of these three tetraspanins can be linked to the stage of tumour progression and can be used as prognostic factors. Moreover, it was found that the rate of survival of patients with non-small-cell carcinomas of the lung is higher at 5 years when the two antigens CD9 and CD82 are coexpressed (86%, versus 31% when these two antigens are not expressed) (Ref. 77). In addition, a new tetraspanin, C4.8 (identical to Tspan-1/NET-1), has been identified as a possible marker of progression of the cervix tumours induced by papillomavirus (Ref. 78).

The expression of CD9 in breast (Ref. 79) and oesophagus (Ref. 80) carcinomas, and of CD82 in prostate (Ref. 73), pancreas (Refs 74, 81), hepatocellular (Refs 74, 82), oesophagus (Ref. 80) and colon carcinomas (Refs 76, 83), was found to be frequently lower on metastasis compared with the primary tumour. An inverse correlation between the expression of CD9 in the primary

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tumour and the appearance of metastases in melanomas, colon, lung and breast cancers has been reported (Table 2). In lung cancer (Ref. 77) and breast cancer (Ref. 84), the reduction in CD9 expression associated with that of CD82 was correlated with an increased metastatic potential compared with when the expression of only one of these two antigens is reduced. Similarly, the level of CD9 was lower in cell lines derived from metastasis of colon carcinoma as compared with cell lines derived from the primary tumour (Ref. 85).

Experimentally, it was shown that transfection of CD9 or CD63 into melanoma cells induced a reduction of the metastatic potential of these cells (Refs 25, 86). This phenomenon was also observed following the transfection of CD82 into prostate cancer cells (Ref. 72). With regard to the tetraspanin CO-029, induction of its expression was observed in the metastasising subclones of rat cell lines derived from carcinomas of the colon or pancreas (Ref. 87). Furthermore, transfection of CO-029 into low-metastasising cells induces an increase in their metastatic potential (Ref. 87).

Putative relationship between tetraspanin complexes and metastasis

Malignant transformation is associated with changes in cell adhesion and motility. Many cell-surface molecules involved in cell–ECM or cell–cell interactions have been described, including cadherins, selectins, immunoglobulin-like receptors, integrins and proteoglycans. These molecules act in a complex and coordinated way to hold the cells in place or, alternatively, to sustain cell movement. Adhesion molecules function in bidirectional signalling pathways required for many cellular functions, such as transcription, cytoskeletal organisation and proliferation. Among the adhesion molecules, integrins are major ECM receptors and are also involved in cell–cell interactions. There have been many reports showing the critical role of integrins in tumour development, invasion or metastasis (for reviews see Refs 27, 88, 89), and it is possible that this is somehow linked to tetraspanins.

Anti-tetraspanin antibodies have major effects on cell migration (as listed above) and tetraspanins are known to form large complexes that include integrins. It is tempting to speculate that a link exists between tetraspanin expression and metastasis potential, involving effects on cell motility and molecular associations with integrins. A direct or indirect effect of tetraspanins on integrin function might lead to alterations of adhesion or migration properties of the malignant cells that could modify their metastatic potential. The possible influence of tetraspanins on integrin function has been suggested by the observation that ligation of anti-tetraspanin antibodies can affect tyrosine phosphorylation of focal adhesion kinase (FAK), either positively or negatively depending on the experimental conditions (Ref. 54). Another interesting aspect is the topology of tetraspanins during cell motion: they tend to be localised at the leading edge of spreading cells, like integrins, and also in intracellular vesicles. These data suggest that tetraspanins might interfere with integrin movements in cell motility. This hypothesis is supported by the association of tetraspanin–integrin complexes with phosphatidylinositol 4-kinase (PI 4-kinase) (Ref. 90), which is involved in the production of phosphatidylinositol-4,5-bisphosphate (4,5-PIP2), a regulator of cytoskeletal architecture. Finally, anti-tetraspanin antibodies, such as anti-α_3 integrin antibodies, induce PI 3-kinase-dependent production of matrix metalloproteinase 2 (MMP-2) in MDA-MB231 breast carcinoma cells (Ref. 23); this indicates that tetraspanins are involved in the control of a matrix proteinase that allows malignant cells to invade adjacent tissues by destruction of the ECM.

However, the mechanisms underlying the effects of anti-tetraspanin antibodies on cell migration are unknown and it has not been demonstrated clearly that integrin functions can be directly modulated by tetraspanins, despite the experimental data on cell migration described above suggesting that this could be the case. It should also be noted that the choice of substrates for in vitro experiments might not have been appropriate since, in vivo, the composition of the ECM can vary considerably in a tumoural environment (Refs 91, 92). For instance, the appearance of new isoforms of fibronectin or laminin, particularly embryonic isoforms, has been reported and no tetraspanin studies with these substrates have been performed. The importance of the substrate is supported by experiments demonstrating that integrin $\alpha_3\beta_1$-dependent neurite outgrowth on laminin-5 (the ligand for $\alpha_3\beta_1$) was strongly inhibited by anti-CD151 and anti-CD81 antibodies, whereas it was not inhibited on laminin-1 or fibronectin, on which $\alpha_3\beta_1$ is not engaged (Ref. 16).

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Regulation of tetraspanin gene expression in tumours

The observation that a reduced level of expression of tetraspanins is associated with an increased metastatic potential of tumours raises the question of how they are downregulated. In cancer of the oesophagus, in which prognosis is linked to the expression of CD82, no mutations have been observed in the gene encoding CD82 (Ref. 93); thus, downregulation is not a result of mutation. For CD82/Kai1, it has been suggested that the gene encoding this protein is controlled by the p53 tumour suppressor such that, in the presence of mutant p53, CD82/Kai1 gene expression is downregulated (Ref. 94). Although this was not confirmed by other reports (Refs 95, 96), the frequent differential expression of tetraspanins in metastatic and non-metastatic cells should encourage studies on the regulation of tetraspanin gene expression in these cirumstances.

Despite the vast amount of work devoted to the relationship of tetraspanin expression with the prognosis of tumours, tetraspanins are not used as markers in routine practice for assessing the prognosis of cancer in patients. Thus, there are no protocols that correlate tetraspanin status with the results of different treatment regimens. The difficulty is technical since the quantification of tetraspanins relies on subjective evaluation of the intensity of labelling in frozen tissue sections. Furthermore, the epitopes recognised by the available antibodies do not resist the fixation procedures used in pathology laboratories. On the other hand, the use of reverse transcriptase polymerase chain reaction (RT-PCR) to quantify tetraspanin mRNA is usually not applicable since the tumour cells are contained in an environment of stromal and inflammatory cells, which express tetraspanins themselves and bias the dosage of the tetraspanin mRNA detected. However, these difficulties could be overcome by the production of new reagents more adapted to clinical use.

Another aspect of research into tetraspanins and malignancy would be to investigate the development of drugs that might positively or negatively modulate biological properties of tetraspanins such as the formation of cell-surface molecular complexes. However, it is perhaps too early to propose this type of therapeutic research since some of the basic mechanisms underlying the ability of tetraspanins to modulate cellular functions still require elucidation. Nevertheless, the search for therapeutic methods/agents that might mimic the effect of tetraspanin loss on tumour cells could become a new area of research for anticancer drugs.

Conclusions

Data summarised in this review clearly indicate that the tetraspanins are involved in important biological functions. The abundance of experimental and biochemical data, together with clinical observations, provides hints that might help to fill the conceptual gap between the observation of molecular associations and how they affect basic cellular or organ functions. Among the avenues of possible research, it seems essential to determine what keeps the tetraspanins together and how a loss of one tetraspanin might affect the function of its partner molecule. Even if the hypothesis of a conformational change was confirmed for proHB-EGF, the difficulty of demonstrating a similar effect for integrins suggests that tetraspanins could indirectly affect the functions of these molecules. A closer look at how tetraspanin complexes could modulate the integration of signalling pathways or regulate the traffic of cell-surface molecules will certainly provide some insights into membrane biology.

A better understanding of tetraspanin function could lead to improved methods for prognosis prediction or even treatment of malignant tumours. New tools, such as mAbs that are usable in fixed tissues, need to be developed to assess more easily, by routine analysis, the level and pattern of tetraspanin expression by tumour cells. The mechanism that links the level of tetraspanin expression to tumour progression needs to be further investigated. There are some indications that it could be mediated by modulation of integrin functions but other hypotheses, such as a link with the regulation of growth factor receptor signalling, have to be explored. Further insights into these mechanisms could pave the way to new treatments for the prevention of metastasis.

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Further reading, resources and contacts

The Mutation Database of Retina International's Scientific Newsletter provides updated information about mutations observed in the RDS/peripherin gene. Maps of the mutations are also available.
http://www.retina-international.org/sci-news/mutation.htm

Tetraspan Central is a new website designed to be a central hub for information about the tetraspanin superfamily (TM4SF). It provides useful information and entries related to the field and should be developed rapidly.
http://www.ksu.edu/tetraspan/thepage.htm

Protein Reviews on the Web (PROW) is an online resource that features PROW Guides. The Guides are authoritative, short, structured reviews on proteins and protein families and provide approximately 20 standardised categories of information (abstract, biochemical function, ligands, references, etc.) for each protein.
http://www.ncbi.nlm.nih.gov/prow/

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Tables

Table 1. Tetraspanins and cell migration (tab001cbv).
Table 2. Tetraspanins and cancer: correlations (tab002cbv).

Figures

Figure 1. Schematic drawing of the predicted structure of the tetraspanins (fig001cbv).
Figure 2. Dendrogram comparing amino acid identity of human tetraspanins (fig002cbv).
Figure 3. Detergent-dependent co-immunoprecipitation of two different types of tetraspanin complexes (fig003cbv).

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