Membrane proteases and tetraspanins

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Abstract

TEMs (tetraspanin-enriched microdomains) are specialized platforms in the plasma membrane that include adhesion receptors and enzymes. Insertion into TEMs dictates the local concentration of these molecules, regulates their internalization rate, their interaction and cross-talk with other receptors at the plasma membrane and provides links with certain signalling pathways. We focus on the associations described for tetraspanins with membrane proteases and their substrates, reviewing the emerging evidence in the literature that suggests that TEMs might be essential platforms for regulating protein shedding, RIP (regulated intramembrane proteolysis) and matrix degradation and assembly.

Tetraspanins and ADAMs (a disintegrin and metalloproteinases; α-secretases)

Proteolytic release of the ectodomains of transmembrane proteins (‘shedding’) plays a key role in important cell processes such as adhesion, migration, invasion, proliferation and signalling with crucial relevance in physiological and pathological phenomena, including development, leucocyte extravasation and inflammatory responses, tumour cell growth and dissemination and brain pathology. The common feature of this cleavage is that it occurs at extracellular sites proximal to the cell membrane. Two closely related members of the ADAM (a disintegrin and metalloproteinase) family of metalloproteinases, namely ADAM10 (also known as Kuzbanian from its orthologous gene -kuz- in Drosophila) and ADAM17 (also termed TACE [TNFαε-CONVERTING ENZYME], HB-EGF, TGFα-CONVERTING ENZYME), are responsible for the shedding of a growing number of shared substrates that include growth factors, cytokines, receptors and adhesion proteins, therefore influencing the behaviour of different cell types (see (1,2) for excellent recent reviews), their activities being sometimes complementary or redundant (1,3). ADAM10 is involved in the shedding of EGF (epidermal growth factor) and betacellulin EGFR (EGF receptor) ligands, while ADAM17 is largely responsible for cleavage of TGFα (transforming growth factor), HB-EGF, epiregulin and amphiregulin (4). ADAM17−/− mice display developmental defects similar to those observed in TGFα−/−, HB-EGF−/−, amphiregulin−/− or EGFR-deficient animals (5,6), clearly evidencing the in vivo relevance of ADAM17 in EGFR signalling. Moreover, it has been observed that activation of a variety of GPCRs (G-protein-coupled receptors) by agonists such as thrombin, angiotensin II or endothelin-1 induces ADAM-mediated transactivation of EGFR signalling, through a cross-talk mechanism termed TMPS (triple membrane-passing signalling) (1).

The physiological and pathological relevance of ADAM10 and ADAM17 substrates suggests that their proteolytic activities must be finely controlled; however, the underlying regulatory mechanisms remain largely unknown. The current view supports that the constitutive release of the ectodomains of most substrates is mainly mediated by ADAM10, while the dramatic enhancement that occurs after stimulation with phorbol esters is mostly dependent on ADAM17. Calcium ionophores activate predominantly ADAM10-mediated shedding, whereas activation of PKC (protein kinase C) by short-term treatment with phorbol esters stimulates ADAM17-dependent release of EGFR ligands (7). However, the mechanisms by which phorbol esters activate ADAM17 are not clear. Several studies have reported that the cytoplasmic domain of ADAM17 becomes phosphorylated on cell treatment with PMA (8–10), but other studies have shown that a truncated form of ADAM17 lacking the cytoplasmic tail can still be activated by phorbol esters (7,11). There is evidence that PMA stimulation also increases the transport of ADAM17 to the cell surface and its processing and maturation by furin (10,12), whereas other studies suggest that increased transport to the cell surface is not likely to be a requirement for ADAM17 activation (7).

Interactions with specific regulatory proteins involved in cell signalling or substrate accessibility have been proposed as regulatory mechanisms of ADAM activity (reviewed in (13)). As we discuss in the present mini-review, tetraspanins are emerging as important regulatory proteins that can bind to both ADAMs and their substrates, therefore modulating their efficient cleavage rates.

Arduise et al. (14) reported that a large fraction of ADAM10 is associated with several tetraspanins, including

Key words: a disintegrin and metalloproteinase (ADAM), regulated intramembrane proteolysis (RIP), α-secretase, shedding, tetraspanins.

Abbreviations used: Aβ, amyloid β-peptide; ADAM, a disintegrin and metalloproteinase; APP, amyloid precursor protein; EGFR, epidermal growth factor; EGF, EGF receptor; ERK, extracellular signal-regulated kinase; GPCR, G-protein-coupled receptor; ICAM-1, intercellular adhesion molecule 1; IEL, large extracellular loop; MMP, matrix metalloproteinase; PKC, protein kinase C; RIP, regulated intramembrane proteolysis; TACE, TNFαε-CONVERTING ENZYME, TBM, tetraspanin-enriched microdomain; TGFα, transforming growth factor, VCAM-1, vascular cell adhesion molecule 1.

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Biochem. Soc. Trans. (2011) 39, S41-S46; doi:10.1042/BST0390S41

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Membrane proteases are functionally regulated by insertion into TEMs

Figure 1

Tetraspanin-enriched microdomains include both ADAM-10 and ADAM-17 α-secretases. Moreover, several receptors susceptible to shedding by ADAMs, including Ig molecules ICAM-1 and VCAM-1 and EGFR ligands, also associate with tetraspanins. In addition, the γ-secretase complex is found associated at the plasma membrane with different members of the tetraspanin family. Finally, by means of a ternary complex that includes integrins, tetraspanins and membrane metalloproteinase MT1-MMP, TEMs spatio-temporally regulate extracellular matrix (ECM) degradation and assembly.

CD9, CD81 and CD82, indicating that this metalloproteinase is a component of the tetraspanin web (see Figure 1). Interestingly, engagement of these tetraspanins, but not of tetraspanin CD53, with specific antibodies stimulated the ADAM10-mediated release of TNFα and EGF present on the same cells, without altering ADAM10 interaction with tetraspanins. One possibility for explaining the regulatory effects of tetraspanins on ADAM10 activity could be the stimulation of certain signalling pathways by anti-tetraspanin antibodies. These authors found that the stimulatory effects of anti-tetraspanin antibodies required neither tyrosine phosphorylation nor PKC signalling, but an intact MEK [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase]/ERK pathway [14]. Tetraspanins might also directly regulate the enzymatic capacity of ADAM10 via their association at the membrane. Stimulation by anti-tetraspanin antibodies is not, however, a consequence of disruption of the ADAM10–tetraspanins associations but instead seems to correlate with their ability to induce redistribution of ADAM10 into patches enriched in tetraspanin molecules. These authors propose that this increase in ADAM10 local concentration may stimulate its sheddase activity, thus overcoming the inhibitory effect of associated tetraspanins.

The association of ADAM10 with different tetraspanins, and preferentially with Tspan12, has also been reported by the group of Martin Hemler [15]. Using MS to search for Tspan12-associated proteins these authors identified peptides corresponding to both ADAM10 and ADAM17. The association of ADAM10 with Tspan12 was further confirmed by reciprocal co-immunoprecipitation. Through the use of three different molecular approaches, overexpression, silencing and mutation, these authors demonstrated that Tspan12–ADAM10 complexes are functionally important, since Tspan12 enhances the maturation of ADAM10 and accordingly increases the α-secretase (ADAM10)-dependent shedding of APP (amyloid precursor protein) in different human tumour cell lines. Three different regions of the Tspan12 molecule, namely LEL (large extracellular loop), intracellular C-terminal domain and palmitoylation sites, contributed to its association with ADAM10. These authors postulate that this association results in stabilization of active ADAM10 on the cell surface and/or in acceleration of ADAM10 activation by prodomain convertases. Since ADAM10 is a major α-secretase responsible for APP processing along the non-amyloidogenic pathway, it is assumed that ADAM10 stimulation should lead to therapeutically relevant reduction in brain deposits of Aβ (amyloid β-peptide) [16], so that soluble LELs of Tspan12 or specific anti-Tspan12 antibodies could potentially be therapeutically beneficial in Alzheimer’s disease.

In these previous reports, the authors did not observe any association of ADAM17 with tetraspanins in the cell types they tested, which presented a lower level of expression of TACE compared with ADAM10. Our group has observed that tetraspanin CD9 associates with ADAM17 on the surface of leucocytes and endothelial cells [17] (Figure 1). Our results demonstrate, through overexpression and silencing, that CD9 exerts negative regulatory effects on ADAM17-mediated shedding of TNFα and ICAM-1 (intercellular adhesion molecule 1) in different cell types. These results are consistent with the observed increase in membrane TNFα on different human colon carcinoma cells after neoexpression of CD9 or treatment with anti-CD9 mAbs [18]. ICAM-1 and VCAM-1 (vascular cell adhesion molecule-1), two key endothelial adhesion molecules mediating leucocyte extravasation, are also shed by ADAM17 [19,20], and elevated soluble levels of these proteins have been found in several
inflammatory and tumoral pathologies [21]. Moreover, the adhesive function of ICAM-1 and VCAM-1 during leucocyte transendothelial migration is functionally regulated by endothelial tetraspanins [22]. Hence, CD9 silencing on stimulated HUVECs (human umbilical vein endothelial cells) results in a marked decrease in the membrane level of ICAM-1, reflecting the release from the inhibitory effect exerted by CD9 on ADAM17 activity. These results also provide a mechanism by which CD9 regulates the levels of ICAM-1 on endothelial cells and reinforces the subsequent consequences in terms of leucocyte adhesion and transmigration [22,23].

As pointed out above, the number of substrates for ADAM10 and ADAM17 is growing rapidly, and many of these substrate proteins can be cleaved in vitro by both ADAM10 and ADAM17. An attractive possibility is that in vitro tetraspanins may control the substrate specificity for each ADAM on the cell surface by regulating the segregation of substrates into distinct tetraspanin microdomains and antibody engagement could bring different microdomains together, promoting substrate cleavage. Interestingly, many substrates of ADAM10 or ADAM17 are in turn associated with tetraspanins (Figure 1). The tetraspanin CD9 has been shown to interact with the precursors of HB-EGF, TGFα, epiregulin and amphiregulin, markedly enhancing the juxtacrine signalling mediated by these transmembrane ligands of EGFR [24,25]. In fact, CD9 has been reported to strongly decrease the PMA-induced proteolytic conversion of transmembrane into soluble TGFα [24]. The inhibitory effect of CD9 on ADAM17 activity would explain the important role of this tetraspanin as an enhancer of EGFR juxtacrine signalling by increasing the surface levels of these transmembrane ligands. CD9 could also potentially inhibit the reported cross-talk between GPCRs and EGFR signalling, which requires release of EGFR ligands by ADAM10 and ADAM17, and has been shown to contribute to tumorigenesis, migration and invasion in different tumour cell lines (reviewed in [1,3]). In this regard, our results are consistent with the well-established tumour- and metastasis-suppressor role of CD9 in a variety of carcinoma types [26].

Tetraspanins and γ-secretases: RIP (regulated intramembrane proteolysis)

The proteolytic cascade termed RIP controls the processing and subsequent intracellular signalling mediated by key transmembrane proteins. The processing of Notch and APP represents paradigms for RIP, but the list of transmembrane proteins that are undergoing RIP is growing and includes different adhesion molecules such as CD44, N-cadherin, E-cadherin and L1, as well as members of the EGFR (m80-ErbB-4) family, the p75 neurotrophin receptor (p75NTR) and ligands for Notch itself (Delta, Jagged). RIP involves an initial ectodomain shedding by an α-secretase activity, followed by subsequent intramembrane cleavage, which generates an ICD (intracellular domain fragment) usually involved in signal transduction (see [1,3,4] for reviews). ADAM10 and ADAM17 are the main sheddases responsible for the α-secretase activity in the initial proteolytic step of RIP [27–30], while subsequent intramembrane cleavage of the remaining truncated transmembrane stub can be carried out by the γ-secretase (also termed presenilin) complex acting on truncated type 1 transmembrane proteins, signal peptide peptidases (acting on truncated type II transmembrane proteins), S2P or rhomboids [31].

Given its pathological relevance in Alzheimer’s disease, the study of APP RIP has attracted much interest (for reviews see [32,33]). Aβ, the main component of amyloid or senile neuritic extracellular plaques deposited in the brains of Alzheimer’s disease patients, comprises 38–43 amino acids derived from the processing of APP through sequential cleavage by β- and γ-secretases. Alternative sequential cleavage of APP by α- and γ-secretase activities generates a different peptide (P3), precluding generation of Aβ, and constitutes a non-amyloidogenic pathway. Type I transmembrane asparyl protease BACE1 (β-site amyloid precursor protein-cleaving enzyme) is responsible for the β-secretase activity in the brain. Major goals of current research in Alzheimer’s disease are aimed to inhibit β- and γ-secretases in order to reduce the generation of Aβ and to enhance α-secretase activity to stimulate the production of P3 peptide. In fact, ADAM10 overexpression has been shown to prevent amyloid plaque formation in a transgenic murine model of Alzheimer’s disease [16].

By means of a proteomic approach, Wakabayashi et al. [34] found different tetraspanins (CD81, CD9 and UpK1b) as well as tetraspanin-associated proteins (EWI-2, EWI-F, integrins and CD98) in a complex with presenilin1 and presenilin2 (Figure 1). Knockdown of tetraspanins, EWI-F or CD98 inhibited γ-secretase activity and Aβ release, while their overexpression enhanced Aβ secretion. γ-Secretase activity was also significantly impaired in cells derived from CD81- and CD9-knockout mice. In a second report [35], a subgroup of tetraspanins has been shown to facilitate Notch signalling by enhancing γ-secretase activity. Thus tsp-12 small interfering RNA or a null allele of these tetraspanin was able to rescue the defects of a missense allele of Notch protein GLP-1 (glucagon-like peptide-1) in Caenorhabditis elegans, while it synergized with a loss-of-function mutant of this gene. In parallel, in human tumours, Tspan33 silencing inhibited Notch signalling depending on the γ-secretase-mediated release of the intracellular signalling portion of Notch. In that report, tsp-12 and tsp-14 were found to have redundant functions in C. elegans, while Tspan33 and Tspan5 synergistically regulated Notch in human cancers. In contrast, CD81 or CD9 tetraspanins did not seem to play any role in these processes.

In summary, tetraspanin-based specialized microdomains regulate the sequential cleavage by α- and γ-secretases of different transmembrane receptors. This regulation seems to be cell-type- and tetraspanin-specific.
Tetraspanins and MMPs (matrix metalloproteinases): MT1-MMP

Alterations in the expression levels of tetraspanins are a common feature of cell malignant transformation [36]. Anti-tetraspanin antibodies have also been reported to inhibit tumour cell growth [18] or invasion [37]. In several contexts, induction of soluble metalloproteinase gene expression by tetraspanins has been reported. Anti-tetraspanin antibodies induce phosphoinositide 3-kinase–dependent production of MMP2 [37], cross-linking of CD81 by hepatitis C virus E2 glycoprotein up-regulates MMP2 in hepatoma cell lines [38], and CD151 homophilic interactions stimulate integrin-dependent MMP9 expression in human melanoma cells [39]. CD9 expression induces MMP2 in melanoma [40], while its deletion augments MMP2 secretion in small-cell lung cancer cells [41] and mouse blastocysts [42]. Overexpression of CD81 or CD82 in several myeloma cells reduces MMP2 expression [43]. Moreover, CD63 interacts with the soluble factor TIMP-1 (tissue inhibitor of metalloproteinases–1), a metalloproteinase inhibitor involved in the regulation of proliferation, cell survival, differentiation and renewal of extracellular matrix proteins [44].

In addition, membrane-anchored metalloproteinase MT1-MMP/MMP14 has been demonstrated to be included in TEMs (tetraspanin–enriched microdomains) in different cell types [45,46] (Figure 1). MT1-MMP regulates pericellular matrix proteolysis in cancer invasion and angiogenesis by initiating a metalloproteinase cascade via the enzymatic maturation of pro-MMP2 [47]. In endothelial cells, MT1-MMP was found to be directly associated with CD151, as demonstrated by co-immunoprecipitation and FRET (fluorescence resonance energy transfer) experiments [46]. Knockdown of this tetraspanin, but not of CD9, resulted in an increase in MMP2 maturation. However, endothelial cells depleted of CD151 or derived from CD151-deficient mouse presented an aberrant collagenolysis. Insertion of MT1-MMP into TEMs was shown to be crucial for its association and functional co-ordination with integrins. Hence, a ternary CD151–MT1-MMP–α3β1 integrin complex spatio-temporally directs pericellular proteolysis during endothelial cell migration and angiogenesis [46]. This effect is consistent with the angiogenesis defect observed in CD151-deficient mice [48]. In tumour cells, MT1-MMP was found associated with CD81, CD9 and Tspan12. In these cells, tetraspanin deletion also caused an impairment of matrix degradation by reducing MT1-MMP expression levels at the plasma membrane via lysosomal degradation [45]. Previous reports had shown an increment in MT1-MMP degradation at lysosomes by overexpression of tetrascopan CD63, which was also found associated with the haemopexin domain of the metalloproteinase [47]. The stoichiometries of tetraspanin–MT1-MMP complexes can be in turn regulated by EW1-2. EW1-2 is a member of the Ig superfamily receptor, tightly associated with tetraspanins CD9 and CD81, whose re-expression in glioblastoma cells causes an increase in CD9–CD81 association and disturbs that of tetraspanins with MT1-MMP. This molecular reorganization impairs tumour cell invasiveness [49]. Tetraspanins might also influence not only invasion, but also matrix deposition [50] and degradation. Supporting this notion, some phenotypes of tetraspanin-deficient mice, such as the disorganized basement membrane in the renal glomeruli [51] and skin [52] of CD151-knockout mice, are consistent with a defect in extracellular matrix organization.

Conclusions

Together, this emerging evidence points to tetraspanin-enriched microdomains as potential regulators of membrane protein shedding, RIP and matrix degradation and rearrangement. These functions might occur through a compartmentalization of both the enzymes and their substrates into specialized membrane platforms, so that dynamic regulation of their association with tetraspanins would allow a very rapid regulation of their accessibility to substrates. Regarding matrix degradation, TEMs regulate both subcellular localization and association with other receptors such as integrins, spatio-temporally directing the enzymatic degradation. All these aspects will have to be further explored in future research.

Funding

This work was supported by the Ministerio de Ciencia e Innovación [grant number BFU2007-66443/BMC], a grant from Fundación de Investigación Médica Mutua Madrileña (to C.C.), and Instituto de Salud Carlos III [grant number PI080794 (to M.Y.-M.)].


Received 30 August 2010
doi:10.1042/BST0390541