Tetraspanins-Structural and Signalling Scaffolds that Regulate Platelet Function

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Abstract: There are several tetraspanins present in platelets including CD9, CD151, TSSC6, and CD63. Recent studies in knockout mouse models have revealed that CD151 and TSSC6 are physically and functionally involved in regulation of the 'outside-in' signalling properties of the major platelet integrin, integrin $\alpha_{IIb}\beta_3$ and thrombus stability *in vivo*.

Key Words: Tetraspanin, integrin $\alpha_{IIb}\beta_3$, outside-in signalling, platelet thrombus formation.

PLATELET OVERVIEW

Platelets represent small anuclear terminally differentiated blood cells that are derived as fragments from megakaryocyte blebbing. They play an important role in hemostasis, and wound healing, but in diseased vessels can also be involved in thrombotic events such as ischemic stroke and heart attack [1]. They are crucial for the regulation of hemostasis by preventing blood loss *via* the formation of a thrombus following vascular injury. However, platelets are also involved in pathological situations, where loss of the strict controls regulating hemostasis results in hemorrhage or alternatively, thrombosis. Indeed, thrombosis resulting in acute myocardial infarction or ischemic stroke is a leading cause of death and disability in developed nations. Where there is an underlying disease state, for instance in the case of atherosclerosis, a number of factors will increase the thrombogenic potential of ruptured plaques, including the role of increased shear in narrowed blood vessels, and the heightened responsiveness of platelets to activation. Understanding some of the fundamental mechanisms of receptor interplay, signalling events and regulation of platelet responsiveness will not only improve our understanding of platelet thrombus formation, but provide the basis for interventive therapies to prevent or treat disease.

There are a number of steps that lead up to thrombus formation. Upon injury to a blood vessel wall, the initial interaction of platelets with the damaged subendothelium involves the tethering of von Willebrand Factor (vWF), which binds exposed collagen, to the GPIb-IX-V complex on the platelet surface [2]. This event not only initiates the adhesion of platelets to the exposed collagen of an injured vessel, but also leads to activation of GPIb-IX-V signalling that potentiates the conversion of integrins $\alpha_{IIb}\beta_3$ and $\alpha_2\beta_1$ to a high-affinity state. Integrin $\alpha_{IIb}\beta_3$ is a major platelet integrin, and once activated it binds to fibrinogen, leading to the stable adhesion and spreading of platelets, as well as recruitment of further platelets to the growing thrombus to arrest bleeding. At later points, integrin $\alpha_{IIb}\beta_3$ is involved in clot retraction, a process which merges into wound healing [3](Fig. **1**).

The engagement of collagen receptors, GPIb-IX-V complex, integrin $\alpha_2\beta_1$ and glycoprotein VI (GPVI) triggers phosphorylation events by Src family kinase members, Fyn and Lyn. This is followed by recruitment of Syk tyrosine kinase, LAT, SLP-76 and PLC-γ2. Phosphorylated LAT forms a scaffold that includes SLP-76, as well as the PI-3 Kinase and PLC-γ2 effectors. In this context, PI-3 Kinase generates secondary messenger phosphatidylinositol 3,4,5-triphosphate (PtdI3) which recruits IP_3 and diacylglycerol (DAG)[4]. This leads to calcium mobilization, activation of protein kinase C and conversion of integrin $\alpha_{IIb}\beta_3$ to an active conformation where it can bind its soluble ligand, fibrinogen (Fig. **1**).

The integrin, $\alpha_{IIb}\beta_3$, is critically important in the process of maintaining hemostasis. This integrin is a type I transmembrane receptor, composed of a heterodimeric complex consisting of an α_{IIb} and a β_3 subunit. Each subunit contains a large extracellular domain, single-pass transmembrane domain and a short cytoplasmic tail. While platelets express several other integrins including $\alpha_v\beta_3$, $\alpha_2\beta_1$, $\alpha_5\beta_1$, and $\alpha_6\beta_1$, integrin $\alpha_{IIb}\beta_3$ is the most highly expressed (approximately 80,000 copies). It also represents the major platelet integrin that is absolutely required for integrin $\alpha_{IIb}\beta_3$-mediated platelet aggregation, platelet spreading, clot retraction and stable platelet adhesion [5]. In humans, Glanzmann thrombasthenia is a naturally occurring example of the importance of integrin $\alpha_{IIb}\beta_3$ in platelet function. Where integrin $\alpha_{IIb}\beta_3$ is either absent or present but dysfunctional, platelets fail to aggregate, bind fibrinogen and retract fibrin clots [6].

Recent crystallographic studies of $\alpha_v\beta_3$, in conjunction with the head of integrin $\alpha_{IIb}\beta_3$ have shown that in the inactive state, the integrin heterodimer is bent over and does not bind to its extracellular ligand [8]. Upon activation, the integrin heterodimer is stabilized either by its extracellular ligand, and/or by intracellular cytoskeletal proteins such as talin. Lateral clustering of integrins by homo-oligomerization of the beta and alpha transmembrane domains also contributes to its active, extended conformation [9]. Tyrosine phosphorylation of integrin $\alpha_{IIb}\beta_3$ is followed by focal adhesion cytoskeletal reorganization and platelet aggregation.

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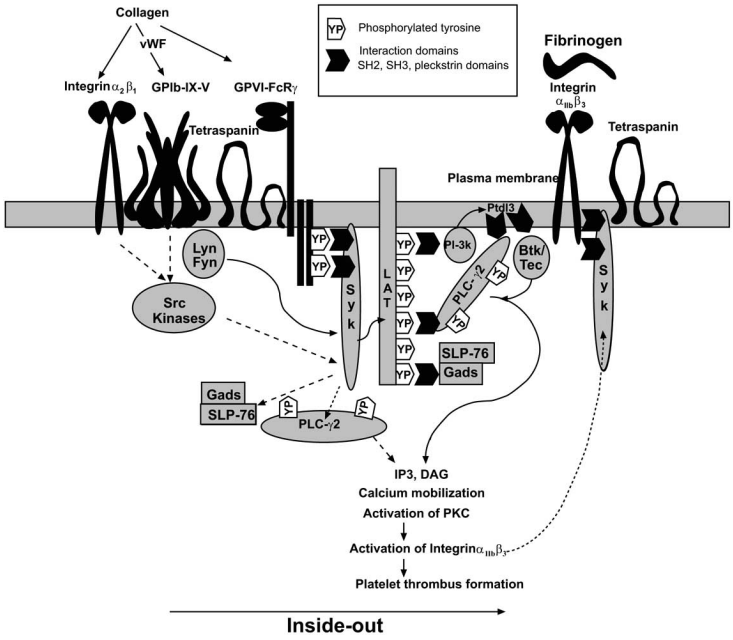

Fig. (1). Collagen binding to receptor GPVI leads to phospho-tyrosine labeling of the ITAM motif on the FcRγ chain by Src kinases Fyn and Lyn. This leads to the recruitment and tyrosine phophorylation of Syk, which leads in turn to phophorylation of LAT. LAT supports the assembly of signaling complex that includes SLP-76 and effectors PI-3K and PLC-γ2. PI-3K generates second messenger phosphatidylinositol 3,4,5 triphosphate (Ptdl3) which recruits the Tec family kinases and PLC-γ2 to the membrane. PLC-γ2 is activated by Btk and Tec and cleaves membrane lipids to generate inositol 1,4,5 triphosphate (IP3) and diacylglycerol (DAG). The generation of these second messengers lead to calcium mobilization, cytoskeletal rearrangements, activation of integin $\alpha_{IIb}\beta_3$ and ultimately, platelet thrombus formation. The engagement of collagen-receptors GPIb-IX-V and integrin $\alpha_2\beta_1$ also triggers the recruitment and phospho-tyrosine labeling of Syk, SLP-76 and PLC-γ2 although the signaling pathways involved (represented by broken arrows) are still poorly defined at present. Modified from Wee and Jackson, 2006 [7].

Agonist-induced activation of integrin $\alpha_{IIb}\beta_3$ leads to a conformational change and clustering of the receptor, converting it from a resting ('bent') low-affinity state, to an active ('extended') high-affinity state, allowing binding of its soluble ligand, fibrinogen. When activated, integrin $\alpha_{IIb}\beta_3$ has the ability to transmit bi-directional signalling *via* 'inside-out' (agonist induced activation of integrin $\alpha_{IIb}\beta_3$) or 'outside-in' (fibrinogen-occupied integrin $\alpha_{IIb}\beta_3$) signalling events. Post-ligand binding signalling that is mediated by integrin $\alpha_{IIb}\beta_3$ leads to irreversible stable platelet adhesion, cytoskeletal reorganization required for platelet spreading, clot retraction, microvesicle formation, platelet aggregation and subsequent thrombus growth [5,10].

TETRASPANIN OVERVIEW

Tetraspanins are present in many cell types and are involved in a number of cellular processes. These processes include modulation of the immune system, cell migration, cancer progression and metastases [11-13], as well as wound healing [14] and infectious processes such as viral budding and infectivity [15-16] [17-19]. Tetraspanins constitute a protein superfamily, consisting of over 30 members. They are integral membrane proteins containing four transmembrane domains with several conserved residues. This sets them apart from other four transmembrane domain receptors. For example, TM4SF1, TM4SF4 and TM4SF5 lack the conserved residues associated with tetraspanins and therefore do not qualify for inclusion in the tetraspanin protein superfamily [20]. The four transmembrane domains (which are significantly conserved between molecules) with two extracellular (EC) loops (highly variable), several conserved residues (CCG, PxSC and two cysteines that contribute to EC2 sulphide bonds) and two disulphide bridges that stabilize the molecule and contribute to its structure. Nearly all tetraspanins contain putative intracellular cysteines associated with palmitoylation. Indeed, the highly variable region of the large extracellular loop (EC2) of domain 2 is thought to be important for protein-protein interactions at the cell surface. Furthermore, the EC2 loop of CD151 contains a $QRD^{194\text{-}196}$ conserved site that is required for tetraspanin-integrin interactions, as well as a constant region that is proposed to mediate tetraspanin-tetraspanin homo- and hetero-dimerization [21,22]. Association with other tetraspanins and cell surface molecules, so called intra- and inter-molecular interactions, essential in the formation of TEMs, is possible *via* the four transmembrane domains. Even though tetraspanins associate with multiple integrins, including $\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_6\beta_4$, $\alpha_7\beta_1$, $\alpha_2\beta_1$, $\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$ in various cells, they are not found in focal adhesions [23]. Several studies have shown that tetraspanin interactions with integrins leads to regulation of adhesion strengthening but not cell adhesion to the extracellular matrix [18,24-25]. This suggests that tetraspanins regulate the functional properties of integrin extracellular matrix interactions but not attachment to immobilized extracellular matrix. Therefore, these findings have led to the conclusion

that tetraspanins regulate post-ligand binding integrin-dependent signalling events [18,23]. Indeed, studies have found that tetraspanins influence adhesion-dependent downstream signalling events such as tyrosine phosphorylation of cellular proteins [26] and activation of serine-threonine kinase PKB/cAkt [27].

Apart from associating with kinases, tetraspanins also exert their influence on cellular function by organising cell surface molecules (by virtue of lateral association) and assembly of signalling complexes into tetraspanin-enriched microdomains (TEMs). These tetraspanin-enriched microdomains are distinct from a lipid raft due to their detergent solubility to Triton X-100 and resistance to cholesterol depletion. A recurring theme is that tetraspanins serve as structural molecules, acting as spacers (keeping molecules apart), linkers (bringing molecules together) and supports (for example by keeping molecules, such as integrins in their extended, activated state for longer). This results in the assembling of multi-molecular complexes, which act as a node for various cellular processes. A further way they are postulated to exert influence is as supports or 'pillars in a sea of lipids' [28], and so may, in terms of integrins, structurally support them in their extended, activated conformation [29] augmenting post-ligand binding and downstream signalling. Thus, tetraspanins assemble multi-molecular complexes, which can then acts as nodes for various cellular processes. Furthermore, by association with partner proteins, tetraspanins can also play crucial roles in protein biosynthesis, protein trafficking and cell surface association. Examples of this include CD81 association with CD19, and CD151 association with integrin $\alpha_3\beta_1$ [30,31]. Indeed, CD81-deficient mice show reduced CD19 cell surface expression [31].

Apart from tetraspanins, several additional proteins are recruited into TEMs. These include G-protein coupled receptors (GPCRs), cadherins, epidermal growth factor receptor (EGFR) and immunoglobulin superfamily members. In this context, tetraspanins mediate homophilic and heterophilic ligand interactions that involve lateral clustering to regulate cell signalling and cell-adhesion events. As a result, several functional secondary relationships between tetraspanins and GPCRs, cadherin-catenin complexes, growth factor receptor, EGFR and EW1-2 immunoglobulin superfamily members have been defined [34-37]. Biologically, these tetraspanin interactions may prove to be important in regulating post-endocytic trafficking, compartmentalization, and epidermal polarisation.

TETRASPANIN SIGNALLING

The association of tetraspanins with integrins, especially CD151 and TSSC6 with $\alpha_{IIb}\beta_3$ in platelets, regulates not only 'outside in' signalling, but also adhesion-dependent cell signalling [36-38]. In nucleated rat fibroblasts, CD151 was found to act as a negative regulator in the adhesion-dependent activation of Ras [36]. Not only was activation of Ras diminished in the presence of CD151, but so were the downstream targets of Ras, PKB/c-Akt and ERK1/2. While adhesion-dependent activation of FAK and c-Src were not affected [36]. Interestingly, the C-terminal domain of CD151 was found to be critical for the activity of CD151 towards Ras, although, no biochemical signalling events have previously been ascribed to the activity of CD151 [36]. CD151 appears to exhibit its activity towards Ras only when integrins are disengaged from their ligands. This underscores the complexity of the tetraspanin interactions, where they exist in different microdomains so that in one context they can act as a negative regulator [36], but in another potentiates 'outside-in' signalling [39]. Indeed, evidence that the same tetraspanins can exist in different TEMs has been discovered by several groups [40-42]. This implies that it is the overall balance of a system that determines an outcome, rather than being an 'all' or 'none' phenomenon. Indeed, if tetraspanins do really modulate cell surface molecular interactions, they would be predicted to modulate different processes at different times, (by virtue of existing in these different multi-molecular complexes). This would depend on the cell and organ type, and the demands being made on a particular organ at a particular time. When considering experimental results in a specific cell type or system, this must be taken into consideration when interpreting results.

There is little mechanistic information on how tetraspanins influence cell signalling pathways. However, at least in nucleated cells, several candidates signalling molecules have been found to interact with the tetraspanin superfamily members CD151, CD81 and CD9. Upon palmitoylation, CD151 is known to associate with Type II phosphatidylinositol 4-kinase (PI4-K) [11], and protein kinase C (PKC) can be co-immunoprecipitated with a number of tetraspanins [39-41](Fig. **2**). In addition, CD81, CD63 and CD9 have been shown to associate with PI4-K to produce local phosphoinositides such as phosphatidylinositol-4,5-bisphosphate (PtdIns $(4,5)P_2$). Following these interactions, Shc is recruited and activated leading to downstream linkage with Ras/MAPK pathway, Akt/protein kinase B (PKB), and Jun Kinase (JNK) pathway. The C-terminal tails of CD151, CD81 and CD9 has been suggested to contain PDZ-domain-binding motifs, although putative PDZ binding proteins are still to be defined. It is possible that these putative PDZ-binding proteins provide a pivotal connection between tetraspanins and the cytoskeleton that may be important for modulation of cell adhesion strengthening.

In the context of platelets, tetraspanin superfamily members, CD151 and TSSC6 act by augmenting 'outside-in' (ligand-occupied) integrin $\alpha_{IIb}\beta_3$ signalling but not agonist-induced activation of 'inside-out' integrin $\alpha_{IIb}\beta_3$ signalling. Fibrinogen binding to the active conformer of integrin $\alpha_{IIb}\beta_3$ leads to 'outside-in' integrin $\alpha_{IIb}\beta_3$ signalling, and consequent tyrosine phosphorylation of signalling molecules, cytoskeletal reorganization and platelet aggregation (Fig. **2**). The presence of tetraspanins in tetraspanin-integrin $\alpha_{IIb}\beta_3$ complexes appears to be important for maintaining stable platelet adhesive interactions in platelet thrombus formation. At this stage, mechanistic information of signalling cross-talk between tetraspanins and integrin $\alpha_{IIb}\beta_3$, as well as other members in TEMs in platelets is lacking but it is likely to involve tyrosine phosphorylation signalling pathways. As platelets are terminally differentiated and anucleate, signalling cross-talk between tetraspanins and integrin $\alpha_{IIb}\beta_3$ can be defined without influence of nuclear signalling pathways.

TETRASPANINS IN PLATELETS

A number of tetraspanins have been identified in platelets including CD9, CD151, TSSC6, and CD63, whose function

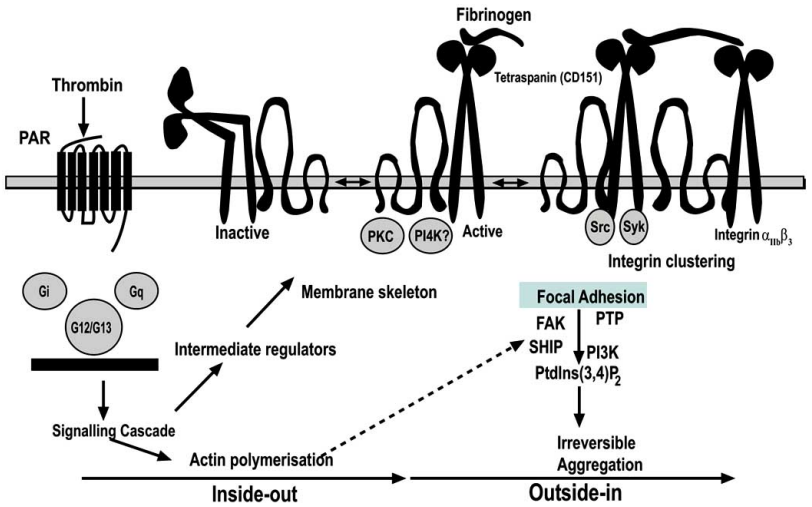

Fig. (2). 'Inside-out' signaling involves agonists acting via G protein coupled or tyrosine kinase-linked pathways converting integrin $\alpha_{IIb}\beta_3$ from a resting (bent) to an active (extended conformation). This facilitates its interaction with soluble fibrinogen, as well as consitituents of exposed subendothelium such as collagen, and likely, laminin. 'Outside-in' signaling occurs when fibrinogen (or other ligand) binding results in integrin cross-linking. The resulting integrin clustering stimulates tyrosine phosphorylation of specific platelet proteins, leading to cell adhesion, cytoskeletal reorganization and platelet aggregation. 'Inside-out' signaling feeds into 'outside-in' signaling tetraspanins such as CD151 and TSSC6 acting to augment 'outside-in', but not 'inside-out' signaling.

has been investigated [42,43]. The presence and function of novel tetraspanins in platelets has yet to be fully determined (Table **1**). To this end, Mike Tomlinson and colleagues have taken a proteomics approach to systematically identify the tetraspanins present in platelets [44]. Using this approach, these workers report Tspan9 and Tspan33 tetraspanins are also present in platelets together with some novel tetraspanins. Some of these tetraspanin members have been identified as an mRNA transcript but need to be proven to be expressed at a protein level in platelets. This information will provide a basis on which to determine associating proteins, hierarchies of association through immunoprecipitations in different detergents and fluorescence microscopy, as well as functional studies in knockout mice.

CD9

Many members of the tetraspanin superfamily form physical supramolecular complexes with other tetraspanin family members and transmembrane receptors including integrins. In platelets, CD9 is expressed at approximately 80,000 copies equivalent to the major platelet integrin $\alpha_{IIb}\beta_3$. CD9 has been co-localized with integrin $\alpha_{IIb}\beta_3$ on activated platelet pseudopods and alpha granule membranes [45]. Several reports indicate that CD9 is functionally linked with the low-affinity IgG receptor, FcγRIIa in human platelets [46-48], while others suggest that CD9 may regulate platelet function independent of FcγRIIa [49-51]. CD9 has been demonstrated to be physically associated with the major platelet integrin $\alpha_{IIb}\beta_3$ and CD36 on the surface of activated platelets [52-55]. This CD9:integrin $\alpha_{IIb}\beta_3$:CD36 and GPIb-IX-V complex is thought to be influenced by integrin $\alpha_{IIb}\beta_3$ conformational changes [56].

CD151

Like other members of the tetraspanin family, CD151 forms highly specific direct lateral interactions with other tetraspanin family members and transmembrane receptors including α_3, α_6, α_7 and α_{IIb} integrins [54-55]. Several studies have suggested that CD151 may not modulate integrin-dependent cell adhesion, but markedly affects integrin-dependent cell spreading and morphogenesis and adhesion strengthening when cells are grown on a Matrigel basement membrane [23,29]. These studies originally suggested that CD151 is capable of modulating the signalling properties of integrins. However, until recently, the role of CD151 in platelet function was unclear. Previous studies have implied a strong dependence between CD151 and FcγRIIa in platelet activation using monoclonal antibody (Mab) 14A2.H1, anti-human CD151 antibody [56]. Mab 14A2.H1 was shown to recognize a restricted conformational repertoire of CD151 excluding its association with certain integrins, and suggesting a role for CD151 in FcγRIIa-mediated signalling responses [38]. In this study, CD151 was reported to be expressed at approximately 1,000 copies/platelet [56]. However, subsequent studies using other alternative CD151 mAbs have demonstrated that CD151 is actually expressed at higher levels (approximately 10,000 copies/platelet) which is equivalent to PECAM-1 [35]. In contrast to previous studies, our recent studies in human platelets have reported that CD151 functions independently of platelet FcγRIIa [57]. Indeed, CD151 appears to be physically and functionally associated with integrin $\alpha_{IIb}\beta_3$. Using CD151 knockout mice (-/-), we demonstrated that the mice have an *in vivo* bleeding defect as shown by a tendency to re-bleed, indicating unstable haemostasis [58]. Platelets derived from $CD151^{-/-}$ mice have impaired 'outside-in' integrin $\alpha_{IIb}\beta_3$ signalling with delayed kinetics of clot retraction *in vitro*. In addition, $CD151^{-/-}$ platelets display normal 'inside-out' integrin $\alpha_{IIb}\beta_3$ signalling properties as shown by normal agonist-induced binding of soluble FITC-fibrinogen, and JON/A antibody binding [34]. Furthermore, *in vivo* intravital microscopy studies suggest a role for CD151 in primary thrombus formation and clot sta-

Table 1. Tetraspanins Identified in Platelets and Megakaryocytes

Tetraspanin	Cellular Origin	Function	Location	Associating/ co-localizing Proteins	References
CD151 (TSPAN24)	Platelets, protein	Regulates 'outside-in' signalling of integrin $\alpha_{IIb}\beta_3$ and stabilization of platelet thrombus	Platelet surface	Integrin $\alpha_{IIb}\beta_3$	[37,57]
TSSC6 (TSPAN32)	Platelets, protein	Regulates 'outside-in' signalling of integrin $\alpha_{IIb}\beta_3$ and stabilization of platelet thrombus	Platelet surface	Integrin $\alpha_{IIb}\beta_3$	[38]
CD9 (TSPAN29)	Platelets, megakaryocytes, protein	?	Alpha-granules Platelet Surface	Integrin $\alpha_{IIb}\beta_3$	[42,45]
CD63 (TSPAN30)	Platelets and megakaryocytes, protein	Cytoskeletal organization, platelet spreading	Dense granules (platelets), alpha granules (platelets and megakaryocytes)		[43, 66, 67]
NET5 (TSPAN9)	Megakaryocyte, transcript	?	?	?	[44]
(TSPAN33)	Megakaryocyte, transcript	?	?	?	[44]

bilization. These findings are consistent with other cellular models where tetraspanins were shown to be important in potentiating keratinocyte migration, wound healing and in adhesion strengthening. Overall, these cellular processes are central to the formation of an effective platelet plug to stop bleeding, and in later stages, the resolution of tissue damage through wound healing.

Recently, patients found to have a rare MER2 negative blood group antigen status (found in approximately 8% population) were also found to be associated with a mutant CD151 gene. This rare mutation (G383 in exon 5) in human CD151 has been described in Indian Jewish people where a premature in-frame stop codon at position 140 results in a truncated CD151 protein lacking the integrin contact site. Patients bearing this rare mutation display end stage hereditary nephritis and a skin-blistering disease known as pretibial epidermolysis bullosa [63]. At this stage evidence of any bleeding diasthesis has not been reported and it will require further studies to determine if these patients with a mutant CD151 gene show any features of unstable haemostasis.

In contrast to the human phenotype, the mouse phenotype does not appear to be as severe. This could be because the mouse genome contains a gene with significant homology to CD151, termed TSPAN11, that may be lacking in the human genome (Mark Wright, personal communication). The expression level of this gene in mice, perhaps dictated by epigenetic factors, could account for the difference in penetrance observed between the human and mouse systems. This, along with differences in genetic background, may also account for the differences observed in the penetrance of the CD151 phenotype where it has been knocked out of mice by three independent groups.

TSCC6

Unlike other tetraspanin family members, TSSC6 expression is restricted to all three hematopoietic lineages [64], including megakaryocytes [65]. Recent studies have demonstrated that TSSC6 is expressed both on the surface and in intracellular pools in human and murine platelets. In this context, as for CD151, TSSC6 is physically and functionally associated with the major platelet integrin, integrin $\alpha_{IIb}\beta_3$. Again, as for CD151, using TSSC6 knockout mice (-/-), we demonstrated that the mice have an *in vivo* bleeding defect as shown by a tendency to re-bleed, indicating unstable haemostasis. As for CD151, platelets derived from TSSC6$^{-/-}$ mice were also found to have impaired 'outside-in' integrin $\alpha_{IIb}\beta_3$ signalling with delayed kinetics of clot retraction *in vitro*. In addition, TSSC6$^{-/-}$ platelets also displayed normal 'inside-out' integrin $\alpha_{IIb}\beta_3$ signalling properties as shown by normal agonist-induced binding of soluble FITC-fibrinogen, and JON/A antibody binding [38]. However, in contrast to CD151, *in vivo* intravital microscopy studies demonstrated a role for TSSC6 in secondary (rather than primary) thrombus stabilization. These studies provide further direct evidence that multiple tetraspanin superfamily members including CD151 and TSSC6 are essential for normal integrin $\alpha_{IIb}\beta_3$-mediated platelet function, and hence normal clot initiation, formation, and stabilization. This work also illustrate that the loss-of-function of an individual tetraspanin such as CD151 or TSSC6 is sufficient to bestow a phenotype and cannot be compensated by the presence of other tetraspanins in platelets.

CD63

A striking feature of CD63 is the presence of an intracellular C-terminal tyrosine-based sorting motif G-Y-E-V-M that targets CD63 to lysosomal storage compartments. In resting platelets, CD63 has been demonstrated to be present in dense granule and lysosomal membranes and following platelet activation is translocated to the plasma membrane [66]. Under conditions of platelet activation, translocated CD63 becomes associated with integrin $\alpha_{IIb}\beta_3$-CD9 complex on the surface of platelets [66]. Recent studies suggest that

CD63 may modulate integrin $\alpha_{IIb}\beta_3$-dependent cytoskeletal reorganization and platelet spreading [67]. This is consistent with the notion that CD63 either alone or in conjunction with CD9 may modulate the 'outside-in' integrin $\alpha_{IIb}\beta_3$-mediated signalling events. While in nucleated cells, CD63 has been found to associate with syntenin-1, a PDZ containing protein that can bind phosphatidylinositol 4,5-biphosphate [PI(4,5) P2] *via* its dual PDZ domains [68]. Syntenin-1 can in turn interact with cytoplasmic molecules involved in signalling such as Ulk1 and GTPases [69]. In nucleated cells, the interaction of syntenin-1 with the C-terminal cytoplasmic domain of CD63 controls CD63 internalization, implying syntenin-1 is a regulator of endocytosis [70].

FUTURE DIRECTIONS

Future directions of research include determining the presence of novel tetraspanins in platelets. Although there are some technical problems in detecting proteins of low abundance, novel proteins are being identified by proteonomic and transcriptional profiling studies on megakaryocytes and platelets. Information generated by these means provides a starting point to determine tetraspanin function in platelets. Ultimately though, this involves experiments aimed at identifying the molecules that tetraspanins associate with by immunoprecipitation and immunofluorescence (including FRET and confocal microscopy), and function by generating knockout mouse lines. It will be interesting to identify downstream signalling molecules involved in platelet activation and aggregation [44] with different agonists, in different knockout and knockdown mouse and cell lines. This will lead not only to a greater understanding of the basic biology of tetraspanins and their role in platelet function, but also, potentially, novel therapeutic strategies. A further useful avenue of investigation would be to determine the role of mutation and polymorphism(s) in disease. Specifically, the role of tetraspanin defects in disease, such as has been found for CD151 truncation in humans.

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