Kindlins: essential regulators of integrin signalling and cell–matrix adhesion

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Integrin-mediated cell–ECM (extracellular matrix) adhesion is a fundamental process that controls cell behaviour. For correct cell–ECM adhesion, both the ligand-binding affinity and the spatial organization of integrins must be precisely controlled; how integrins are regulated, however, is not completely understood. Kindlins constitute a family of evolutionarily conserved cytoplasmic components of cell–ECM adhesions that bind to β -integrin cytoplasmic tails directly and cooperate with talin in integrin activation. In addition, kindlins interact with many components of cell–ECM adhesions—such as migfilin and integrin-linked kinase—to promote cytoskeletal reorganization. Loss of kindlins causes severe defects in integrin signalling, cell–ECM adhesion and cytoskeletal organization, resulting in early embryonic lethality (kindlin-2), postnatal lethality (kindlin-3) and Kindler syndrome (kindlin-1). It is therefore clear that kindlins, together with several other integrin-proximal proteins, are essential for integrin signalling and cell–ECM adhesion regulation.

Keywords: cell adhesion; extracellular matrix; integrin; kindlin

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See Glossary for abbreviations used in this article.

Introduction

Cell–ECM (extracellular matrix) adhesion is essential for embryonic development, and for the maintenance of tissue integrity and function in adults. At the molecular level, cell–ECM adhesion is mediated primarily by integrins, which constitute a family of $\alpha\beta$ heterodimeric transmembrane receptors (Hynes, 2002). Integrin-mediated cell–ECM adhesion can be regulated through two distinct mechanisms: integrin activation and cytoskeletal reorganization. The cytoplasmic tails of integrins are essential for their regulation and bind to several proteins. Talin—a protein that contains an amino-terminal FERM domain—was the first protein found to bind integrin cytoplasmic tails and is perhaps the best characterized (reviewed by Critchley, 2005; Critchley

& Gingras, 2008). The FERM domain, which consists of three modules (F1, F2 and F3 subdomains) that form a clover-shaped structure (Pearson *et al*, 2000), was initially identified in band 4.1, and was subsequently found in many cytoskeletal and signalling proteins that associate with the plasma membrane. A strong and substantial body of evidence demonstrates that talin is essential for integrin regulation (Calderwood *et al*, 1999, 2002; Garcia-Alvarez *et al*, 2003; Nieswandt *et al*, 2007; Petrich *et al*, 2007a,b; Qin *et al*, 2004; Tadokoro *et al*, 2003; Vinogradova *et al*, 2002; Wegener *et al*, 2007). Nonetheless, substantial evidence from theoretical considerations (Iber & Campbell, 2006), mutational data (Ma *et al*, 2006) and human *in vivo* data (Chen *et al*, 1992, 1994) suggest that other crucial integrin regulators also exist. This hypothesis has been borne out by recent studies that demonstrate that kindlins are essential regulators of integrin activation and cytoskeletal reorganization.

Domain structure and molecular activities of kindlins

Kindlins constitute a family of evolutionarily conserved and structurally related multidomain proteins. Despite being encoded by different genes, the three mammalian kindlins—kindlin-1 (also known as kindlerin and FERMT1), kindlin-2 (also known as MIG-2) and kindlin-3 (also known as URP2)—exhibit identical domain architecture (Fig 1A) and high sequence similarities. Human kindlin-2 and kindlin-3 are approximately 65% and approximately 59% identical to human kindlin-1 at the amino-acid sequence level, respectively, and UNC-112—the *Caenorhabditis elegans* orthologue of kindlins—is approximately 41% identical to kindlin-2 at the amino-acid sequence level (Rogalski *et al*, 2000). A crucial structural feature of kindlins is that they contain a FERM domain (Fig 1A). Unlike talin and most other FERM-domain-containing proteins, which have FERM domains in the N-terminal region, the kindlin FERM domains are located at the carboxyl terminus. In addition, the kindlin FERM domains are split in the F2 subdomain by the insertion of a PH domain (Fig 1A). Among all FERM proteins, the kindlin FERM domains exhibit the highest sequence similarity with that of talin (>50%). Structural modelling suggests that the F3 subdomains of kindlin-1 and kindlin-2 fold into a canonical FERM PTB fold resembling that of talin (Fig 1B; Kloecker *et al*, 2004; Shi *et al*, 2007).

Kindlins do not contain catalytic domains and therefore their primary function is to mediate protein interactions. So far, four kindlin-binding proteins have been identified, all of which are components

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Fig 1 | Kindlin domain structure and binding partners. (A) Domain architecture of kindlins and talin. All members of the kindlin protein family show identical domain architecture (top). Arrows indicate the regions of kindlins that interact with $\beta 1$ -integrin and $\beta 3$ -integrin, ILK or migfilin. The interactions with ILK and migfilin are based mainly on the studies of kindlin-2 and UNC-112 (see text). (B) Overlay of the kindlin-2 F3 subdomain model structure (blue) and the talin F3 subdomain structure (green) in complex with the $\beta 1$ -integrin peptide (red) showing a conserved PTB fold. Panel reproduced with permission from Shi *et al*, 2007. ILK, integrin-linked kinase; PTB, phosphotyrosine binding; UNC-112, uncoordinated protein 112.

Glossary

CH-ILKBP	calponin homology domain-containing integrin-linked kinase-binding protein
CHO	Chinese hamster ovary
FERM	band 4.1, ezrin, radixin and moesin
FERMT1	fermitin family homolog 1
ILK	integrin-linked kinase
MIG-2	mitogen-inducible gene 2
PAT	paralysed, arrested elongation at twofold
PH	pleckstrin homology
PINCH	particularly interesting new Cys–His protein
PTB	phosphotyrosine binding
Talin-H	N-terminal head of talin
TGF- $\beta 1$	transforming growth factor- $\beta 1$
UNC-112	uncoordinated protein 112
URP2	UNC-112 related protein 2
VASP	vasodilator-stimulated phosphoprotein

of cell–ECM adhesions: ILK (Mackinnon *et al*, 2002; Tu *et al*, 2003; Montanez *et al*, 2008), migfilin (Tu *et al*, 2003; Wu, 2005), and $\beta 1$ -integrin and $\beta 3$ -integrin (Kloeker *et al*, 2004; Shi *et al*, 2007; Montanez *et al*, 2008; Moser *et al*, 2008; Fig 1A). Consistent with the structural similarity between the talin and kindlin F3 subdomains (Fig 1B), binding studies using recombinant proteins and mutational analyses have shown that the F3 subdomains of kindlin-1, kindlin-2 and kindlin-3 can also interact directly with the $\beta 1$ -integrin and $\beta 3$ -integrin cytoplasmic tails (Kloeker *et al*, 2004; Shi *et al*, 2007; Montanez *et al*, 2008; Moser *et al*, 2008). Both kindlin-2 and UNC-112 can bind to ILK (Mackinnon *et al*, 2002; Tu *et al*, 2003; Montanez *et al*, 2008), although whether kindlin-1 and kindlin-3 can also interact with it remains to be determined. Kindlin-1 and kindlin-2 can also bind to migfilin (Tu *et al*, 2003; Wu, 2005; Lai-Cheong *et al*, 2008), but whether kindlin-3 can do so is still unknown.

Although some kindlins can probably localize to other subcellular sites—where they could interact with yet-to-be identified proteins—they are highly concentrated at sites of cell–ECM adhesion (Fig 2),

where they mediate their primary function. A fraction of kindlin-1 is phosphorylated in keratinocytes (Herz *et al*, 2006); casein kinase-2—a serine–threonine kinase that is involved in cytoskeleton regulation—is at least partly responsible for this modification, as its inhibition leads to a reduction in kindlin-1 phosphorylation. These results suggest that kindlins could potentially act as targets of intracellular kinase signalling. It will therefore be interesting to characterize the sites and the precise function of kindlin phosphorylation in order to understand more thoroughly the processes that are regulated by these proteins.

Kindlins are crucial components of cell–ECM adhesions

Several lines of evidence have led to the identification of kindlins as crucial cell–ECM adhesion proteins. First, the characterization of UNC-112 in *C. elegans* shows that it localizes to integrin-rich muscle-attachment structures (Rogalski *et al*, 2000). Furthermore, the null mutation of UNC-112 results in an embryonic-lethal PAT phenotype resembling that induced by the lack of several other crucial focal-adhesion components, including PAT-2/ α -integrin, PAT-3/ β -integrin, PAT-4/ILK, PAT-6/actopaxin/CH-ILKBP/parvin and UNC-97/PINCH (Rogalski *et al*, 2000). UNC-112 interacts with PAT-4/ILK (Mackinnon *et al*, 2002), and its loss alters PAT-3/ β -integrin spatial organization (Rogalski *et al*, 2000) and PAT-4/ILK (Mackinnon *et al*, 2002).

The second line of evidence comes from the characterization of kindlin-2 (Tu *et al*, 2003). Kindlin-2 localizes to cell–ECM-adhesion sites, and interacts directly with the focal-adhesion proteins ILK and migfilin; depletion of kindlin-2 or migfilin impairs cell spreading (Tu *et al*, 2003). These and other studies suggest that kindlin-2 and migfilin constitute an important connection between cell–ECM adhesions and the actin cytoskeleton (Wu, 2005; Zhang *et al*, 2006).

The third line of evidence comes from the studies on kindlin-1. The loss of kindlin-1 causes Kindler syndrome (Jobard *et al*, 2003; Siegel *et al*, 2003), which is a rare autosomal recessive skin-fragility disorder (Kindler, 1954). This finding is consistent with a role for kindlin-1 in cell–ECM adhesion, as skin-fragility disorders are frequently a consequence of compromised ECM adhesion of keratinocytes. Indeed, molecular analyses have shown that kindlin-1 localizes to focal adhesions in cultured keratinocytes (Kloeker *et al*, 2004; Siegel *et al*, 2003).

Fig 2 | Subcellular localization of kindlin-1 and kindlin-2. (A) Human HaCaT keratinocytes stained with monoclonal antibodies that recognize kindlin-1. (B) The cells were dual-stained with rhodamine-phalloidin (red) and anti-kindlin-1 (green). Note that kindlin-1 was concentrated at focal adhesions where actin stress fibres were anchored (arrows). (C) Human HaCaT keratinocytes stained with monoclonal antibodies against kindlin-2. Images kindly provided by G. Owen.

Furthermore, it interacts directly with $\beta 1$ -integrin and $\beta 3$ -integrin cytoplasmic tails, and is required for normal keratinocyte spreading in culture (Kloeker *et al*, 2004).

Kindlins in cell–ECM adhesion assembly

Kindlins are required for the correct assembly of integrin-rich cell–ECM adhesions. Kindlin-2 is localized to cell–ECM adhesions by its direct interaction with the cytoplasmic tails of β -integrin (Shi *et al*, 2007). In turn, kindlin-2 binds to migfilin and recruits it to these sites (Tu *et al*, 2003). The presence of migfilin in cell–ECM adhesions facilitates the accumulation of other migfilin-binding proteins such as VASP and filamin (Zhang *et al*, 2006). Therefore, kindlin-2 is immediately downstream of integrins in the hierarchy of cell–ECM-adhesion assembly (Fig 3). The localization of ILK to cell–ECM adhesions also requires kindlin-2 (Mackinnon *et al*, 2002; Montanez *et al*, 2008); however, unlike migfilin, which is not essential for kindlin-2 localization to cell–ECM adhesions (Tu *et al*, 2003), ILK is essential (Mackinnon *et al*, 2002). Therefore, the assembly of kindlin-2 and ILK into cell–ECM adhesions seems to be interdependent.

The functional importance of kindlins in mediating reorganization of the cytoskeleton at cell–ECM adhesions was shown in mammalian cells. Expression of kindlin-2 in kindlin-2-deficient colon cancer cells was shown to promote focal-adhesion formation and to enhance cell–ECM adhesion through a mechanism dependent on cytoskeletal reorganization (Shi *et al*, 2007).

Kindlins in integrin activation

In addition to promoting cytoskeletal reorganization, kindlins act as crucial co-activators in integrin activation. Various structural (Vinogradova *et al*, 2002; Wegener *et al*, 2007), cellular (Kim *et al*, 2003), mutational (Han *et al*, 2006; Hughes *et al*, 1996) and biophysical (Haas & Plow, 1996) approaches have indicated that the cytoplasmic tails of α -integrin and β -integrin interact with one another to form a weak clasp. This clasp maintains the integrin in a resting state, such that the extracellular domains have low affinity for ligands, and its disruption triggers integrin activation, leading to a substantial increase in ligand affinity.

Fig 3 | Assembly of kindlin-2-containing cell–ECM adhesions. Model for the assembly of kindlin-2-containing cell–ECM adhesions, which occurs through multiple protein–protein interactions (double arrows). ILK is recruited to cell–ECM adhesions as part of a ternary protein complex (the PINCH–ILK–Parvin complex; Wu, 2004). The interaction between kindlin-2 and ILK (open double arrows) facilitates their localization to cell–ECM adhesions, where they contribute to integrin clustering (filled double arrow) and cytoskeletal organization. ECM, extracellular matrix; ILK, integrin-linked kinase; PINCH, particularly interesting new Cys–His protein.

A function of kindlins in integrin activation was first observed in a study using CHO cells that express $\alpha 1 \beta 3$ -integrin (Shi *et al*, 2007). Kindlin-2 was shown to interact directly with the $\beta 3$ -integrin cytoplasmic tail, and its overexpression was shown to induce a modest activation of the $\alpha 1 \beta 3$ -integrin. Structural modelling identified Gln 614 and Trp 615, within the kindlin-2 PTB domain, as crucial residues at the integrin-binding interface. Substitution of these residues with two alanines abrogated integrin-binding and concomitantly abolished the ability of kindlin-2 to activate integrin (Shi *et al*, 2007). Therefore, kindlin-2 seems to regulate integrin activation through, at least in part, direct interaction with the integrin. These results provided the initial evidence for a role of kindlin binding in integrin activation.

The second breakthrough came when it was found that co-transfection with talin-H and kindlin-2 resulted in substantially greater integrin activation than that obtained with talin-H or kindlin-2 alone (Ma *et al*, 2008). Mutation of Gln 614 and Trp 615 on kindlin-2 abolished the direct interaction with integrin (Shi *et al*, 2007; Montanez *et al*, 2008) and resulted in a complete loss of co-activator activity (Ma *et al*, 2008), indicating that the capacity of kindlin-2 to activate $\alpha 1 \beta 3$ -integrin together with talin-H depends on its direct binding to residues in the C-terminal region of the $\beta 3$ cytoplasmic tail. Despite the homology between the PTB domains of kindlins and talin (Fig 1B), they have been shown to bind to two distinct sites of the $\beta 3$ cytoplasmic tail. The NPXY motif in the mid-segment of the $\beta 3$ cytoplasmic tail—which includes Tyr 747 and is crucial for talin binding—is not required for kindlin-2 binding (Shi *et al*, 2007), whereas a proline substitution

Fig 4 | Clinical manifestations of skin and oral mucosal lesions in Kindler syndrome. (A) A 3-year-old male Kindler syndrome patient with healing ulcers (arrows). (B, C) A 13-year-old male Kindler syndrome patient with acute skin blister (B, arrow) and oral mucosal lesions (C, arrows indicate red, swollen and spontaneously bleeding gingiva). Photographs courtesy of C. Wiebe.

at Ser 752 impaired kindlin-2 binding (Ma *et al*, 2008; Shi *et al*, 2007). This substitution is found in a variant of Glanzmann thrombasthenia—a congenital bleeding disorder caused by a lack of functional $\alpha\text{IIb}\beta_3$ -integrin in platelets—which is known to suppress $\alpha\text{IIb}\beta_3$ -integrin activation without affecting talin binding, thereby emphasizing the importance of kindlins for correct integrin functionality.

The third breakthrough came from studies in kindlin-knockout mice and human primary cells. Moser and colleagues characterized platelet function in kindlin-3 knockout mice (Moser *et al*, 2008); platelet aggregation—a response dependent on the activation of $\alpha\text{IIb}\beta_3$ -integrin—was absent and platelet adhesion to immobilized ligands of the integrin was blunted. These mice also failed to form a thrombus *in vivo*. The same residues that were involved in kindlin-2 binding to the β_3 -integrin cytoplasmic domain were implicated in the binding of kindlin-3 (Moser *et al*, 2008). Therefore, kindlin-2 and kindlin-3 both have co-activator activity, and can probably cooperate with talin-H in a similar manner. In studies using human primary cells (Ma *et al*, 2008), knockdown of kindlin-2 markedly altered endothelial cell adhesion to fibrinogen and vitronectin. In addition, impairments in β_1 -integrin activation, basement-membrane organization and cell-ECM adhesion were observed in embryoid bodies derived from kindlin-2-null embryonic stem cells (Montanez *et al*, 2008). Taken together, these studies demonstrate a crucial role of kindlins in integrin regulation. The phenotypes observed in the absence of kindlins probably arise, at least in part, from a loss of the integrin co-activator activity of the kindlins.

Kindlins in development and disease

Kindlin-1 and kindlin-2 are widely expressed in murine and human tissues, including skin, heart, lung, liver, kidney, colon, prostate, ovary and pancreas (Siegel *et al*, 2003; Ussar *et al*, 2006); the expression patterns of kindlin-1 and kindlin-2, however, are not identical. For example, kindlin-1 is strongly expressed in the epidermis and only weakly expressed in the dermis, whereas the level of kindlin-2 in the dermis is higher than that in the epidermis (Ussar *et al*, 2006). Unlike kindlin-1 and kindlin-2, the expression of kindlin-3 is restricted to haematopoietic tissues, where it is the dominant form of kindlin expressed, although relatively low expression levels of kindlin-1 and kindlin-2 have also been detected (Ussar *et al*, 2006). These differential expression patterns might explain, at least in part, the distinctive phenotypes that result from the loss of different kindlins, which are discussed below.

In *C. elegans*, UNC-112 is essential for embryonic development (Rogalski *et al*, 2000). The phenotypes resulting from the loss of individual kindlins have also been characterized in vertebrates. Loss of kindlin-2 in mice results in peri-implantation embryonic lethality (Dowling *et al*, 2008; Montanez *et al*, 2008) and knockdown of kindlin-2 in zebrafish reveals a strong dependence of cardiac development and function on kindlin-2 (Dowling *et al*, 2008). Consistent with the restricted expression of kindlin-3 in haematopoietic tissues, mice lacking kindlin-3 show severe osteopetrosis, haemorrhage and defects in the erythrocyte membrane skeleton, and die within one week of birth (Kruger *et al*, 2008; Moser *et al*, 2008).

Loss of kindlin-1 in humans causes Kindler syndrome (Jobard *et al*, 2003; Siegel *et al*, 2003). The characteristic clinical features of this disease include congenital skin blistering and poikiloderma with extensive atrophy (Fig 4; Kindler, 1954). Most reported Kindler syndrome patients also have oral symptoms such as restricted mouth opening, gingivitis, gingival atrophy and aggressive periodontal disease (Wiebe *et al*, 2008). Kindlin-1 deficiency does not seem to affect many tissues beyond the skin and oral mucosa, with the exception of the colon (Kern *et al*, 2007). Unlike in the skin, two forms of kindlin-1—arising from alternative splicing—can be detected in the colon (Siegel *et al*, 2003). However, the histological manifestations of colon blisters in Kindler syndrome are similar to those of the skin and oral mucosa, presenting epithelial detachment from the underlying tissue.

The most characteristic histological abnormality found in the skin and oral mucosa of Kindler syndrome patients is the reduplication of the lamina densa (Shimizu *et al*, 1997; Wiebe & Larjava, 1999). Breaks in the basement membrane are often also present, but the localization of most basement membrane proteins is normal (Wiebe & Larjava, 1999). Another notable histological feature is an abnormal deposition of type VII collagen (Wiebe & Larjava, 1999). Kindlin-1-deficient keratinocytes show reduced cell adhesion, migration and proliferation, and increased apoptosis (Herz *et al*, 2006; Kloecker *et al*, 2004); the periodontal disease phenotype is associated with a reduced ability of the epithelial cells to attach and seal the gingiva to the tooth (Wiebe *et al*, 2008). Given the crucial roles of integrins in cell-ECM adhesion, proliferation, survival, cytoskeleton organization and ECM deposition, many of the defects observed in the absence of kindlins could arise from abnormal integrin regulation.

Sidebar A | In need of answers

- (i) How do kindlins cooperate with talin to elicit integrin activation?
- (ii) What other proteins interact with kindlins besides integrin, migfilin and integrin-linked kinase?
- (iii) Are there unique activities associated with different members of the kindlin family?
- (iv) What are the functions of the splice variants of kindlins?
- (v) Do kindlins function outside of cell–extracellular matrix adhesion?
- (vi) How are the expression and activities of kindlins regulated?
- (vii) Can kindlins act as targets for therapeutic intervention of human diseases such as cancer and cardiovascular diseases?

Although the loss of kindlins is often deleterious, an aberrantly high level of kindlin-1 might also be harmful. Kindlin-1, but not kindlin-2, is overexpressed in colorectal and lung tumours (Weinstein *et al*, 2003). The expression of kindlin-1 is upregulated by TGF- β 1 (Kloeker *et al*, 2004), which is known to increase the motility of tumour cells. This raises the interesting possibility that kindlin-1 overexpression mediates the increase in motility, thereby contributing to tumour invasion and cancer progression. By contrast, overexpression of kindlin-2 suppresses mesenchymal cancer cell invasion (Shi & Wu, 2008). Therefore, although kindlin-1 and kindlin-2 share common activities, their functions might not be entirely redundant.

Conclusions and future directions

Although kindlins have been studied for only a few years, they have been firmly established as an important family of integrin regulators. Two mechanisms whereby kindlins regulate integrins have emerged. First, kindlins bind to the β -integrin cytoplasmic domains, cooperate with talin and consequently activate integrins. Second, kindlins interact not only with integrins, but also with other components of cell–ECM adhesions—such as migfilin and ILK—thereby regulating cytoskeletal reorganization and strengthening cell–ECM adhesion. These findings put kindlins, together with several other integrin-proximal proteins, at the centre stage of integrin signalling and cell–ECM adhesion regulation. There are currently several unanswered questions regarding kindlin function and regulation (Sidebar A). Addressing these questions will not only provide new mechanistic insights into integrin regulation, but also could lead to a better understanding of the molecular basis underlying the pathogenesis and progression of several human diseases, including cancer and cardiovascular disease.

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