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COMMENTARY

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The kindlin family: functions, signaling properties and implications for human disease

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ABSTRACT

The kindlin (or fermitin) family of proteins comprises three members (kindlin-1,-2 and -3) of evolutionarily conserved focal adhesion (FA) proteins, whose best-known task is to increase integrin affinity for a ligand (also referred as integrin activation) through binding of β-integrin tails. The consequence of kindlin-mediated integrin activation and integrin-ligand binding is cell adhesion, spreading and migration, assembly of the extracellular matrix (ECM), cell survival, proliferation and differentiation. Another hallmark of kindlins is their involvement in disease. Mutations in the KINDLIN-1 (also known as *FERMT1*) gene cause Kindler syndrome (KS) – in which mainly skin and intestine are affected, whereas mutations in the KINDLIN-3 (also known as *FERMT3*) gene cause leukocyte adhesion deficiency type III (LAD III), which is characterized by impaired extravasation of blood effector cells and severe, spontaneous bleedings. Also, aberrant expression of kindlins in various forms of cancer and in tissue fibrosis has been reported. Although the malfunctioning of integrins represent a major cause leading to kindlin-associated diseases, increasing evidence also point to integrin-independent functions of kindlins that play an important role in the pathogenesis of certain disease aspects. Furthermore, isoform-specific kindlin functions have been discovered, explaining, for example, why loss of kindlins differentially affects tissue stem cell homeostasis or tumor development. This Commentary focuses on new and isoform-specific kindlin functions in different tissues and discusses their potential role in disease development and progression.

KEY WORDS: Disease, Integrins, Kindlins

Introduction

The first of the kindlin proteins to be discovered – in 1994 – was kindlin-2. It was detected in a screen for epidermal growth factor (EGF)-induced mRNAs and initially named mitogen-inducible gene 2 (Mig-2) protein (Wick et al., 1994). The function of Mig-2/kindlin-2 was not further characterized in that study. At the same time the kindlin ortholog UNC112 was identified in a genetic screen in *Caenorhabditis elegans* as an essential component for muscle assembly (Williams and Waterston, 1994). A few years later, UNC112 was described as a so-far-unknown component of dense bodies – integrin-based cell-ECM adhesion structures that resemble vertebrate focal adhesions (FAs) and firmly attach muscle to the hypodermis (Rogalski et al., 2000). In 2003, it was shown that mammalian Mig-2/kindlin-2 binds to a newly described LIM domain-containing protein termed migfillin that links Mig-2/kindlin-2 to filamin and, so, to the actin cytoskeleton (Tu et al., 2003). In the same year, a genetic study identified kindlin-1, a homolog of kindlin-2, to be mutated in and the cause of Kindler syndrome (KS), a skin-blistering disease combined with pigmentation defects, increased photosensitivity, skin atrophy and high risk of skin cancer (Siegel et al., 2003).

Integrin activation was, for a long time, thought to be mediated by talin only (Tadokoro et al., 2003). The first hints pointing to the existence of an integrin-activating protein that is required in addition to talin came from the analyses of cells and mice, in which the two tyrosine residues in the β1 integrin cytoplasmic domain had been replaced with alanine residues (Czuchra et al., 2006; Meves et al., 2011; Wennerberg et al., 2000). The mutation of the proximal tyrosine residue that is required to bind talin (Calderwood et al., 1999), as well as the mutation of the distal tyrosine residue whose function was unknown at the time, resulted in peri-implantation lethality in mice (Meves et al., 2011). Both mutations impaired cell adhesion and spreading, and abrogated the activation of β1 integrins. In search for a binding partner for the tyrosine residue within the distal integrin tail, Mig-2/kindlin was identified (Moser et al., 2008). The comparison of the human and mouse genomes revealed the existence of three Mig-2/kindlin isoforms (Siegel et al., 2003), and expression analyses revealed that the three Mig-2/kindlin family members are expressed in different cells and tissues (Ussar et al., 2006). Kindlin-1 is mainly expressed in epithelial cells, kindlin-2 is broadly expressed but absent in all blood cells analyzed so far (Ussar et al., 2006), and kindlin-3 is found in hematopoietic cells and, possibly, also at low levels in endothelial cells (Bialkowska et al., 2010) and in solid cancers, such as breast cancer and melanoma (Sossey-Alaoui et al., 2014; Djaafri et al., 2014; Delyon et al., 2015). It is unclear whether the expression of kindlin-3 in solid tumors is caused by *de novo* activation of the KINDLIN-3 gene or by infiltration of hematopoietic cells. Genetic evidence in mice, as well as biochemistry and cell biology experiments of cells derived from mice or performed with the αIIbβ3-complemented Chinese hamster ovary (CHO) cells, revealed that kindlins bind the distal NxxY motif of several β-integrin tails and trigger, in concert with talin, the activation of integrins (Moser et al., 2008, 2009a; Montanez et al., 2008; Ma et al., 2008). Subsequent studies carried out in several laboratories identified also kindlin-3 as a so-far-unknown 'disease gene', and all three kindlin proteins as prominent and essential regulators of integrin-mediated adhesion and signaling.

In this Commentary, we summarize and discuss recent advances in kindlin research for integrin signaling, subcellular localization and post-translational modifications, and highlight specificities of kindlin family members. Furthermore, we highlight functional differences between kindlin isoforms, integrin-independent functions of kindlin proteins and their role in disease.

Kindlins and their interaction partners

Phylogeny analysis of the kindlin paralogs suggests that a single ancestral kindlin protein in earliest metazoan underwent duplication events in insects and a genomic duplication in vertebrates, leading to

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the sub-functionalized kindlin family, in which each member displays its distinct expression pattern (Ussar et al., 2006; Khan et al., 2011). The kindlins consist of ∼680 amino acids and have a size of ∼75 kDa. The human kindlins are encoded by three different genes, with KINDLIN-1 (also known as *FERMT1*) located on human chromosome 20p12.3, KINDLIN-2 (also known as *FERMT2)* on chromosome 14q22.1 and KINDLIN-3 (also known as *FERMT3*) on chromosome 11q13.1 (Siegel et al., 2003). They share considerable amino acid sequence and protein structural similarities. For example, kindlin-1 has ∼62% homology with kindlin-2 and ∼49% with kindlin-3 (Siegel et al., 2003; Weinstein et al., 2003).

As noted above, a major task of kindlins is to regulate the activation of integrins. Integrins belong to a large family of cell-surface receptors, which bind adhesion molecules on endothelial cells, such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecules 1 and 2 (ICAM-1/2), as well as ECM proteins, such as collagen, laminin and fibronectin. Integrins consist of non-covalently associated α and β subunits that consist of large ectodomains, single-span transmembrane domains and short cytoplasmic domains. In mammals there are 18 α and eight β subunits that assemble 24 distinct integrins heterodimers with specialized function and ligand-binding activity (reviewed in Humphries et al., 2006; Hynes, 2002).

Integrins reversibly switch from a low to a high ligand affinity conformation, referred to as integrin activation. The consequence of integrin activation is ligand binding, adhesion, the assembly of a large molecular network and the linkage to the actin cytoskeleton (Moser et al., 2009b; Shattil et al., 2010). The integrin adhesion to the ECM can be strengthened by lateral, non-covalent association of integrins at an adhesion site, referred to as integrin clustering (Cluzel et al., 2005; Shattil et al., 2010). The coordination of multiple weak binding integrin allows the formation of a strong adhesion bond in an additive manner, strengthening integrin signaling induction (Bunch, 2010; Zhu et al., 2007). But how clustering is mechanistically regulated, remains largely unclear.

Integrin activation requires the binding of kindlin and talin to the cytoplasmic tails of the β subunit. Both, talin and kindlins contain a 4.1 protein, ezrin, radixin, moesin (FERM)-like domain, consisting of three subdomains (F1, F2 and F3) (Shi and Wu, 2008; Kloeker et al., 2004). The F3 subdomain harbors a phosphotyrosine-binding (PTB)-fold that can directly bind to the NPxY motifs in β-integrin subunits. Kindlin and talin interact with the cytoplasmic tail of β integrin at the plasma membrane but it is still unclear whether they bind consecutively or jointly and control integrin activation. Furthermore, it is also unclear whether talin and kindlin are responsible for different steps during integrin activation. For example, one report suggests that talin mediates unbending and kindlin unclasping of integrins (Lefort et al., 2012). Finally, it has been shown that talin fully activates αIIbβ3, whereas kindlin-3 stabilizes integrin-ligand complexes by stimulating αIIbβ3 clustering (Ye et al., 2013). Similarly, αLβ2 integrin clustering seems to be induced by kindlin-3 that, subsequently, interacts with the scaffolding protein receptor for activated C kinase 1 (RACK1) to promote outside-in signalling (Feng et al., 2012).

It is believed that talin requires an activation step prior to binding of the integrin tail. Whether kindlins also require an activation step *in vivo* in order to be able to bind β-integrin tails is unclear. Although recent studies suggest that binding of integrin-linked-kinase (ILK) to kindlin supports its localization to FAs and kindlin-mediated integrin activation, the underlying mechanisms remain unclear. A study in *C. elegans* proposed that binding of ILK to kindlin induced a conformational change of kindlin that promotes its binding to integrin tails (Qadota et al., 2012). This mechanism, however, has not been observed with mammalian kindlin orthologues (Huet-Calderwood et al., 2014; Fukuda et al., 2014).

In contrast to talin, the kindlin FERM domain contains a pleckstrin homology (PH) domain that is inserted in the F2 subdomain (Kloeker et al., 2004; Shi and Wu, 2008). The PH domain of kindlin interacts with multiple phosphoinositides, especially with phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3] and phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] (Qu et al., 2011; Legate et al., 2013), which supports FA targeting, integrin-meditated adhesion and fibronectin deposition (Qu et al., 2011). In contrast to talin, kindlins contain no obvious actin-binding sites; therefore, linkage to actin is mediated through their binding to the ILK–PINCH–parvin (IPP) complex and/or migfilin (also known as FBLIM1) (Mackinnon et al., 2002; Tu et al., 2003). Whereas the migfilin-binding site remains unknown, the ILK-binding site has been mapped to a short leucine-rich, amphipathic α-helix between the F2 and PH domain of mammalian kindlins (Huet-Calderwood et al., 2014; Fukuda et al., 2014). The differential binding of kindlins to β-integrin tails is discussed in Box 1 and the binding sites for kindlin-interacting proteins are shown in Fig. 1.

Subcellular localisations of kindlins

Kindlins are found in different subcellular compartments. How they are recruited to their sites of action is not known. In cultured cells, kindlins accumulate in all types of integrin adhesion (nascent adhesions, focal adhesions, fibrillar adhesions) and are diffusely present throughout the cytoplasm (Herz et al., 2006; Kloeker et al., 2004; Lai-Cheong et al., 2008; Siegel et al., 2003; Ussar et al., 2006; Tu et al., 2003). In keratinocytes, kindlin-1 and kindlin-2 colocalize to integrin adhesions, and in cardiac muscle cells, kindlin-2 is also present in adherens junctions (Dowling et al., 2008a,b). Staining of other tissues revealed that also colon epithelial cells (Ussar et al., 2008) and keratinocytes (He et al., 2014) contain substantial amount of kindlin-2 – but not kindlin-1 – in cell–cell junctions. Kindlin-1 is also found outside of integrin adhesions; for example, it has been shown to translocate in an integrin- and phosphorylation-dependent manner to centrosomes, where it participates in the assembly of the mitotic spindle (see Box 2) (Patel et al., 2013). In osteoclasts, kindlin-3 is predominantly found in podosomes, which seal a membrane pocket that contains proteases and proteins that are essential for bone resorption (Schmidt et al., 2011). Kindlin-2, probably with the help of its nuclear localization signal (NLS) (Ussar et al., 2006), can localize to the nucleus of smooth muscle cells (Kato et al., 2004) and breast cancer cells (Yu et al., 2012). However, it is not known how nuclear trafficking of kindlin-2 is regulated and which function(s) are executed in the nucleus.

Kindlins and disease

Kindlin-1 in KS and cancer

KS was first described in 1954 by Theresa Kindler as a new subtype of bullous skin disease that is characterized by skin blistering, hyperkeratosis, skin atrophy, photosensitivity and poikiloderma in sun-exposed areas. It took almost 50 years until loss-of-function mutations in the KINDLIN-1 gene were identified as cause of KS (Jobard et al., 2003; Kindler, 1954; Siegel et al., 2003). It is also interesting to note that, since 1954, only around 170 KS patients have been reported. Because the clinical symptoms resemble that of other skin-blistering diseases, it is believed that KS is frequently misdiagnosed and the true occurrence of KS is likely to be much higher (Intong and Murrell, 2012). To date, over 73 distinct

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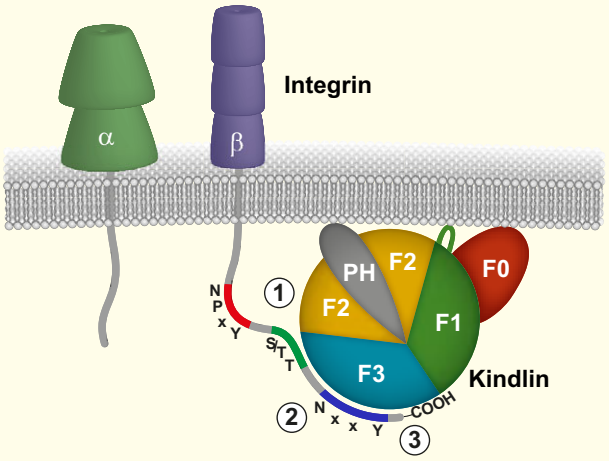
Box 1. Integrin-binding specificity of kindlins

By using pull-down assays with β-integrin-tail peptides, kindlin-1 has been shown to bind β1, β3 and β6 integrins (Bandyopadhyay et al., 2012; Rognoni et al., 2014; Harburger et al., 2009), and kindlin-2 and -3 to bind β1-, β2- and β3-integrin tails (Böttcher et al., 2012; Bledzka et al., 2012; Harburger et al., 2009; Montanez et al., 2008; Moser et al., 2009a, 2008; Ma et al., 2008) at their distal NxxY motifs and adjacent threonine and/or serine residues. Mutational analysis in the kindlin F3 subdomain revealed that a conserved (Q)W motif is essential for binding integrin tails (Fitzpatrick et al., 2014; Harburger et al., 2009; Moser et al., 2009a, 2008; Rognoni et al., 2014). It is believed that phosphorylation of the tyrosine residue of the NxxY motif inhibits binding of kindlin (Bledzka et al., 2010). However, whether the phosphorylation is an important mean to regulate interactions between kindlin and the integrin β-tail *in vivo* is unclear because mice that carry a tyrosine-to-phenylalanine mutation in β1-integrin tails do not display phenotype(s) that resemble a defect in the activation of β1 integrins (Czuchra et al., 2006; Meves et al., 2011). In addition to the (Q)W motif, a carboxylate-binding motif (h-G-h) in the kindlin-F3 subdomain binds the C-terminus of β1-integrin tails. Furthermore, this kindlin-F3 binding site in β1 tail might be responsible for the high affinity of kindlin-2 to the β1 tail and its lower affinities to β3 and β2 tails (Fitzpatrick et al., 2014). It has been shown that the distance between the threonine/serine residues, the NxxY motif and the carboxylate-binding site also influence the affinity of kindlins to β-integrin tails (Bledzka et al., 2012).

Another remarkable finding is that the β6-integrin tail binds kindlin-1 but not kindlin-2 (Bandyopadhyay et al., 2012; Rognoni et al., 2014). The consequence in disease situations – such as in KS that lacks kindlin-1, or in cancer cells that present with high levels of αvβ6 integrin and low levels of kindlin-1 (see below; Rognoni et al., 2014; Sin et al., 2011) – is the inability of kindlin-2 to compensate for loss of kindlin-1 and, hence, to activate αvβ6 integrin. The differential binding specificities of kindlin-1 and -2 for αvβ6 integrins are due to evolutionary splitting of the kindlin family, allowing specific regulation of tissue-specific integrin classes and associated signaling pathways, and even differential regulation of integrins when multiple integrin isoforms are expressed in a cell. Similarly, different affinities of coexpressed kindlins to integrins might represent an mean to opt for those integrins with higher ligand-binding affinity during early cell spreading, or might allow to regulate the subcellular localization of kindlin isoforms. The figure below illustrates binding of kindlins to phosphoinositides through their PH domain and the negatively charged membrane through positively charged regions in their F1 and F0 domains. The β-integrin cytoplasmatic tail binds the FERM F3 subdomain through a serine/threonine motif (1), the membrane distal NxxY motif (2) and the C-terminus (3).



mutations of the KINDLIN-1 gene have been identified, including deletions of parts or the entire gene, splice-site mutations, nonsense and frame shift mutations, which produce premature termination codons and, consequently, lead to nonsense-mediated mRNA decay and a lack of kindlin-1 protein in epithelial cells (Lai-Cheong et al., 2010; Has et al., 2011). Although the course of the disease varies extensively between patients, it is not possible to correlate the manifestation of specific symptoms or the severity of disease to specific mutations in the KINDLIN-1 gene or additional gene mutations. Furthermore, kindlin-2, which is expressed in keratinocytes and shares functional properties with kindlin-1, is unable to reverse the disease, suggesting – in addition to the discovery of the KINDLIN-1 gene as disease causing gene – that kindlin-1 and -2 not only execute similar but also different cellular functions (He et al., 2011a; Ussar et al., 2008; Margadant et al. 2013).

The first symptoms of KS manifest during infancy and are characterized by trauma-induced skin blistering, which is due to the impaired integrin function in basal keratinocytes (Fassihi et al., 2005; Lai-Cheong et al., 2009; Penagos et al., 2004) (Fig. 2A). With age, KS patients develop further skin abnormalities, including hyperkeratotic palms and soles, pronounced skin atrophy – especially on the dorsal side of the hands – and symptoms of premature skin aging, such as cigarette-paper-like wrinkling and webbing between fingers. These symptoms point to a severely perturbed skin homeostasis that is normally maintained throughout life by cutaneous stem cells (SCs), which reside in specialized niches of the epidermis where they are kept in a non-proliferative (quiescent) state. They become periodically activated to self-renew and to provide a pool of transient amplifying or progenitor cells that expand and differentiate into distinct cutaneous cell lineages, such as interfollicular epidermis, sebaceous gland and hair follicle (HF) cells (Fuchs, 2008). The activation of cutaneous SCs is controlled by opposing signaling pathways; on one hand, BMP and TGFβ signaling inhibit proliferation and induce SC quiescence and, on the other hand, Wnt signaling promotes SC activation and differentiation (reviewed in Alonso and Fuchs, 2003; Woo and Oro, 2011). Deregulation of either signaling pathway affects cutaneous SC homeostasis and promotes initiation and development of skin cancer (Arwert et al., 2012; Owens and Watt, 2003).

A comparison between skin phenotypes of mice that lack either expression of kindlin-1 or β1 integrin, or express a kindlin-binding-deficient β1 integrin in cutaneous epithelial cells revealed that all three mouse strains show skin blisters and impaired integrity of the epidermal-dermal basement membrane, indicating that these defects are caused by defective β1-integrin-mediated adhesion of epidermal keratinocytes to the underlying basement membrane (Fig. 2A). These defects are accompanied by tissue-repair-induced inflammation and, as expected, visible in each of the three mouse mutants. However, mice that lack kindlin-1 expression developed additional defects, including aberrant HF cycles and hair development, enlarged SC compartments and cutaneous SC numbers that concomitantly decreased with age, elevated cutaneous SC proliferation and an increased susceptibility to develop skin tumors. None of these defects were apparent in mice that lack β1 integrins or express a kindlin-binding-deficient β1 integrin (Brakebusch et al., 2000; Rognoni et al., 2014; Frank et al., 2005).

Intriguingly, colony-forming efficiency assays of serially cultured primary keratinocytes that had been isolated from KS patients indicated accelerated depletion and premature senescence of SCs, suggesting that, similar to those mice that lack kindlin-1 in keratinocytes (Rognoni et al., 2014), the enhanced SC proliferation eventually leads to SC exhaustion, which is accompanied by a loss of SC-marker expression in the patient skin (Lai-Cheong et al., 2009; Piccinni et al., 2013). Two main defects have been identified in the KS mouse model that contribute to the hyperproliferation of

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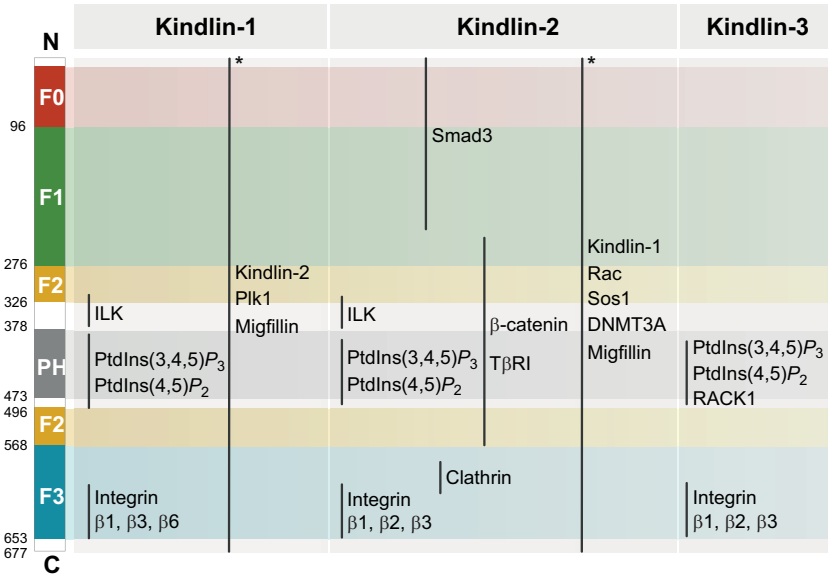

Fig. 1. Kindlin-binding proteins. The domains of kindlins and their amino acid numbers are depicted at the left (from top, N-terminus to bottom, C-terminus). Binding regions of kindlin-interacting proteins are indicated by black vertical bars. Asterisks at bars that span the entire kindlin protein indicate unknown binding regions.

cutaneous SCs. First, activation of αvβ6 integrins was severely impaired and could not be rescued by kindlin-2, which is unable to bind β6-integrin cytoplasmic domains (Bandyopadhyay et al., 2012; Rognoni et al., 2014). αvβ6 integrins on cutaneous SCs have been shown to bind the tripeptide Arg-Gly-Asp (RGD) motif of the TGFβ1 latency-associated peptide (LAP), which results in the release of TGFβ1 (Fig. 2A) and the inhibition of SC proliferation (Rognoni et al., 2014). Second, loss of kindlin-1 leads to an excessively enhanced transcription of Wnt ligands and receptors, resulting in increased canonical Wnt/β-catenin signaling, SC commitment, ectopic HF development and aberrant cycling of HFs (Fig. 2B) (Rognoni et al., 2014). The consequence of reduced TGFβ1 and increased Wnt/β-catenin signaling is an expansion of the cutaneous SC compartment, which is followed by loss of SCs with age. Initial rescue experiments with kindlin-1-deficient mouse keratinocytes indicate that the transcriptional control of Wnts and Wnt receptors can be achieved by a kindlin-1 that is deficient in integrin binding and resides in the cytoplasm (Fig. 2B) (Rognoni et al., 2014). Hence, it is conceivable that kindlin-1 can bind to integrin tails at the plasma membrane and, in addition, binds to and retains transcriptional activators in the cytoplasm. Which transcriptional activators are bound and how they are released will have to be addressed in future studies. Importantly, aberrant TGFβ and Wnt signaling also occurs in KS patients (Rognoni et al., 2014), although ectopic HF formation has, so far, not been reported in KS patients.

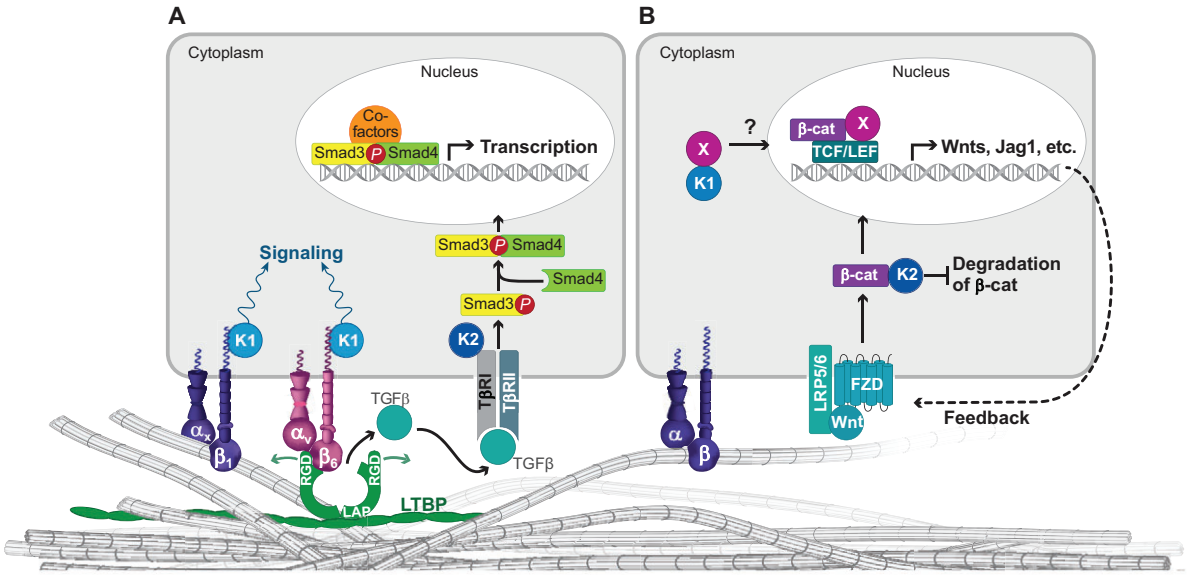

Fig. 2. Kindlins regulate Wnt and TGFβ signaling. (A) Kindlins and TGFβ signaling. Besides mediating adhesion and integrin signaling by activating various β1-class integrins (shared by all kindlin-1), kindlin-1 binds and activates αvβ6 integrin (not shared by kindlin-2). αvβ6 integrin binds the RGD motif of the latency-associated peptide (LAP) that is associated with the latent TGFβ-binding protein (LTBP), and induces the release of TGFβ. Free TGFβ binds to the transforming growth factor beta receptors I and II (TβRI and TβRII), which triggers phosphorylation of Smad3, association with Smad4, translocation into the nucleus and together with cofactors the transcription of TGFβ target genes. Kindlin-2 binds TβRI directly to promote Smad3 phosphorylation and TGFβ signaling. (B) Kindlins and Wnt signaling. Kindlin-1 suppresses Wnt signaling in the cytoplasm in an integrin-independent manner, potentially, by retaining transcriptional cofactors in the cytoplasm. Loss of kindlin-1 leads to Wnt ligand transcription, induction of canonical Wnt signaling and increased expression of Wnt target genes. Kindlin-2 directly binds β-catenin, which inhibits its degradation.

An additional hallmark of KS is the development of poikiloderma, which is defined by the existence of areas of hyperpigmentation, hypopigmentation and erythema (redness of the skin). It is believed that KS-associated poikiloderma is due to an increased photosensitivity and, in line with this hypothesis, it mainly develops in sun-exposed skin areas. The molecular basis for poikiloderma is unknown (Ashton et al., 2004; Penagos et al., 2004). It has been reported that melanocytes of KS patients are highly proliferative; they detach from the epidermis and accumulate in the dermis where they deposit melanin that subsequently becomes engulfed by resident macrophages that, thereby, convert to melanophages (Papa and Kligman, 1965). Interestingly, the KS mouse model also develops severe pigmentation defects (Rognoni et al., 2014) although kindlin-1 is not expressed in melanocytes. This observation suggests that kindlin-1-deficient keratinocytes are responsible for the induction of poikiloderma in KS. In line with this hypothesis, it has been shown that melanocyte homeostasis in skin is crucially dependent on Wnts and TGFβ1 (Nishimura et al., 2010; Rabbani et al., 2011), which are aberrantly released by kindlin-1-deficient epithelial SCs (Fig. 2).

A direct consequence of the SC hyperactivation in KS is that patients also have a higher risk of developing skin cancer – mainly squamous cell carcinomas (SCCs) and often at an early age (Arita et al., 2007; Emanuel et al., 2006; Lotem et al., 2001; Mizutani et al., 2012). Although an impaired β1-integrin function has been shown to prevent the formation of skin tumors (Janes and Watt, 2006; Frank et al., 2005), aberrant Wnt and TGFβ signaling caused by loss of kindlin-1 is probably a important reason for the increased risk of tumor development (Beronja et al., 2013; Malanchi et al., 2008; Guasch et al., 2007).

Although kindlin-1 appears to have a tumor suppressor function in cutaneous epithelial cells, KINDLIN-1 mRNA levels are increased in the majority of lung, breast and colon cancers (Sin et al., 2011; Weinstein et al., 2003), and in several pancreatic cancer cell lines, where it promotes carcinogenesis by inducing migration and invasion (Mahawithitwong et al., 2013a) (Table 1). In these cancers, kindlin-1 exerts an oncogenic effect that appears to be strongly dependent on TGFβ signaling (Sin et al., 2011). Hence, it is tempting to speculate that the increased TGFβ signaling in these tumors is caused by an enhanced kindlin-1-mediated activation of αvβ6 integrin, with subsequent TGFβ1 release and TGFβ-receptor signaling that leads to epithelial-to-mesenchymal transition (EMT) and increased tumor cell invasion and metastasis. Notably, expression of kindlin-1 and αvβ6 integrin is regulated by TGFβ1, pointing to a possible feedback loop that reinforces the pro-migratory properties of kindlin-1 (Kloeker et al., 2004; Wang et al., 1996) (Fig. 2A). Thus, the dual role of kindlin-1 during tumor development resembles the paradoxical effects of TGFβ1 signaling in cancer (reviewed in Chaudhury and Howe, 2009; Massagué, 2008); although kindlin-1 suppresses the formation of early tumors by inducing the release of TGFβ1 and inhibiting the transcription of Wnts, it can promote tumor progression through the same signaling pathways as well as by increasing integrin activity and signaling. In future studies it will be important to elucidate whether the tumor-promoting function of kindlin-1 mainly depends on coexpression with αvβ6 integrin, or whether further signaling pathways are affected.

Although KS has initially been described as a skin disorder, KS patients also suffer from an ulcerative colitis-like condition (Kern et al., 2007; Ussar et al., 2008). The colitis-like condition is much more severe in mice compared with humans; in the latter symptoms are usually mild and the condition is rarely lethal. The reason for this species difference is unclear. Kindlin-1 is also highly expressed in the kidney epithelial cells (Ussar et al., 2006) but no kidney defects have been reported in KS patients and in the KS mouse model, suggesting that, in this tissue, loss of kindlin-1 is probably compensated for by kindlin-2.

Kindlin-2 is involved in multiple diseases

Kindlin-2 is widely expressed and can be found in several cell types, such as primitive endoderm, mesenchymal cells, and others that do not have other kindlin isoforms. Therefore, it is not unexpected that loss of kindlin-2 in mice leads to peri-implantation lethality (Dowling et al., 2008a; Montanez et al., 2008). Studies of kindlin-2-deficient embryoid bodies revealed that the main defects at the peri-implantation stage include detachment of the primitive endoderm and epiblast from the basement membrane, lack of cavitation and defective cell survival (Montanez et al., 2008). Kindlin-2 is highly expressed in cardiac and skeletal muscle (Dowling et al., 2008a; Ussar et al., 2006) where it promotes muscle elongation and muscle cell fusion in an integrin-dependent manner (Bai et al., 2008; Dowling et al., 2008a,b). The ability of kindlin-2 to regulate the expression of the myogenic regulatory factor myogenin implicated the protein in muscle cell differentiation. Mechanistically, kindlin-2 was shown to form a complex with β-catenin and Tcf4 and to bind the myogenin promoter, thereby enhancing myogenin expression (Yu et al., 2013a).

In skin, kindlin-2 promotes dermal fibroblast adhesion and formation of cell–cell contacts between keratinocytes. The role in keratinocytes has been addressed by using organotypic cultures of human keratinocytes, which revealed an accumulation of kindlin-2 at adherens junctions where kindlin-2 regulates Rho GTPases and the cytoskeleton (He et al., 2014). The role of kindlin-2 in dermal fibroblasts was analyzed in healing wounds. Kindlin-2 becomes highly expressed in activated myofibroblasts, where it regulates FA formation, the organization of α-smooth muscle actin into stress fibers and the transmission of force (He et al., 2011b).

Elevated kindlin-2 expression was also observed in tubular intestinal fibrosis (TIF) of the kidney. This kidney fibrosis is characterized by massive expansion of the cortical interstitium, conversion of fibroblasts into myofibroblasts and progressive EMT of tubular epithelial cells (Bielesz et al., 2010). In affected mouse and human tubular epithelial cells, kindlin-2 is highly expressed and promotes EMT by increasing Erk1/2, Akt and TGFβ signaling. It has been demonstrated that kindlin-2 induces Ras activation through the recruitment of Son of sevenless homolog 1 (Sos1), which, subsequently, activates Erk1/2 and Akt signaling (Wei et al., 2014). Furthermore, kindlin-2 has been shown to activate the TGFβ signaling pathway through direct binding of its C-terminal FERM domain to transforming growth factor β receptor I (TβRI; also known as TGFBR1) and of its N-terminus to Smad3 (Wei et al., 2013). How these distinct kindlin-2-binding events are regulated and whether they occur at the same time in tubular epithelial cells remains elusive.

Kindlin-2 is also highly expressed in endothelial cells and is required for angiogenesis and blood vessel homeostasis. These functions are achieved by promoting integrin-mediated adhesion and migration during angiogenic sprouting (Pluskota et al., 2011). In these cells, kindlin-2 triggers endocytosis and recycling of cell-surface enzymes, such as adenosine triphosphate diphosphohydrolase (CD39) and Ecto-5′-nucleotidase (CD73), through interaction with the clathrin coat, thereby, modulating ATP/ADP catabolism, which indirectly affects platelet aggregation and hemostasis (Pluskota et al., 2013).

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Table 1. Role of kindlins in tumor development and progression

Kindlin isoform	Tumor type	Effect	Molecular mechanism	Reference
Kindlin-1	Breast cancer	Increased expression promotes tumor growth and invasion	Induces TGFβ signaling and controls TGFβ induced EMT	Sin et al., 2011
	Colon cancer	Increased expression	Unknown	Weinstein et al., 2003
	Hepatocellular carcinoma	Increased expression	Unknown	Ma et al., 2015
	Lung cancer	Increased expression inhibits EMT	Unknown	Weinstein et al., 2003; Zhan et al., 2012
	Pancreatic cancer	Increased expression promotes migration and invasion	Unknown	Mahawithitwong et al., 2013a,b
	Skin cancer	Loss of expression promotes tumor formation	Induces αvβ6-integrin-mediated TGFβ release and Wnt ligand expression	Rognoni et al., 2014
Kindlin-2	Bladder cancer	Highly expressed in invading tumor and stromal cells	Unknown	Talaat et al., 2011
	Breast cancer	Increased expression promotes genome instability	Unknown	Zhao et al., 2013
		Increased expression promotes cell migration and cancer progression	Promotes EGF signaling by interact with EGFR kinase domain inhibiting its degradation	Guo et al., 2015
	Colon cancer	Reduced expression promotes tumor growth and migration	Kindlin-2 promotes β-catenin degradation via GSK3β phosphorylation	Ren et al., 2015
	Esophageal squamous cell carcinoma	Increased expression promotes tumor invasion	Expression is regulated by miR-200b promoting migratory phenotype	Zhang et al., 2014
	Gastric cancer	Increased expression promotes migration and proliferation	Unknown	Shen et al., 2012, 2013
	Hepatocellular carcinoma	Expression increased	Unknown	Ge et al., 2015
	Lung cancer	Increased expression promotes EMT	Unknown	Zhan et al., 2012
	Malignant mesothelioma	Increased expression promotes cell proliferation and invasion	Unknown	An et al., 2010
	Mesenchymal tumors	Increased expression suppresses invasion	Inhibits secretion of uPA	Shi and Wu, 2008
	Pancreatic ductal adenocarcinoma	Increased expression in peritumoral stroma	Unknown	Mahawithitwong et al., 2013a,b
		Increased expression promotes tumor progression	TGFβ induced kindlin-2 promotes TβRI and inhibits HOXB9 and E-cadherin expression	Zhan et al., 2015
	Prostate cancer	Increased expression promotes cell survival	Positively regulates Gli1 expression in a feedback loop	Gao et al., 2013
	Serous epithelial ovarian cancer	Reduced expression promotes tumor progression through MET inhibition	Induces up regulation of estrogen receptor α which enhances E-cadherin expression	Ren et al., 2014
Kindlin-3	B-cell malignancies	Increased expression	Unknown	Boyd et al., 2003
	Breast cancer	Reduced expression promotes metastasis formation	Reduces β3 integrin activity decreasing cell attachment	Djaafri et al., 2014
	Melanoma	Reduced expression promotes metastasis formation	Reduces β3 integrin activity decreasing cell attachment	Djaafri et al., 2014
		Loss of expression increases cell invasion	EMMPRIN regulates kindlin-3 and β1 integrin expression	Delyon et al., 2015

Kindlin-2 is also involved in tumor development and progression (Table 1). Similarly to kindlin-1, kindlin-2 can exert tumor-promoting or tumor-inhibiting functions. These opposing outcomes appear to be tumor-type-dependent (Table 1). For example, in breast cancer (Gozgit et al., 2006), pancreatic ductal adenocarcinomas (Mahawithitwong et al., 2013b), malignant mesothelioma (An et al., 2010) and bladder cancer (Talaat et al., 2011), kindlin-2 expression levels correlate with tumor invasion, lymph node metastasis and poor disease outcome. One main oncogenic effect of kindlin-2 appears to be the promotion of EMT by activating Erk1/2, Akt and TGFβ signaling, which also play a role in TIF (see above). Kindlin-2 has been shown to stabilize EGF receptors in breast cancer cells, which could provide an explanation for its ability to induce activation of Erk1/2 and Akt (Guo et al., 2015). Interestingly, kindlin-2 also triggers EMT and invasion of breast cancer cells by either promoting Wnt signaling by stabilizing β-catenin and facilitating the formation of a nuclear Tcf4–β-catenin complex (Yu et al., 2012), or by silencing the expression of the microRNA-200 (miR-200) family member miR-200b through binding to DNA methyltransferase 3A (DNMT3A) and by hypermethylating CpG islands in the miR-200b promoter region (Yu et al., 2013b). Interestingly, in esophageal SCC, kindlin-2 itself might be a target for miR-200b, resulting in decreased kindlin-2 levels and tumor cell invasion (Zhang et al., 2014). In addition to regulation of EMT and invasion, kindlin-2 has been shown to promote cell survival through stimulation of Hedgehog signaling by inducing the expression of

Gli1 and anti-apoptotic Bcl2 proteins (Gao et al., 2013; Gong et al., 2010; Shen et al., 2013). Kindlin-2 has also suggested to enhance genome instability, a hallmark of cancer formation, although it is not clear how this is achieved at the molecular level (Zhao et al., 2013).

It has also been reported that, in mesenchymal tumor cells, high kindlin-2 levels suppress invasion through inhibition of urokinase-type plasminogen activator (uPA) secretion (Shi and Wu, 2008) and impair tumor progression of serous epithelial ovarian cancer through up-regulation of estrogen receptors, which leads to mesenchymal-to-epithelial transition (MET) and, as a consequence, to reduced tumor cell dissemination (Ren et al., 2014).

It should be noted that many tumor cells express more than one kindlin isoform and that tumors can increase the expression of kindlin-2 in stromal cells (Talaat et al., 2011) (Table 1). Furthermore, the presence of several kindlin isoforms in a tumor cell can either result in functional cooperation or neutralization of their respective functions. Indeed, it has been reported that kindlin-1 and kindlin-2 have opposite effects in the progression of lung cancer; whereas kindlin-1 inhibits EMT and is associated with the differentiation status of non-small-cell lung cancer cells, kindlin-2 expression correlates with invasiveness and poor disease prognosis (Zhan et al., 2012).

Kindlin-3 in hematopoietic dysfunctions and LAD III

Kindlin-3 is highly expressed in all hematopoietic cells (Ussar et al., 2006; Ruppert et al., 2015). The analyses of the kindlin-3-deficient mouse strain eventually uncovered the role of kindlin-3 in integrin activation and placed kindlins into the center stage of integrin research. Kindlin-3-deficient mice die shortly after birth and suffer from severe hemorrhages, anemia, marked leukocytosis, loss of hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs) from the bone marrow (BM), as well as from pronounced osteopetrosis (Fig. 3) (Moser et al., 2008, 2009a; Schmidt et al., 2011; Ruppert et al., 2015). *In vitro* studies of kindlin-3-deficient platelets, neutrophils and osteoclasts revealed a complete functional abrogation of integrins. The role of kindlin-3 in the activation and functions of leukocyte integrins was corroborated with intravital imaging of TNF-α-stimulated cremaster-muscle venules, which revealed that adhesion of leukocytes to inflamed endothelial cells was dramatically impaired (Moser et al., 2009a). In effector T cells, the kindlin-3-mediated integrin activation step appears to be particularly important when the expression levels of integrin ligands on endothelial cells are low (Moretti et al., 2013; Morrison et al., 2013). Hence, in experimental autoimmune encephalitis (EAE), a model for multiple sclerosis with a high expression of integrin ligand in the inflamed tissue, inhibition of kindlin-3 is probably not sufficient to block extravasation of auto-reactive T cells and disease progression (Moretti et al., 2013). Kindlin-3 is also required for B-cell adhesion under flow (Willenbrock et al., 2013), trafficking into lymph nodes and immunoglobulin expression (Morrison et al., 2015). Furthermore, dendritic cells display enhanced granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor/Syk signaling associated with an accumulation of mature, migratory dendritic cells in lymphoid organs and an increased Th1 immune responses *in vivo* when the kindlin-3 interaction with β2 integrins is impaired (Morrison et al., 2014).

The presence of hemorrhages, leukocyte adhesion defects, loss of HSCs and HPCs in the BM and osteopetrosis are all hallmarks of leukocyte adhesion deficiency type III (LAD III). The similarity of the defects prompted several laboratories to investigate whether LAD III patients carry mutations in their KINDLIN-3 genes (Kuijpers et al., 2009; Malinin et al., 2009; Svensson et al., 2009; Mory et al., 2008). Indeed, in all investigated cases so far, LAD III was caused by loss of KINDLIN-3 expression. The symptoms reported in LAD III patients are primarily caused by defective integrin functions. In addition to LAD III, two further subtypes of LAD (LAD I and LAD II) exist in humans, each caused by different gene mutations and affecting different aspects of the

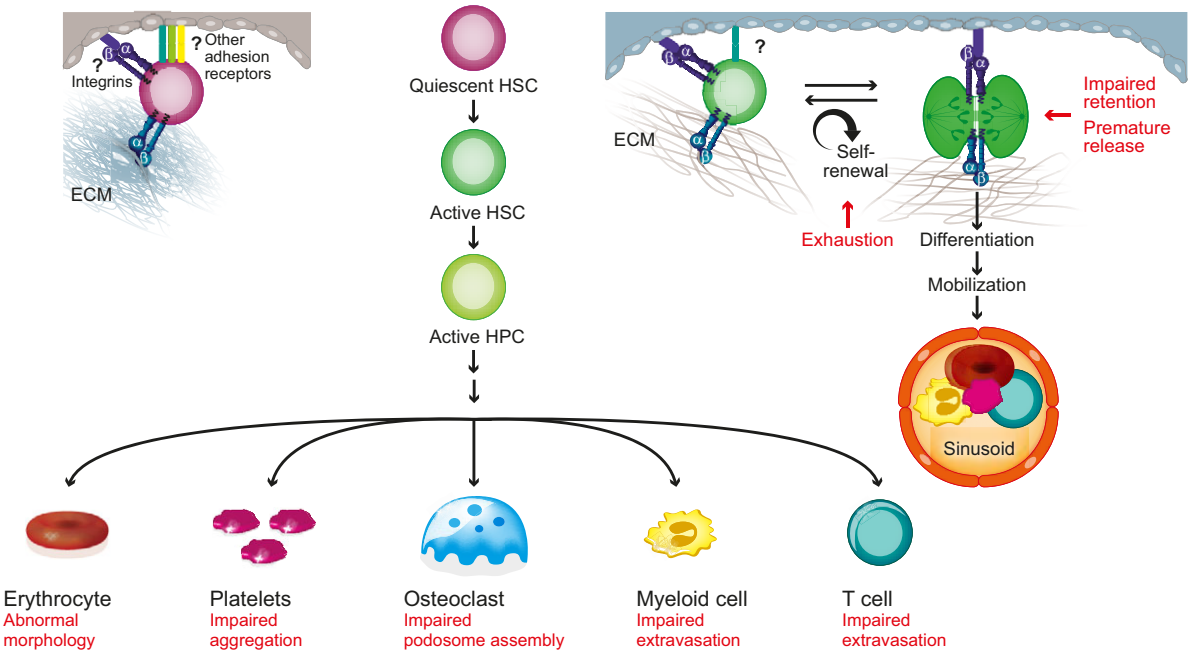

Fig. 3. Defects in kindlin-3-deficient hematopoietic cells. Activation of integrins through kindlin-3 is not required to maintain quiescent HSCs in their niche (top left), whereas activated (proliferating) HSPCs require kindlin-3 for their retention and to prevent their premature release into the blood circulation (top right). Hematopoietic effector cells also require kindlin-3 for their functions (see main scheme). Effects of kindlin-3 loss are indicated in red.

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leukocyte adhesion cascade. LAD I is due to loss of β2-integrin expression, which prevents firm adhesion of leukocytes but does not interfere with platelet function. LAD II is caused by mutations in a gene encoding a specific Golgi GDP-fucose transporter whose absence leads to a loss of selectin ligands and defective leukocyte rolling. Although LAD I, II and III are caused by mutations in different genes, they all lead to impaired wound healing, marked leukocytosis and recurrent bacterial infections owing to a severely compromised inflammatory response (Hanna and Etzioni, 2012).

Although kindlin-3 is highly expressed in all hematopoietic cells, including quiescent and active HSCs and HPCs, loss of kindlin-3 expression impairs retention of active, proliferating HSCs and HPCs in the BM but is dispensable for retention of the small pool of quiescent HSCs (Fig. 3) (Ruppert et al., 2015). Quiescent HSCs localize to specific niches and ensure a functional hematopoietic system throughout the life of an organism. Why the absence of kindlin-3 expression does not affect their BM retention or their quiescence is unclear. It is possible that other adhesion molecules, such as CD44, selectins or N-cadherin, compensate for the absence of active integrins and retain quiescent HSCs in their specific BM niche. It is also conceivable that the niches of quiescent and active HSCs are different (Morrison and Scadden, 2014), and that only the latter niche is equipped with integrin ligands. Finally, it is also possible that, during mitosis, HSCs have low adhesion strength, particularly if integrins – similar to what has been shown for non-hematopoietic cells – can translocate to the mitotic furrow in order to enable cell rounding and cell division (Yamaguchi et al., 1998; Pellinen et al., 2008). The consequence of the impaired retention of kindlin-3-deficient activated HSCs and HPCs is an increase of their numbers in the circulation, which was also observed in a LAD III patient (Ruppert et al., 2015).

It is interesting to note that the absence of β1-integrin expression on HSCs abrogates extravasation and homing of HSCs to the BM (Potocnik et al., 2000), whereas the homing of kindlin-3-deficient HSCs into the BM was significantly diminished but not abolished. Therefore, it appears that the few integrins that adopt an active conformation in the absence of kindlin-3 are sufficient to allow the low number of kindlin-3-defcient HSCs to bind to the vascular wall and allow homing to their BM niches.

The analyses of kindlin-3 in HSCs disclosed remarkable differences in the role of kindlins in the homeostasis of tissue and HSCs. In cutaneous SCs, kindlin-1 regulates the Wnt/β-catenin and TGFβ signaling pathways to maintain their quiescence (Rognoni et al., 2014), whereas – in HSCs – kindlin-3 does not appear to regulate either Wnt/β-catenin or TGFβ signaling. A reasonable explanation for these differences could be that kindlins do not share their integrin-independent functions. Alternatively, the TGFβ1-releasing integrin αvβ6 could be expressed on cutaneous SCs but not on HSCs (Klimmeck et al., 2012; Yamazaki et al., 2011).

Beside the important function of kindlin-3 in HSC homeostasis, to date there are only few reports that point to a role of kindlin-3 in blood cell malignancies (Table 1). A proteomic screen revealed high expression of kindlin-3 in various B-cell malignancies, including B-cell lymphoma, chronic lymphocytic leukemia and Hodgkin lymphoma (Boyd et al., 2003). However, the impact(s) of the elevated kindlin-3 levels are elusive. Another study reported increased levels of kindlin-3 in human breast tumors that result in increased activity of β1 integrin and Twist-induced expression of vascular endothelial growth factor (VEGF), which, in turn, promotes primary tumor growth, angiogenesis and lung metastasis (Sossey-Alaoui et al., 2014).

Conclusions and future perspectives

After the discovery of the kindlin family, research has mainly focused on the proteins prime and common functions in integrin activation, clustering and outside-in signaling. Despite the numerous papers that implicated kindlins in integrin activation, the molecular mechanisms of integrin-tail binding, the mode of cooperation with talin, mechanism(s) leading to kindlin activation and deactivation, and their involvement in mechano-signaling are far from understood.

The different subcellular localizations of kindlins and their association with tumor development point to functions that operate in an integrin-independent manner. The dissection of integrin-independent functions are at their beginnings, and questions regarding the regulation of signaling pathways, such as Wnt and TGFβ signaling, how kindlins are recruited and regulated in different subcellular compartments, and how post-translational modifications impact their functions and localizations, have – so far – not been satisfactorily addressed. It is clear that kindlins can become phosphorylated at multiple sites and are subject to calpain- and caspase-mediated cleavage in different cellular contexts. However, it is unlikely that phosphorylation is the only post-translational modification and, therefore, the search for additional modifications, such as sumoylation or ubiquitylation, will help to uncover further regulation of kindlin functions, trafficking and localization (see Box 2).

Further interesting questions to explore in the future are kindlin-isoform-specific mechanisms and/or functions, whether specific kindlin functions are conserved between different cell types and how two mechanisms that affect the same signaling pathway are controlled within a cell. It is still unclear why so many polarized cells, such as keratinocytes, express two kindlin isoforms that cannot compensate for each other. Is it because the kindlin protein levels are too low, or do they carry out very different functions that did not permit evolvement of compensatory mechanisms? Clearly, an exciting time lies ahead for all kindlin researchers!

Box 2. Post-translational modifications of kindlins

The function and, possibly, also the subcellular localization of kindlins are regulated by post-translational modifications, such as phosphorylation and proteolytic cleavage. Bioinformatic analysis of the kindlin sequence predicted multiple highly conserved phosphorylation sites (Herz et al., 2006) and, in addition, evidence is accumulating that kindlins are prominent targets of multiple signaling kinases. For example, in keratinocytes, a fraction of kindlin-1 is phosphorylated by casein kinase-2, a serine/threonine kinase that is involved in the regulation of the cytoskeleton (Herz et al., 2006). In breast cancer cells, recruitment of kindlin-1 to the centrosome is controlled through phosphorylation of threonine-30 by the Polo-like kinase 1 (PLK1) (Patel et al., 2013). In platelets and leucocytes, the integrin–kindlin-3 interaction appears to be regulated by calpain-mediated cleavage of kindlin-3 at tyrosine-373, which is located in the N-terminal part of the kindlin-3 PH domain (Zhao et al., 2012). Expression of a calpain-resistant kindlin-3 leads to stronger adhesion and inhibits cell migration by stabilizing the association of kindlin-3 with the integrin tail. Thus, it is possible that calpain-mediated cleavage promotes the removal of kindlin-3 from activated integrins in order to free the binding site for other integrin-binding partner(s); alternatively, it could promote integrin inactivation to terminate integrin–matrix interactions. In addition to calpain, also caspase-3 has recently been reported to cleave kindlin-3. The cleavage site has been mapped to the N-terminus at aspartic acid 344 and was detected in wound exudates where it, potentially, can promote apoptosis of hematopoietic cells leading to suppression of the inflammatory response (Sabino et al., 2015).

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Competing interests
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