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When integrins fail to integrate

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Three studies implicate Kindlin-3, a molecule that mediates signaling through integrins, in a rare disorder characterized by spontaneous bleeding and susceptibility to infection

The function of integrins, a major class of cell adhesion molecules, can rapidly switch on blood cells from a resting low-affinity state to an active high-affinity conformation. Tight regulation of integrin activity ensures normal blood flow and leukocyte trafficking and mediates rapid adhesion during events such as the formation of a platelet plug after vessel injury.

Several naturally occurring mutations can inactivate the platelet β3 integrin, leading to Glanzmann's thrombasthenia characterized by spontaneous bleeding due to defective platelet aggregation, whereas patients with homozygous mutations in the β2 integrin (CD18) have recurrent infectious complications due to leukocyte adhesion deficiency type 1 (LAD1)[1]. More recently, patients exhibiting both Glanzmann's- and LAD-like symptoms have been described in which the expression of integrins is normal but integrin activity on leukocytes and platelets is defective[2]. This rare but devastating syndrome is termed LAD1-variant or LAD3.

The syndrome was thought to result from altered signal transduction downstream from G protein-coupled receptors (GPCRs), since their chemokine ligands, major integrin activators, are unable to activate integrins on leukocytes and platelets isolated from people affected with this condition. One potential culprit has been the diacylglycerol-regulated guaninenucleotide exchange factor I (CalDAG-GEF1, encoded by *Rasgrp2*); mice lacking this protein exhibit an LAD3-like phenotype[3] and a homozygous splice junction mutation in the *Rasgrp2* gene has been observed in people with the disease[4].

In this issue of *Nature Medicine*, three different groups point to a more likely culprit. They provide strong evidence that a deficiency in Kindlin-3, a molecule downstream of GPCRs that helps coax integrins into an active conformation, underlies LAD1-variant / LAD3 (refs).

The Kindlins comprise three members named after Theresa Kindler who first described a congenital skin blistering condition that combined clinical features of hereditary epidermolysis bullosa and poikiloderma congenitale[5]. The Kindler syndrome is caused by

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loss-of-function mutations in Kindlin-1 (encoded by *FERMT1*) which participates in the integrin-dependent anchorage of the actin cytoskeleton within focal adhesions. In mice, deficiency in Kindlin-2 (*Fermt2*$^{-/-}$) leads to a failure of the early embryo to implant in the uterus, due to impaired integrin activation in embryonic stem cells[6].

Kindlin-3 is expressed in leukocytes and mice lacking the molecule (*Fermt3*$^{-/-}$) have defects in platelet aggregation and resistance to arterial thrombosis[7]. These observations led to the question of whether Kindlin-3 is involved in the LAD1-variant / LAD3 syndrome.

The report by *Svensson et al.* indeed describes *FERMT3* mutations in three subjects diagnosed with LAD3, resulting in undetectable expression of the protein(REF). Whereas two of these subjects also presented a mutation in *RASGRP2*, no such mutation was found in the third patient.

In the study by *Malinin et al.(REF)*, two siblings displaying classical LAD3 symptoms were found to harbor a distinct mutation in the *FERMT3* gene and no Kindlin-3 protein expression, whereas expression and function of CalDAG-GEF1 was normal. Importantly, complementation studies in both reports revealed that the enforced expression of Kindlin-3, but not CalDAG-GEF1, restored the adhesive deficiencies in cells lines derived from the patients.

Interestingly the two subjects studied by *Malinin et al.* both had ostepetrosis (e.g. a condition producing abnormal thickening of the bones). (REF of *Malinin et al.*), which was suggested to originate from an enhanced capacity of mesenchymal stem cells to generate bone. However, because Kindlin-3 expression is restricted to the hematopoietic lineage[7], the cause of the osteopetrosis might lie with the osteoclast, a hematopoietic cell mediating αVβ3 integrin-dependent bone resorption[8].

Experiments in mice by *Moser et al.*(REF) delved into the mechanism by providing insight into how Kindlin-3 deficiency might lead to disease. As with the LAD3 human cells, leukocytes from mice deficient in Kindlin-3 exhibited impaired adhesion to and spreading on ligands for β2 integrins. This translated into defective arrest of myeloid leukocytes in inflamed venules *in vivo*, and impaired extravasation during inflammation, defects which persisted even after stimulation with chemokines. The phenotype of *Fermt3*$^{-/-}$ mice recapitulated that of mice deficient in adhesion receptors required for leukocyte binding (e.g., selectins or β2-integrins), and explains the increase in leukocyte counts and human immunodeficiency reported in the other studies.

The findings in Nature Medicine dovetail with two other recent studies. These have described mutations in the *FERMT3* gene in all LAD3 subjects tested (total of 12 patients)[9,10]. Whereas the majority of the patients also harbored the same mutation in *RASGRP2*, two patients did not.

Despite the wealth of information, these studies do not completely exclude a role for *RASGRP2*. Since the *RASGRP2* and *FERMT3* gene loci are closely positioned on human chromosome 11, Kuijpers et al. suggested that the *RASGRP2* mutation may represent an innocuous intronic SNP transmitted through an ancestral haplotype derived from affected

Turkish families[9]. Nonetheless, it remains possible that *RASGRP2* dysfunction may indeed produce LAD3-like symptoms since both proteins operate in the same signaling axis (Figure 1) and mice lacking *Rasgrp2* have an LAD3-like phenotype.[3] Owing to their chromosomal proximity (within ~ 600 Mbp on mouse chromosome 19), it is also possible that the knockout mutation in the mouse *Rasgrp2* locus might have affected the expression of neighboring genes, although these effects are usually detectable within shorter chromosomal distances[11]. Analyses of the Kindlin-3 expression levels in *Rasgrp2*$^{-/-}$ mice will help resolve such issues.

A major challenge will be to elucidate how Kindlin-3 regulates integrin activation. *Moser et al.*(REF), set the stage for future experiments, showing that Kindlin-3 interacts with the cytoplasmic tails of β1, β2 and β3 integrins at a distal NxxY/F motif, distinct from the membrane proximal NxxY/F motif recognized by Talin (Figure 1). Importantly, all *FERMT3* mutations reported by *Svensson et al.* and *Malinin et al.(REF)* affect the subdomain that mediates the interaction with these motifs. Previous studies with Kindlin-2 support a mechanism in which Kindlin-3 binding to the integrin cytoplasmic tail promotes Talin binding to the proximal motif, thus leading to integrin activation[6]. Furthermore, Moser et al. found that both Kindlin-2 and -3 are required for transducing signals upon integrin engagement (*outside-in* signaling) that allow lamellipodia formation, spreading over a substrate and, in the case of neutrophils, generation of oxygen radicals[6]+*Moser*(REF). Therefore, interactions of Kindlin-3 with the integrin cytoplasmic tail may allow the transmission of signals in both directions.

It is intriguing that Moser et al. have found that *Fermt3*$^{-/-}$ fetal liver-derived hematopoietic stem cells could efficiently reconstitute the hematopoietic system of lethally irradiated mice (*Moser et al. REF*), a process thought to require β1 and β2 integrin functions. This finding suggests the possibility of differential requirements of integrin subtypes for Kindlin-3 and or the presence of compensatory mechanisms. As the analyses thus far have focused on myeloid cells, studies of other leukocyte subsets, such as lymphoid and progenitor cells, may shed new light in this regard.

The discovery of this cytoplasmic link provides an important piece in the puzzle of integrin activation, and broadens our understanding of the role of integrin modulation in health and disease.

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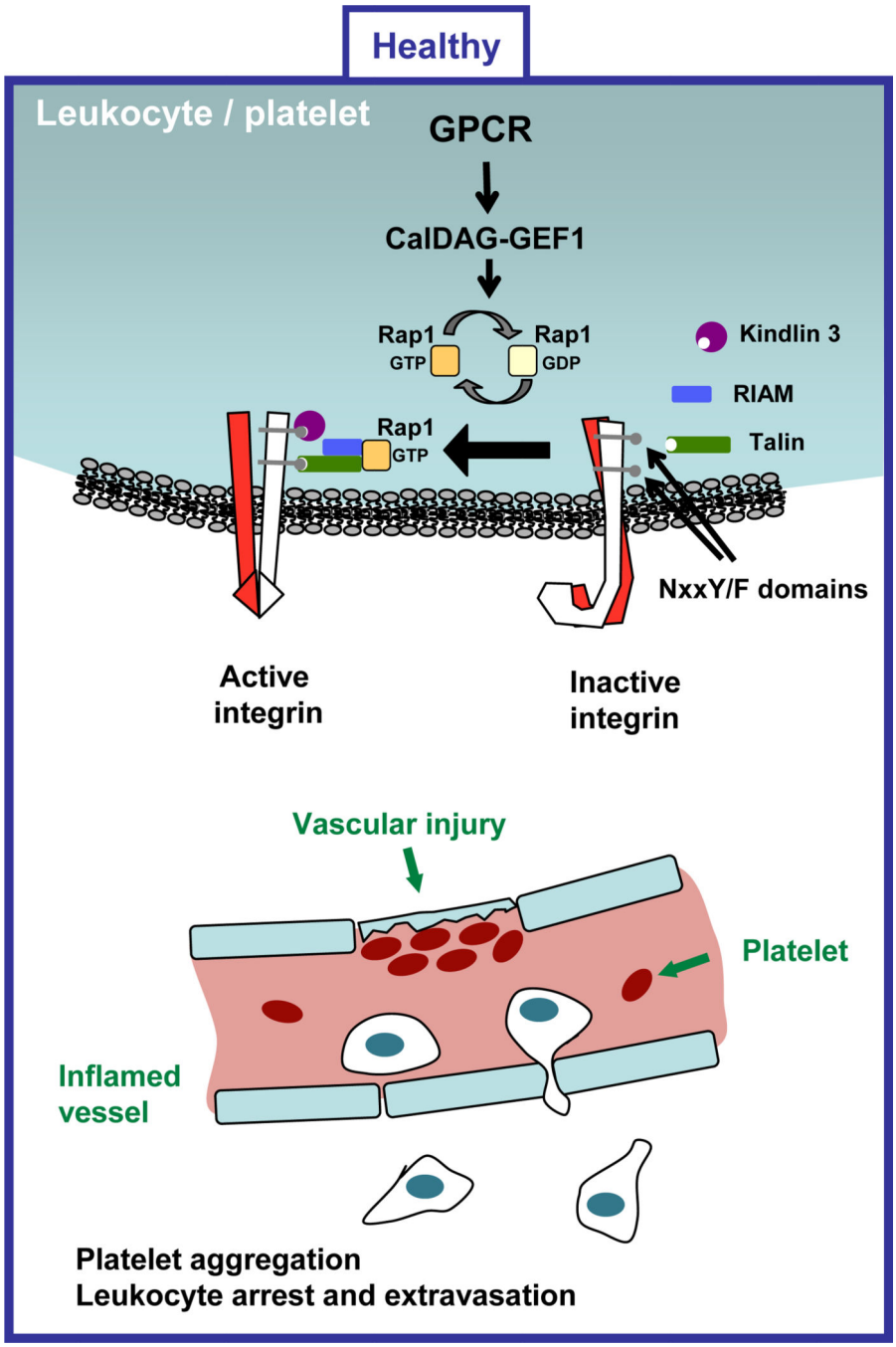

Figure 1.

In healthy individuals, integrin activation in leukocytes and platelets is induced by extracellular stimuli through G protein-coupled receptors (GPCR) and transduced by the CalDAG-GEF1 and Rap1 pathway, which triggers the binding of a complex containing activated Rap1, Rap1-GTP–interacting adaptor molecule (RIAM), and Talin to NxxY/F motifs within the cytoplasmic tail of the β integrin chain. This allows the switch from low (inactive, bent form) to high affinity conformation (active, extended form) of integrins, mediating leukocyte arrest or platelet aggregation in inflamed or injured vessels (left).

Kindlin-3 also binds to NxxY/F motifs, and in its absence, leukocyte and platelet integrins remain in low affinity conformation (LAD1-variant / LAD3, right). Kindlin-3 was clearly identified by three independent studies in this issue as the molecular basis of the known cases of LAD1-variant / LAD3. Nonetheless it remains possible that defects in other molecules involved in the inside-out integrin activation cascade, such asCalDAG-GEF1l, could produce a similar syndrome.