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New insights into the versatile roles of platelet FlnA

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Abstract

Recent findings have identified critical roles for the actin filament-crosslinking protein filamin A (FlnA) in platelets and megakaryocytes. This short review focuses on the structure of FlnA and its interaction with the Von Willebrand Factor (VWF) receptor GPIb-IX-V complex and the fibrinogen receptor, the integrin α IIB β 3 in platelets.

Keywords

FlnA; GPIb-IX-V complex; integrin α IIB β 3; Syk

Introduction

Filamin A (FlnA, previously known as actin-binding protein 280, ABP-280) plays a critical role in cell motility and signaling, as it cross-links actin filaments, tethers membrane glycoproteins, and serves as a scaffold for signaling intermediates (1). In platelets, FlnA links the Von Willebrand Factor (VWF) receptor GPIb-IX-V complex and integrins to the underlying actin cytoskeleton.

FlnA is encoded by the *FLNA* gene located on chromosome Xq28. *FLNA* mutations causing early truncation of FlnA are typically embryonic lethal in males, due to cardiovascular defects and hemorrhage, and lead to the brain malformation disorder, periventricular heterotopia (PH) in females. Missense mutations cause otopalatodigital (OPD) syndrome spectrum disorders, primarily affecting skeletal development (2).

In mice, FlnA deficiency is embryonic lethal in hemizygous males, due to pericardiac and visceral hemorrhage, severe cardiac structural defects, and aberrant vascular patterning (3, 4). Heterozygous *FLNA*^{+/-} females have a mild thrombocytopenia, and 95% of their platelets contain FlnA, indicating a critical role for FlnA in platelet circulation (5).

FlnA structure

FlnA consists of two subunits of 2646 amino acids (aa) and 280 kDa that self-associate (Figure 1A). It contains a tandem calponin-homology actin-binding domain (ABD) at the N-terminus, followed by 24 immunoglobulin-like repeats of 90–100 aa, the C-terminal of

which (repeat 24) mediates dimerization (6). Two calpain-sensitive hinges separate FlnA repeats into rod 1 (repeats 1–15), rod 2 (repeats 16–23), and the self-association domain. Rod 1 is elongated and linear, and binds to actin filaments, independently of the ABD (6). Rod 2 has a more compact structure, due to interaction between repeats, and contains most of the binding sites for FlnA partners, *e.g.* GPIb α and β 3 (7, 8).

Each of FlnA 24 repeats is structurally composed of seven β -strands (A-G), of which strands C and D are particularly important for FlnA partner binding and dimerization. Anti-parallel dimerization of repeat 24 is mediated by hydrophobic stacking of aa 2591–2593 (LVG) in strands C and hydrogen bonding between aa 2602–2608 (EEILVKH) in strands D (9, 10). In repeats 17, 19, 21 and 23, β -strands C and D form a hydrophobic groove for proteins containing an hydrophobic “FlnA-binding motif” of nine aa that also form a β -strand (7, 8) (Figures 1B & 1C). However, repeats 17, 19 and 21 are paired with incompletely folded repeats 16, 18 and 20, respectively (11, 12). Importantly, β -strands A of repeats 18 and 20 occupy CD grooves of repeats 19 and 21, respectively, requiring mechanotransduction for partner binding (13).

FlnA partners in platelets

The GPIb-IX-V complex

In platelets, FlnA constitutively binds to the GPIb-IX-V complex (Figure 1D). The interaction requires FlnA repeat 17 and aa 563–571 (FRSSLFLWV) in the cytoplasmic tail of GPIb α (7).

Our group has generated mice specifically lacking FlnA in megakaryocytes by breeding *FLNA*^{loxP/loxP} females with GATA1-Cre and PF4-Cre males (5, 14). These mice have a severe thrombocytopenia, enlarged platelets, and increased tail bleeding time, similar to GPIb α / β -null mice (15, 16). As expected, GPIb α is not linked to the actin cytoskeleton in FlnA-null platelets, where its surface expression is reduced and altered (5). A time course analysis indicates that the association of GPIb α to the actin cytoskeleton occurs in mature megakaryocytes and depends on FlnA. Conversely, GPIb α modulates the subcellular localization of FlnA (17). FlnA-null megakaryocytes prematurely release large and fragile platelets that undergo microvesiculation, and are cleared rapidly from the circulation by macrophages (14).

The interaction between FlnA and GPIb α positively modulates VWF receptor function. Platelets treated with cell-permeable peptide mimics of GPIb α FlnA-binding motif, which inhibit FlnA binding to GPIb α , have decreased ability to activate their integrin α IIb β 3 and change shape in response to VWF stimulation (18, 19). Chinese hamster ovary (CHO) cells transfected with a double substitution mutant F568A/W570A of GPIb α to disrupt FlnA binding have increased cell detachment from VWF-coated surfaces at high shear (20). Consistently, FlnA-null platelets in whole blood adhere poorly to collagen-coated surfaces under arterial shear conditions (5).

The Jackson group has recently generated a transgenic mouse expressing the double substitution mutant F568A/W570A of human GPIb α (21). Although platelets isolated from

these mice normally contain endogenous GPIIb α , and have normal size and morphology, they adhere poorly onto VWF-coated surfaces and disintegrate under high shear, similar to FlnA-null platelets (5, 14). Thus, the FlnA-GPIIb α linkage not only regulates platelet size, but also maintains the mechanical stability of the platelet plasma membrane.

The integrin α IIb β 3

FlnA binds to the fibrinogen receptor, the integrin α IIb β 3 in resting platelets (Figure 1E). The interaction requires FlnA repeat 21 and β 3 aa 747–755 (YKEATSTFT) (8), raising structurally relevant questions, as 1) β -strand A of repeat 20 normally prevents integrin binding to repeat 21 (11, 12), and 2) β 3 does not have an optimal FlnA-binding motif, *i.e.* it contains an acidic Glu residue in position 749, instead of the neutral Ser residue observed in most FlnA binding partners (Figure 1C). Further studies are required to determine how megakaryocytes overcome these structural challenges to form the FlnA- β 3 linkage.

Platelet activation leads to a conformational change of α IIb β 3 to promote fibrinogen binding and platelet aggregation. α IIb β 3 activation is accompanied by FlnA displacement from β 3, followed by talin binding to β 3 aa 739–750 (WDTANNPLYKEA) (22, 23). FlnA-null platelets have increased β 3 expression, but normally activate α IIb β 3 in response to GPCR agonists and PMA (5). Whether binding of talin to β 3 is increased in FlnA-null platelets remains to be determined.

Talin is a 270-kDa protein composed of an N-terminal head domain binding to β -integrin subunits, followed by an elongated C-terminal actin-binding rod domain. Talin binding to β 3 is required for α IIb β 3 activation, as talin-null platelets have impaired agonist-mediated platelet aggregation (24, 25). Mice specifically lacking talin in platelets exhibit prolonged tail bleeding time and pathological bleeding, similar to β 3-null mice (26). In an elegant study, the Ginsberg group has introduced a substitution L746A in mouse β 3 to selectively disrupt talin, but not FlnA binding (23). Mutant animals exhibit defective α IIb β 3 activation, impaired platelet aggregation, and prolonged tail bleeding time, but normal platelet adhesion onto fibrinogen, intact outside-in signaling, and no spontaneous bleeding, suggesting that α IIb β 3-mediated adhesion and outside-in signaling are sufficient for hemostasis *in vivo*.

Kindlins are another family of proteins that modulate integrin activation. Kindlins bind to β 3 aa 752–759 (STFTNITY), in a region that overlaps with the FlnA binding site (27, 28). Kindlin-3-null platelets exhibit defective α IIb β 3 activation and impaired platelet aggregation (27). However, neither kindlin-1 nor kindlin-2 is able to activate α IIb β 3 in CHO cells in the absence of talin head domain, suggesting that kindlins co-activate α IIb β 3 in synergy with talin (28, 29).

It has been proposed that competitive binding of migfilin to FlnA repeat 21 is necessary to disassociate FlnA from β 3 (30). Cell-permeable peptide mimics of migfilin FlnA-binding motif displace FlnA from β 3, promoting talin binding and α IIb β 3 activation in platelets (31). However, this migfilin-mediated mechanism appears redundant, as platelets contain little to no migfilin, and migfilin-null mice do not exhibit any bleeding phenotype (32). Additional experimental evidence is needed to determine whether kindlins and/or migfilin

promote talin-mediated α IIB β 3 activation by disrupting the FlnA- β 3 linkage in activated platelets.

The tyrosine kinase Syk

FlnA binds to the tyrosine kinase Syk in platelets (5). The interaction requires FlnA repeat 5, and is particularly important for ITAM- and ITAM-like-mediated signaling, as FlnA-null platelets have impaired GPVI and CLEC-2 signaling. FlnA appears to contribute to Syk spatial distribution to the cytoplasmic surface of the platelet plasma membrane, a prerequisite for Syk docking to GPVI and CLEC-2.

Patients with *FLNA* mutations

Thrombocytopenia has been described in patients with *FLNA* mutations (33). The platelets and megakaryocytes of three French female patients with heterozygous *FLNA* mutations have recently been characterized: two with loss-of-function mutations leading to PH, and one with a missense mutation E1803K in repeat 16 (34). All three patients exhibit a bleeding tendency and giant platelets with large numbers of heterogeneously distributed α -granules. One PH patient has normal platelet count, and 80% of her platelets contain full-length FlnA. The two other patients have a more severe phenotype with low platelet count and the presence of multiple FlnA degradation products in their platelets, suggesting that a high proportion of platelets contain rapidly degraded FlnA mutants that negatively modulate platelet circulation. Platelets isolated from all three patients have normal to increased GPIb α expression, and normally activate α IIB β 3 in response to GPCR agonists. Further studies are needed to determine the distribution of mutant platelets in these patients, as the X-linked inheritance of the mutations complicates the interpretation of the phenotype.

A missense mutation V528M in repeat 3 has been reported in a Japanese boy with thrombocytopenia and giant platelets (35). The V528M variant is a functional polymorphism observed in 4.8% of healthy control Japanese, but not Caucasian subjects, suggesting that an alternative defective gene affects platelet count and size in the patient.

Conclusion

Recent structural studies, mouse models, and newly characterized patients with *FLNA* mutations have advanced our understanding of the role of FlnA in platelets and megakaryocytes. However, they also have pointed to new structurally and biologically relevant questions, revealing exciting new directions for research.

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Figure 1.

(A) Schematic structure of FlnA. GPIIb, $\beta 3$ and Syk bind to FlnA repeats 17, 21 and 5, respectively, while repeat 24 mediates dimerization. (B) Structure of FlnA repeat 17 (blue) with GPIIb aa 557–573 (violet). β -strands C and D involved in partner binding are highlighted. Model was generated in Jmol by using Protein Data Bank entry 2BP3 (7). (C) FlnA-binding motif of known FlnA partners. Protein names, aa sequences and positions are indicated. Non-polar residues are colored green, neutral violet, basic blue, and acidic red. An asterisk means non-polar or neutral residue. (D) Schematic structure of the platelet GPIIb-IX-V complex. FlnA links GPIIb to the actin cytoskeleton. (E) $\alpha IIb\beta 3$ activation requires disruption of the FlnA- $\beta 3$ linkage and association of talin and kindlins to $\beta 3$. Migfilin competes with $\beta 3$ for FlnA binding. (Insert) $\beta 3$ aa 734–762. Residues involved in the binding to FlnA, talin and kindlins are highlighted.