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Review Article

Mechanosensitivity of integrin adhesion complexes: role of the consensus adhesome

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ABSTRACT

Cell and tissue stiffness have been known to contribute to both developmental and pathological signalling for some time, but the underlying mechanisms remain elusive. Integrins and their associated adhesion signalling complexes (IACs), which form a nexus between the cell cytoskeleton and the extracellular matrix, act as a key force sensing and transducing unit in cells. Accordingly, there has been much interest in obtaining a systems-level understanding of IAC composition. Proteomic approaches have revealed the complexity of IACs and identified a large number of components that are regulated by cytoskeletal force. Here we review the function of the consensus adhesome, an assembly of core IAC proteins that emerged from a meta-analysis of multiple proteomic datasets, in the context of mechanosensing. As IAC components have been linked to a variety of diseases involved with rigidity sensing, the field is now in a position to define the mechanosensing function of individual IAC proteins and elucidate their mechanisms of action.

Contents

1. Cell adhesion, integrin adhesion complexes and mechanotransduction

A requirement for a multicellular existence is the ability of cells to form higher order structures, tissues and organs [1]. To do this, cells must organise and integrate themselves with regard to each other and their external microenvironment, a feat that is achieved in part via cell surface adhesion receptors. Whilst cell-cell adhesion is mediated mainly by cadherins, cell adhesion to the extracellular matrix (ECM) is predominantly orchestrated by integrins. A multitude of integrin-ECM associations have been described [2],

Abbreviations: ECM, extracellular matrix; FAK, focal adhesion kinase; FN, fibronectin; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; GTPase, guanosine triphosphatase; IAC, integrin-associated adhesion complex; ILK, integrin-linked kinase; IPP, ILK-PINCH-Parvin complex; IQGAP1, IQ motif–containing GTPase–activating protein 1; LIM, Lin-11, Isl1, and Mec-3; MS, mass spectrometry; PINCH, particularly interesting new cys-his protein 1; SYNCRIP, synaptotagmin binding, cytoplasmic RNA interacting protein; VASP, vasodilator-stimulated phosphoprotein; VCAM-1, vascular cell adhesion molecule-1

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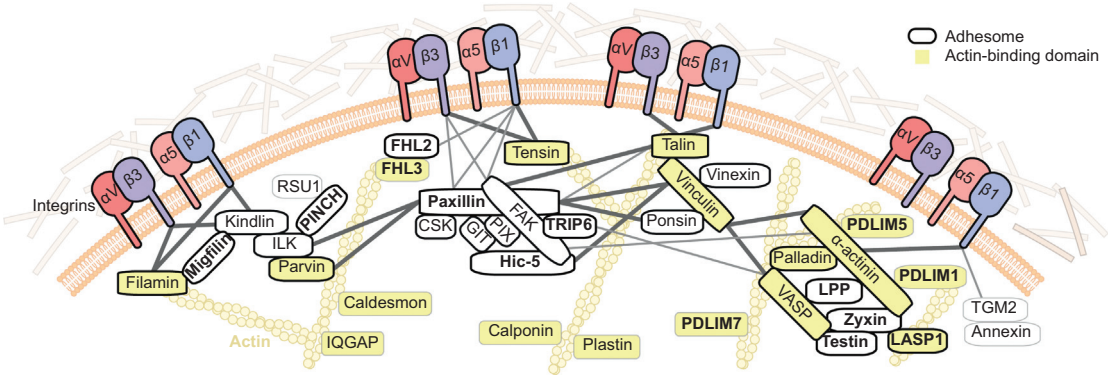

Fig. 1. The consensus integrin adhesome. A schematic representation of the consensus adhesome: proteins that were at least two-fold enriched to FN-induced IACs compared with complexes isolated from negative control ligand conditions in at least five IAC datasets (excluding ECM components). The resulting 60 proteins identified represent the consensus adhesome [41]. The schematic shows the main interactions between 42 proteins that interact with each other, or actin, based upon interaction evidence reported in the literature [41]. Thick black node border indicates literature-curated adhesome protein [16]. Interactions were manually validated and scored (high, medium, low) according to the level of experimental evidence for that interaction shown by the thickness of the grey edges [41]. Bold text indicates LIM domain-containing protein and yellow node indicates actin-binding protein, as reported in InterPro [92]. Actin was not present in the consensus adhesome but is depicted for illustrative purposes. While two α-actinin isoforms (α-actinin-1 and -4) were incorporated in the consensus adhesome, α-actinin is depicted as one node. Unconnected consensus adhesome components not shown are: ALYREF, BRIX1, DDX18, DDX27, DIMT1, DNAJB1, FAU, FEN1, H1FX, HP1BP3, LIMD1, MRTO4, P4HB, POLDIP3, PPIB, RPL23A, SIPA1 and SYNCRIP (see ref. [41] for details).

which provide direct physical connections between the ECM and the intracellular actomyosin cytoskeleton [3]. Integrins rely on the recruitment of multi-protein integrin adhesion complexes (IACs) to their cytoplasmic domains to mediate their functions, and transduce bidirectional signals with wide-ranging effects on development and disease [4–7].

Mechanotransduction, the ability of cells to sense force, whether generated externally via the extracellular matrix or internally by actomyosin-based contractility, is firmly established to play a key role in differentiation and proliferation [8–11]. Variation of cell and tissue stiffness results in altered transcriptional programming, thereby affecting stem cell lineage decision-making, and is also associated with diseases such as cancer and fibrosis [10,12]. Cells sense force via a variety of plasma membrane receptors including integrins [13,14]. As integrins and IACs form linkages between cells and their microenvironment, they can act as mechanochemical signalling centres [14,15]. Indeed many IAC components are linked to a variety of diseases involved with rigidity sensing [16] and proteins such as vinculin, talin and p130Cas transmit forces to actin via conformational changes [17–20]. Moreover IACs relay force to the ECM to regulate durotaxis [21], and integrins themselves act as mechanosensory components of IACs. Force affects the strength of the interaction between α5β1 and its ECM ligand fibronectin (FN) [22], and α5β1 forms force-stabilised catch bonds that undergo cyclic mechanical reinforcement [23,24]. Together, these studies demonstrate that the ECM-integrin-IAC axis is a key regulatory component of the mechanosignalling pathways that determine cell and tissue fate.

2. Proteomic analysis of adhesion complexes – definition of a consensus integrin adhesome

To understand how mechanical links are formed between the ECM and the intracellular environment, the molecular composition of IACs has been studied extensively using candidate-based microscopy and biochemical approaches. The wealth of information gained from these studies, which includes work on different cell types and under different experimental conditions, has been curated into a hypothetical integrin adhesome [16,25,26]. To provide an unbiased view of IAC composition and function [27], the global composition of IACs has recently been characterised using protocols to isolate the adhesion nexus biochemically coupled with mass spectrometry (MS)-based proteomic analysis [28,29]. These methods have been used to analyse IAC composition from a variety of cell types and conditions, including mesenchymal stem cells [30,31], and the effects of different ECM ligands or integrin heterodimers [32–35], microtubule polymerisation inhibition by nocodazole [36,37], and myosin-II inhibition by blebbistatin [35,38,39] on IAC composition. In addition, the phosphorylation profile of isolated IACs has been reported [40], which identified novel phosphorylation sites on IAC components and regulators of adhesion signalling. These datasets provide context-dependent compositional snapshots of IACs at particular time points and have suggested an underestimation of the scale and complexity of IAC organisation at the molecular level.

To create a resource for further analysis of IACs, we have computationally integrated several IAC proteomes. These datasets were generated from multiple cell types, using diverse methods from different laboratories [41]. The resulting experimentally defined 'meta-adhesome' database contains 2412 proteins that are enriched to IACs recruited to FN in at least one of the seven MS datasets [41]. Along with functions classically associated with cell adhesion, the meta-adhesome database contains proteins linked to a wide variety of cellular functions that have not currently been firmly connected to adhesion. Furthermore, an emergent property of the meta-adhesome is the definition of an IAC core of 60 commonly identified proteins, termed the consensus adhesome. Analysis of the protein–protein interaction network of the consensus adhesome identifies four interconnected axes that form the integrin-actin structural connection. These axes centre on the established IAC components of kindlin-ILK (integrin-linked kinase)-PINCH (particularly interesting new cys-his protein 1), FAK (focal adhesion kinase)-paxillin, talin-vinculin and α-actinin-zyxin-VASP (vasodilator-stimulated phosphoprotein) (Fig. 1). Proteins that directly linked integrins with actin are α-actinin, filamin, talin, tensin and, via a low-evidence α5β1 integrin interaction, FHL3. Other associated molecules may function to stabilise the connection of these integrin-actin linkers, such as PDLIM1 and PDLIM5, in facilitating the integrin-α-actinin-actin connection, or migfilin in bridging the connection between kindlin and actin via filamin.

The consensus adhesome represents commonly identified IAC proteins in the context of cellular attachment to FN via the α5β1 and αVβ3 FN-binding integrins (Fig. 1). One outstanding question is whether the composition of the consensus IAC changes in an ECM ligand- or integrin subunit-dependent manner? Since only 10