Composition and function of integrin adhesions

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Appendices

SUMMARY

Multicellular organisms require integrins to connect cells to the surrounding extracellular matrix (ECM) and sense and respond to topographical and mechanical features of the ECM. Integrins are heterodimeric transmembrane receptors that bind to both ECM components and the intracellular cytoskeleton. In order to establish this connection with the cytoskeleton and mediate signal transduction, integrins recruit many intracellular adaptor proteins, as they possess no enzymatic activity of their own and cannot bind the cell cytoskeleton directly. The adhesion complexes formed by integrins and their associated protein network play important roles in a variety of cellular processes, including cell differentiation, migration, proliferation and survival. Not surprisingly, failure to establish cell-ECM adhesions can result in severe human diseases.

Many studies have been undertaken in the last decades to characterize the proteins present in cell-ECM adhesion complexes and study their contribution to diverse cellular processes. Historically, integrins have been described as the key components of adhesion structures called focal adhesions (FAs), which serve as a bridge between the ECM and the intracellular actin cytoskeleton. These studies were mainly based on experiments using fibroblasts. Epithelial cells, on the other hand, can form many different adhesion complexes. To fully understand how these cells mediate cell-ECM adhesion, we conducted microscopy and proximity-dependent biotin identification (BioID) studies to characterize the composition of the distinct adhesion complexes. In addition, we employed BioID assays to study FA composition at nanometer resolution, as previous proteomic studies shared little consensus on the proteins present in this adhesion complex. Next, we interrogated the biological function of poorly studied (proteins in) adhesion complexes and unraveled crosstalk between the different types of adhesion structures.

Chapter 1 provides an overview of the different types of adhesion complexes present in epithelial cells and discusses how crosstalk between these complexes modulates the translation of information about the physical cellular environment into a biochemical response.

In **chapter 2 and 3**, we investigated the mechanisms that regulate the subcellular distribution of the vitronectin receptor integrin αVβ5, which can reside in both FAs and flat clathrin lattices (FCLs). By employing BioID, we demonstrate that the proximitome of integrin αVβ5 consists of the cytoskeletal linker protein talin and many clathrin adaptor proteins, including subunits of the adaptor protein complex 2 (AP2), EPS15L1, and ARH. Disruption of the NPLY motif on the integrin β5 cytoplasmic tail, as well as depletion of the clathrin adaptor proteins ARH, Numb and EPS15/EPS15L1, all impaired the formation of integrin αVβ5-containing FCLs. Moreover, we demonstrate that the clustering of integrin αVβ5 in FCLs requires the β5 cytoplasmic tail, as integrin chimeras containing the extracellular and transmembrane domains of β5 and the cytoplasmic domains of β1 or β3, almost exclusively localize to FAs (**chapter 2**). Most likely, integrin αVβ5 clustering in

FCLs is favored due to the higher affinity of β5 for the clathrin adaptor proteins ARH and Numb than for talin (**chapter 3**). In addition to the clathrin adaptor proteins, we identified the Rho GEFs p115Rho-GEF and GEF-H1 and the serine protein kinase MARK2 as β5 interactors by using mass spectrometry. Depletion of the latter was found to diminish the clustering of β5 in FCLs. Clustering of integrin αVβ5 in FCLs was also promoted by substitution of two serines (S759/762) in the β5 cytoplasmic domain with phospho-mimetic glutamates or by treatment with the phosphatase inhibitor calyculin A (**chapter 3**). In both **chapter 2 and 3**, we demonstrate that low cellular tension dictates the clustering of integrin αVβ5 in FCLs, while an increase in actomyosin-mediated contractility promotes clustering in FAs. Finally, we provide evidence that the biological function of integrin αVβ5 is to mediate cell adhesion to vitronectin and promote cell proliferation, regardless of its localization in FAs or FCLs (**chapter 3**).

We observed that the clustering of integrin αVβ5 in FCLs was favored in keratinocytes that formed hemidesmosomes (HDs), which are specialized adhesion structures that associate with the keratin cytoskeleton. Because we demonstrated that cellular tension dictates the subcellular localization of integrin αVβ5, we wondered whether HDs could play a role in mechanotransduction. In **chapter 4**, we demonstrate that keratinocytes that lack the hemidesmosomal integrin α6β4 or have a disrupted interaction between α6β4 and the intermediate filament network or laminin-332, exhibit increased FA formation, cell spreading, and traction-force generation. In addition, integrin α6β4 regulates the activity of YAP, a mechanosensitive transcriptional regulator, through inhibition of Rho–ROCK–MLC– and FAK–PI3K–dependent signaling pathways. Taken together, these results reveal a novel role for HDs as regulators of cellular mechanical forces and establish the existence of mechanical coupling between distinct adhesion complexes and the cell cytoskeleton.

In **chapter 5**, the interactomes of β1 and β3 integrins were interrogated by BioID and revealed the close proximity of PEAK1 with RGD-binding integrins in FAs. The interaction between PEAK1 and integrins takes place indirectly through Tensin-3, which binds to both the membrane-proximal NPxY motif on the integrin β tail with its PTB-domain and to phosphorylated Tyr-635 on PEAK1 with its SH2 domain. Src mediates phosphorylation of Tyr-635 on PEAK1, which is needed to promote GE11 cell migration. In addition, we find that PEAK1 associates with Shc1 and with a protein cluster that mediates late EGFR/Shc1 signaling. Through interaction with phosphorylated Tyr-1188 on PEAK1, Shc1 is able to localize to FAs. Based on these findings, we propose that PEAK1, through interaction with tensin-3 and Shc1, can converge integrin and growth factor receptor signal transduction to control cell proliferation and cytoskeletal remodeling.

Notably, PEAK1 was found to be more phosphorylated on Tyr-635 in colorectal adenomas than carcinomas in a phosphoproteomic screen that was employed to identify potential drivers of the adenoma-to-carcinoma progression. In **chapter 6**, we investigated

the role of PEAK1 in colorectal cancer (CRC) progression by using different *in vitro* and *in vivo* models that mimic the stepwise pathogenesis of CRC and in which PEAK1 was deleted by CRISPR/Cas9. PEAK1 did not regulate SW480 and HT29 cell proliferation, nor did it affect tumor development in CRC mouse models driven by oncogenic KRAS or loss of PTEN. However, PEAK1 did promote proliferation of Caco-2 cells that were treated with EGF and regulated spheroid polarization and lumenization. These findings indicate that PEAK1 might regulate cell polarity and growth during early adenoma formation, although the protein does not play a major role in CRC progression.
Chapter 7 provides a general discussion, outlook for future research, and limitations of the models used in this thesis, with emphasis on the study of PEAK1 in CRC.

In summary, this thesis characterizes the composition of distinct cell adhesion complexes in epithelial cells and demonstrates that crosstalk exists between FAs and HDs to regulate mechanotransduction. Integrin αVβ5-containing FCLs are mechanosensitive structures that play a role in cell adhesion to vitronectin and cell proliferation, similar to FAs. Finally, PEAK1 was identified as a FA component that can promote epithelial cell migration and possibly enhance colorectal adenoma growth, although it does not seem to drive CRC progression.