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Chapter 2

Integrin switching modulates cell matrix adhesion dynamics.

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Special Focus: Molecular Mechanism of Adhesion Complex Turnover

Integrin switching modulates adhesion dynamics and cell migration

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When cells are stimulated to move, for instance during development, wound healing or angiogenesis, they undergo changes in the turnover of their cell-matrix adhesions. This is often accompanied by alterations in the expression profile of integrins—the extracellular matrix receptors that mediate anchorage within these adhesions. Here, we discuss how a shift in expression between two different types of integrins that bind fibronectin can have dramatic consequences for cell-matrix adhesion dynamics and cell motility.

Cells attach to the extracellular matrix (ECM) that surrounds them in specialized structures termed "cell-matrix adhesions." These come in different flavors including "focal complexes" (small adhesions found in membrane protrusions of spreading and migrating cells), "focal adhesions" (larger adhesions connected by F-actin stress fibers that are derived from focal complexes in response to tension), "fibrillar adhesions" (elongated adhesions associated with fibronectin matrix assembly), and proteolytically active adhesions termed "podosomes" or "invadopodia" found in osteoclasts, macrophages and certain cancer cells. Common to all these structures is the local connection between ECM proteins outside- and the actin cytoskeleton within the cell through integrin transmembrane receptors. The intracellular linkage to filamentous actin is indirect through proteins that concentrate in cell-matrix adhesions such as talin, vinculin, tensin, parvins and others.[1]

Cell migration is essential for embryonic development and a number of processes in the adult, including immune cell homing, wound healing, angiogenesis and cancer metastasis. In moving cells, cell-matrix adhesion turnover is spatiotemporally controlled.[2] New adhesions are made in the front and disassembled in the rear of cells that move along a gradient of motogenic factors or ECM proteins. This balance between formation and breakdown of cell-matrix adhesions is important for optimal cell migration. Several mechanisms regulate the turnover of cell-matrix adhesions. Proteolytic cleavage of talin has been identified as an important step in cell-matrix adhesion disassembly[3] and FAK and Src family kinases are required for cell-matrix adhesion turnover and efficient cell migration.[4,5] Besides regulating phospho-tyrosine-mediated protein-protein interactions within cell-matrix adhesions, the FAK/Src complex mediates signaling downstream of integrins to Rho GTPases, thus controlling cytoskeletal organization.[6,7] The transition from a stationary to a motile state could involve (local) activation of such mechanisms.

Interestingly, conditions of increased cell migration (development, wound healing, angiogenesis, cancer metastasis) are accompanied by shifts in integrin expression with certain integrins being lost and others gained. Most ECM proteins can be recognized by various different integrins. For instance, the ECM protein, fibronectin (Fn) can be recognized by nine different types of integrins and most of these bind to the Arg-Gly-Asp (RGD) motif in the central cell-binding domain. Thus, cell-matrix adhesions formed on Fn contain a mixture of different integrins and shifts in expression from one class of Fn-binding integrins to another will alter the receptor composition of such adhesions. This may provide an alternative means to shift from stationary to motile.

Indeed, we have found that the type of integrins used for binding to Fn strongly affects cell migration. We made use of cells deficient in certain Fn-binding integrins and either restored their expression or compensated for their absence by overexpression of alternative Fn-binding integrins. This allowed us to compare in a single cellular background cell-matrix adhesions containing $\alpha5\beta1$ to those containing $\alpha v\beta3$. Despite the fact that these integrins support similar levels of adhesion to Fn, only $\alpha5\beta1$ was found to promote a contractile, fibroblastic morphology with centripetal orientation of cell-matrix adhesions[8] (Fig. 1). Moreover, RhoA activity is high in the presence of $\alpha5\beta1$ and these cells move in a random fashion with a speed of around 25 mm/h. By contrast, in cells using $\alpha v\beta3$ instead, adhesions distribute across the ventral surface, RhoA activity is low, and these cells move with similar speed but in a highly persistent fashion.[8,9] Finally, photobleaching experiments using GFP-vinculin and GFP-paxillin demonstrated that cell-matrix adhesions containing $\alpha5\beta1$ are highly dynamic whereas adhesions containing $\alpha v\beta3$ are more static.[9]

It has been observed that $\alpha5\beta1$ and $\alpha v\beta3$ use different recycling routes. Interfering with Rab4-mediated recycling of $\alpha v\beta3$ causes increased Rab11-mediated recycling of $\alpha5\beta1$ to the cell surface. In agreement with our findings, the shift to $\alpha5\beta1$ leads to increased Rho-ROCK activity and reduced persistence of migration.[10] One possible explanation for the different types of migration promoted by these two Fn-binding integrins might involve different signaling and/or adaptor proteins interacting with specific amino acids in their cytoplasmic tails. However, this appears not to be the case: $\alpha5\beta1$ in which the cytoplasmic tails of $\alpha5$ or $\beta1$ are replaced by those of αv

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or β3, respectively, behaves identical to wild type α5β1: it promotes a fibroblast-like morphology with centripetal orientation of cell-matrix adhesions and it drives a non-persistent mode of migration.[8,11] Together, these findings point to differences between α5β1 and αvβ3 integrins in the mechanics of their interaction with Fn, which apparently modulates intracellular signaling pathways in control of cell-matrix adhesion dynamics and cell migration.

How might this work? It turns out that although α5β1 and αvβ3 similarly support cell adhesion to immobilized (stretched) Fn, only α5β1 efficiently binds soluble, folded ("inactive") Fn.[11] We have proposed that such interactions with soluble Fn molecules (possibly secreted by the cell itself) may weaken the interaction with the immobilized ligand thereby causing enhanced cell-matrix adhesion dynamics in the presence of α5β1,[11] (Fig. 1). Preferential binding of soluble Fn by α5β1 could be explained by differences in accessibility of the RGD binding pocket between α5β1 (more exposed) and αvβ3 (more hidden) as suggested by others.[12] If this is the case, immobilization ("stretching") of Fn apparently leads to reorientation of the RGD motif in such a way that it is easily accessed by both integrins.

The issue is considerably complicated by the fact that other recognition motifs are present in the Fn central cell-binding domain. In addition to the RGD sequence in the tenth Fn type 3 repeat (IIIFn10), binding of α5β1, but not αvβ3, also depends on the PHSRN "synergy" sequence in IIIFn9.[13-15] The relative contribution of these motifs is controversial and there is structural data pointing either towards a model in which IIIFn9 interacts with α5β1 or towards a model in which IIIFn9 exerts long-range electrostatic steering resulting in a higher affinity interaction without contacting the integrin.[16,17] Cell adhesion studies have suggested that an interaction of α5β1 with the synergy region stabilizes the binding to RGD.[14,18] Such a two-step interaction may facilitate binding to full length, folded Fn for instance by altering the tilt angle between IIIFn9 and IIIFn10 leading to optimal exposure of the RGD loop, perhaps explaining why αvβ3 (which may not interact with the synergy site) poorly binds soluble Fn.

Others have shown that the RGD motif alone is sufficient for mechanical coupling of αvβ3 to Fn whereas the synergy region is required to provide mechanical strength to the α5β1-Fn bond.[19] It appears that the interaction of α5β1 with Fn is particularly dynamic with various conformations of α5β1 interacting with different Fn binding surfaces, including the RGD and synergy sequences as well as other regions in IIIFn9. Thus, besides the above model based on differential binding to soluble Fn molecules, differences in the complexity and dynamics of interactions with immobilized Fn that determine functional binding strength could also underlie the different dynamics of cell-matrix adhesions containing either α5β1 or αvβ3 (Fig. 1).

Precisely how mechanical differences in receptor-ligand interactions result in such remarkably distinct cellular responses is poorly understood. In addition to effects on cell-matrix adhesion dynamics and cytoskeletal organization it is also associated with different activities of Rho GTPases, indicating that mechanical differences between these two integrins must translate into differential activation of intracellular signaling pathways.[8,9,11] Possibly, different adhesion dynamics due to distinct mechanisms of receptor-ligand interaction result in different patterns of F-actin organization, which, in turn, affects the formation of signaling platforms. It is also possible that differences in the extent of integrin clustering have an impact on the conformation of one or more cytoplasmic components of the cell-matrix adhesions containing either α5β1 or αvβ3. This could lead to hiding or exposing binding sites for signaling molecules (e.g., upstream regulators of Rho GTPases) or substrates. Whatever the mechanism involved, altering the integrin composition of cell-matrix adhesions through shifts in integrin expression as observed during development, angiogenesis, wound healing and cancer progression may be a driving force in the enhanced cell migration that characterizes those processes.

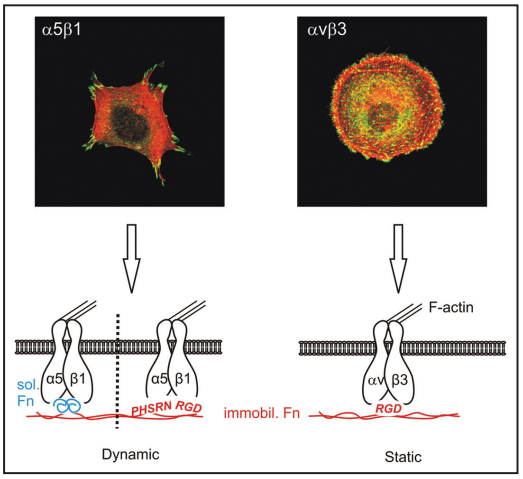

Figure 1. Immunofluorescence images. GE11 cells, epithelial β1 knockout cells derived from mouse embryos chimeric for the integrin β1 subunit endogenously express various αv integrins, including low levels of αvβ3 and αvβ5. Ectopic expression of β1 leads to expression of α5β1 and induced α5β1-mediated adhesion to Fn (left image) whereas ectopic expression of β3 (in the β1null background) leads to strong expression of αvβ3 and induced αvβ3-mediated adhesion to Fn (right image). Adhesions containing either α5β1 or αvβ3 show distinct distribution and dynamics (paxillin; green) and cause different F-actin organization (phalloidin; red). Cartoons: Differences in cell-matrix adhesion dynamics may be explained by differential binding of soluble Fn molecules (blue) or different molecular determinants of the interaction with immobilized Fn (red). See text for details.

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