Membrane tension drives ligand-independent integrin signaling

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Integrins anchor cells to the extracellular matrix (ECM) and control a multitude of essential cellular functions by activating a variety of signaling pathways. In this issue of *The EMBO Journal*, a study conducted by Ferraris and colleagues report that binding of urokinase-type plasminogen activator receptor (uPAR) to vitronectin is sufficient to trigger ligand-independent $\beta 1$ and $\beta 3$ integrin signaling. The coupling of uPAR and integrins occurs independent of lateral interaction between the two receptors, but relies on membrane tension (Ferraris *et al*, 2014).

See also: **GMS Ferraris *et al*** (November 2014)

Integrins require an activation step to switch from an inactive (low affinity) to an active (high affinity) state. The affinity switch is allosteric, affects the conformation of the entire molecule, and is induced upon binding of integrin activators (talins and kindlins) to β integrin cytoplasmic domains (Moser *et al*, 2009). The consequence of integrin activation is ligand binding and cell adhesion to the ECM and the assembly of a large molecular network that regulates multiple cellular processes crucial for development, physiology, and pathology (Legate *et al*, 2009).

When integrins are bound to ligand, they can be modulated through lateral associations with other membrane components, including diverse cell adhesion receptors (Woods & Couchman, 2000). One of these adhesion receptors is uPAR, which binds to distinct ligands including the urokinase-type plasminogen activator (uPA) and the extracellular matrix glycoprotein vitronectin (VN). uPAR is tethered to the plasma membrane with a glycosyl-phosphatidylinositol (GPI)

anchor, lacks transmembrane and cytoplasmic domains, and therefore relies on transmembrane co-receptors for signal transduction. Although there is substantial evidence suggesting that integrins and other membrane proteins such as chemokine receptors and receptor tyrosine kinases (Liu *et al*, 2002; Resnati *et al*, 2002) serve as essential co-receptors for mediating uPAR signaling (Smith & Marshall, 2010), it is still unclear how uPAR and integrins interact functionally and whether a direct interaction or just proximity between the cell adhesion receptors is necessary to initiate signaling. Ferraris and colleagues report in this issue of *The EMBO Journal* that there is no need for a direct interaction between uPAR and integrins, but rather a requirement for a mechanical coupling of the two receptors through membrane tension leading to ligand-independent integrin signaling (Ferraris *et al*, 2014).

In an effort to shed light on the molecular mechanisms of uPAR-mediated signaling, Ferraris and colleagues seeded HEK293 cells expressing either wild-type uPAR (uPAR^{WT}) or vitronectin (VN)-binding deficient uPAR mutants (uPAR^{T54A} and uPAR^{W32A}) onto VN (Ferraris *et al*, 2014). They found that uPAR-induced cell adhesion and spreading requires uPAR engagement with VN and that integrin-inhibitory antibodies blocked uPAR/VN-induced cell spreading and signaling. These findings confirm previous observations showing that integrins are crucial signal transducers downstream of uPAR/VN-induced cell adhesion (Wei *et al*, 1996; Madsen *et al*, 2007). Surprisingly, however, when Ferraris *et al* seeded their cells on a recombinant, integrin-binding-deficient VN variant (VN^{RAD}), neither cell spreading nor p130Cas phosphorylation was

affected (Ferraris *et al*, 2014), indicating that direct binding of integrins to VN was dispensable for uPAR/VN signaling. Moreover, when $\beta 1$ integrins were inhibited with blocking antibodies uPAR/VN^{RAD} signaling was abrogated, showing that unligated $\beta 1$ integrins trigger downstream signaling pathways.

To determine how the uPAR/VN complex achieves ligand-independent integrin signaling, Ferraris and colleagues asked whether certain structural requirements of $\beta 1$ integrin are required for this unusual receptor cross talk. They expressed a ligand binding defective $\beta 1$ integrin^{D130A} and found that it is only able to transduce uPAR-induced cell spreading on VN^{RAD} when $\beta 1$ integrin adopts an active conformation and permits talin and kindlin binding to its cytoplasmic domain (Ferraris *et al*, 2014). Thus, uPAR-induced $\beta 1$ integrin signaling on VN^{RAD} requires the active conformation of $\beta 1$ integrin and the binding of integrin activators to integrin tails, but not binding of the integrin ectodomain to ligand.

To test whether induction of ligand-independent integrin signaling is specific to uPAR and dependent on a direct interaction between uPAR and integrins, Ferraris *et al* expressed structurally unrelated VN binding receptors as well as uPAR-variants tethered to the plasma membrane through the fusion of the uPAR ectodomain with unrelated GPI-anchors or transmembrane domains. They found that also a GPI-anchored PAI-1_{GPI}, which binds VN but differs structurally from uPAR, as well as uPAR-variants with transmembrane tethers can trigger $\beta 1$ integrin-dependent cell adhesion, spreading, and p130Cas phosphorylation when seeded on VN^{RAD}. Interestingly, these integrin-dependent signaling outputs were also induced when uPAR-expressing cells were

Figure 1. Ligand-dependent and ligand-independent integrin signaling.

Canonical integrin signaling requires active integrins binding to specific ligands in the ECM, the assembly of signaling and adaptor proteins at their cytoplasmic tails and the linkage to the actin cytoskeleton. Ligand-independent integrin signaling requires membrane tension generated by the uPAR/VN complex, an active integrin conformation and binding of integrin activators to the β integrin cytoplasmic tail. Whether the integrin-actin linkage is also required for ligand-independent integrin signaling has not been analyzed.

seeded on anti-uPAR antibody-coated surfaces, indicating that not even the uPAR ligand is necessary for signal induction. Furthermore, cells engineered to express a signaling-incompetent $\beta 3$ integrin ($\beta 3^{Y2A}$) and seeded on anti- $\alpha V\beta 3$ antibody-coated surfaces were still able to spread in a $\beta 1$ integrin-dependent manner. Together, these experiments reveal that ligand-independent $\beta 1$ integrin signaling requires integrin activation, which is induced by aggregating uPAR, through binding either to VN, $\alpha V\beta 3$ or to an anti-uPAR antibody.

The authors also noted that induction of ligand-independent integrin signaling required immobilizing the uPAR ligand on a rigid substrate indicating a role of mechanosensing and mechanotransduction in the uPAR/VN signaling process. To more precisely define this functional association, they decided to investigate an involvement of membrane tension in uPAR/VN-integrin crosstalk by decreasing membrane tension with sucrose or deoxycholate (Ferraris *et al*,

2014). This treatment indeed strongly reduced cell spreading on VN^{RAD}, but not on FN, indicating that the molecular mechanisms of ligand-dependent and ligand-independent integrin signaling differ with respect to the requirement for membrane tension.

Ferraris and colleagues show for the first time that the uPAR/VN signaling complex requires $\beta 1$ or $\beta 3$ integrins that do not have to bind ligand. Furthermore, they also show that membrane tension mechanically couples uPAR and integrins and that a direct interaction between uPAR and integrin is not needed for signaling. Although these findings are convincing, one should bear in mind that a direct interaction may well be functionally relevant in other circumstances, for example, for the uPAR-mediated recruitment of $\alpha 5\beta 1$ integrins into lipid rafts (Grove *et al*, 2014).

The observations reported by Ferraris *et al* raise questions regarding the molecular mechanism and physiological relevance of this ligand-independent integrin signaling.

The present study establishes uPAR-induced ligand-independent integrin signaling in cultured cells, which leaves open when ligand-dependent (canonical) and ligand-independent integrin signaling is used under physiological conditions, and to which extent the two modes of integrin signaling co-exist in an *in vivo* context. Furthermore, it will be important to know whether structural constraints to the receptor–ligand pair exist that are capable of inducing ligand-independent integrin signaling as the induction of ligand-independent integrin signaling is not restricted to uPAR/VN in their cell culture experiments. Another unanswered question is how integrins and talin become activated to induce signaling upon uPAR binding to VN. The current model of integrin signaling mandates that integrins and talins undergo a conformational change to induce signaling (Moser *et al*, 2009). It is possible that uPAR binding to VN could directly induce integrin activation as uPAR has been shown to regulate integrin function by inducing conformational changes in $\alpha 5\beta 1$, $\alpha v\beta 3$, and $\alpha M\beta 2$ integrins (Smith & Marshall, 2010). Alternatively, uPAR could activate talin and kindlin or utilize integrins that are already in an active conformation. Finally, it would be interesting to know how ligand-dependent and ligand-independent integrin signaling differs in quantitative and qualitative terms. For example, the ligand-independent integrin signaling described by Ferraris and colleagues occurs independent of acto-myosin pulling forces exerted on integrins. Therefore, the size of integrin clusters should be small and the composition of the adhesome should be quantitatively and qualitatively different from conventional integrin-adhesive sites. Clearly, there is still a lot to do until this unusual integrin signaling is fully understood.

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