

P005 The novel antibody SNAKA51 recognises a distinct activated conformation of integrin $\alpha 5\beta 1$ associated with fibrillar adhesions.

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The use of conformation-dependent monoclonal antibodies has made a major contribution to our understanding of the shape changes that underpin activation of integrin receptors. We describe here a novel activating anti- $\alpha 5$ antibody, SNAKA51, that recognises a distinct, active conformer of $\alpha 5\beta 1$ integrin. The epitope for this antibody lies within the calf domains of $\alpha 5$, some distance away from the ligand-binding pocket. SNAKA51 rescues the effects of point mutations that de-stabilise the $\beta 1$ A-domain, thus binding of the antibody to the $\alpha 5$ leg region can have long-range effects on ligand-binding activity. The ability of SNAKA51 to activate ligand binding requires the leg region of the $\beta 1$ subunit and the C-termini of both subunits to be tethered. Immunocytochemical analyses revealed that SNAKA51 only stains cellular $\alpha 5\beta 1$ when it is present in fibrillar adhesions, not focal adhesions. This suggests that the maturation of focal adhesions to fibrillar adhesions is associated with a distinct shape change in $\alpha 5\beta 1$, possibly the separation of the $\alpha 5$ and $\beta 1$ legs. These findings indicate that function-related conformational changes occur in $\alpha 5\beta 1$ after the initial ligand-binding events occurring in focal adhesions.