

Thy-1: Shade and sun for integrin signaling

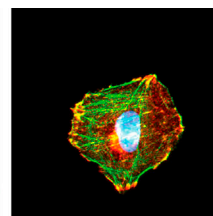
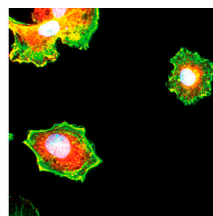
The GPI-anchored protein Thy-1 can both promote and inhibit integrin activity.

Integrins are transmembrane receptors that help link the extracellular matrix (ECM) to the cytoskeleton. They're essential for cell-substrate and cell-cell adhesion, cell locomotion, and for sensing and responding to the extracellular environment (1).

Injury or pathological conditions such as cancer can alter the extracellular environment. For example, lung injuries leave stiff fibrotic lesions (2) that activate fibroblasts via integrins to increase cellular contractility, maintain lung function, and promote tissue repair (3). However, fibrotic lesions sometimes spread uncontrollably, producing a condition called progressive pulmonary fibrosis. Several years ago scientists observed that, although most fibroblasts in the body express the GPI-linked surface protein Thy-1, this marker is lost as fibrotic disease progresses (4). The implications of this finding have remained unclear, but now Fiore et al. show that Thy-1 is an important regulator of integrin mechanotransduction whose loss substantially alters fibroblast contractility (5).

Currently, the first step in integrin activation is thought to involve ligand engagement, followed by integrin clustering. Next comes recruitment of intracellular signaling mediators such as focal adhesion kinase (FAK) and c-Src, and proteins that cross-link integrins to the cytoskeleton—culminating in the formation of focal adhesion complexes (FAs). Application of force to integrins provokes remodeling of cortical actin and actin stress fibers via recruitment of the Src family kinase Fyn and subsequent activation of RhoA (6). In view of this, Thomas Barker's group at the Georgia Institute of Technology, led by graduate student Vincent Fiore, investigated whether loss of Thy-1 affected fibroblast responses to ECM.

As expected, Thy-1-positive mouse lung fibroblasts only developed FAs and actin stress fibers when plated on stiff fibro-



FOCAL POINT

Vincent Fiore (left), Thomas Barker (right), and colleagues found that, compared with control fibroblasts (second from right), Thy-1 deficiency (far right) provokes altered cytoskeletal organization (actin, green), focal adhesion assembly (vinculin, red), and increased cortical stiffness in fibroblasts because of disrupted integrin activity. Nuclei in blue.

PHOTOS COURTESY OF ALISON DOUGLAS (FIORE) AND ROB FELT, GEORGIA TECH (BARKER).

nectin matrices. In contrast, Thy-1-negative fibroblasts plated on soft matrices exhibited stronger RhoA activity and increased cortical stiffness and stress fiber formation compared with Thy-1-positive cells. These responses were unaffected by increased matrix stiffness, indicating the cells were less sensitive to their environment.

The authors next examined force-dependent integrin responses by pulling on fibronectin-coated magnetic beads using magnetic fields. They found Thy-1-negative fibroblasts had higher basal FAK and Src family kinase activity, which may account for the increased basal RhoA activation in these cells. However, whereas Thy-1-positive fibroblasts recruited Fyn

and robustly increased RhoA activation after force application to integrins, Thy-1-negative cells did not. Therefore, Thy-1-negative fibroblasts were more contractile than Thy-1-positive ones, but also less able to respond to changes in their environment—pointing to a complex regulatory role for Thy-1-integrin interactions.

"Thy-1 has a kind of yin-yang behavior in controlling integrin engagement and signaling," explains Barker. On one hand, proximity ligation assays showed that Thy-1 bound to integrins in the plane of the membrane, leading the authors to hypothesize that Thy-1 competes against ECM ligands for integrin binding, interfering with integrin

activation. On the other hand, because Thy-1 is a GPI-anchored cell surface protein, it tends to segregate into special membrane subdomains called lipid rafts. The inner (cytoplasmic-facing) leaflet of lipid rafts are enriched for proteins endowed with lipid modifications, including Fyn, which mediate some aspects of integrin signaling.

Fiore et al. found that Thy-1 hinders integrin interactions with ECM, but simultaneously primes integrins for force-dependent signaling by recruiting Fyn and other raft-associated proteins into proximity with integrins. Loss of Thy-1 results in dysregulated fibroblast behavior that may contribute to fibrotic disease. Indeed, Thy-1-negative fibroblasts isolated from people with progressive pulmonary fibrosis were more contractile and could inappropriately differentiate into highly contractile myofibroblasts when cultured on soft matrices.

It will be important to study the dynamics of Thy-1-integrin interactions to understand these processes better, notes Barker. Yet, he also points out, Thy-1 may not be the only protein to exert this type of regulatory activity upon integrin signaling; it may just be the first example of this class.

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