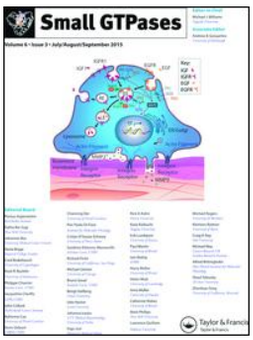

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Rho GEFs and GAPs: Emerging integrators of extracellular matrix signaling

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Investigating cell migration in 3D settings has revealed that specific extracellular matrix environments require differential activities of the Rho GTPases for efficient migration. However, it is largely unknown how the activities of specific Rho GTPases are modulated to direct cell migration in response to different extracellular matrix cues. We have recently reported that extracellular matrix-dependent regulation of a specific Rho GEF is a fundamental mechanism governing cell migration in different microenvironments, providing a direct mechanism for extracellular matrix-specific regulation of Rho GTPase activity directing cell motility. We discovered that the Rho GEF βPix has a unique function during cell migration in fibrillar collagen environments by restraining RhoA signaling through a conserved signaling axis involving Cdc42 and the Rho GAP srGAP1. In this Commentary, we expand upon this new pathway and discuss potential mechanotransductive and therapeutic applications. Additionally, we speculate on a generalized role for Rho GEFs and GAPs in providing localized, context-dependent responses to the cellular microenvironment during cell migration and other cellular processes.

Keywords: extracellular matrix, rho GEF, rho GAP, rho GTPase, 3D migration

Abbreviations: GEF, Guanine nucleotide exchange factor; GAP, GTPase activating protein; ECM, extracellular matrix; T526, Threonine 526; FRET, Fluorescence resonance energy transfer; HUVEC, Human umbilical cord vein endothelial cell.

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During cell migration, cells utilize cell-matrix adhesion in concert with dynamic cytoskeletal rearrangements and myosin II-generated contractility to sense and respond to physical and chemical cues from the extracellular matrix (ECM).[1-3] Cellular responses to these extracellular signals are frequently orchestrated through the activity and function of the Rho family GTPases Cdc42, Rac1, and RhoA, as well as their guanine nucleotide exchange factor (GEF) and GTPase activating protein (GAP) regulators. However, recent investigations in 3D ECM and observations of the plasticity of 3D cell migratory modes have revealed that a cell can exhibit variable dependence on the activity of a particular Rho GTPase, which can be dictated by distinct features of the composition and structure of the surrounding ECM.[4-6] Two key contemporary questions are: What are the major extracellular determinants of cell migration in different 3D ECMs, and how are these distinct environments able to elicit changes in Rho GTPase activity?

Recently, we initiated a study with the hypothesis that cell adhesion to different matrix molecules, such as collagen and fibronectin, would trigger differential regulation of guanine nucleotide exchange factors to regulate migration.[7] To address this question, we developed an unbiased, ECM-based affinity purification screen to isolate and identify novel GEFs uniquely active under collagen or fibronectin-rich ECM conditions.[7,8] We discovered that the GEF βPix has a conserved, extracellular matrix-specific migratory function and is critical for efficient migration in fibrillar collagen environments by restraining the signaling activity of RhoA. Unexpectedly, this suppression occurs through a mechanism of Rho GTPase crosstalk between Cdc42 and RhoA that is mediated by a collagen-specific interaction between the GEF/GAP pair, βPix and srGAP1.

In addition, our model (**Fig. 1**) suggested that the collagen-specific βPix function is dictated by tight phosphoregulation of threonine residue 526 (T526) on βPix. Binding of $\alpha_2\beta_1$ integrin to fibrillar collagen leads, through PP2A, to loss of phosphorylation at T526 on βPix

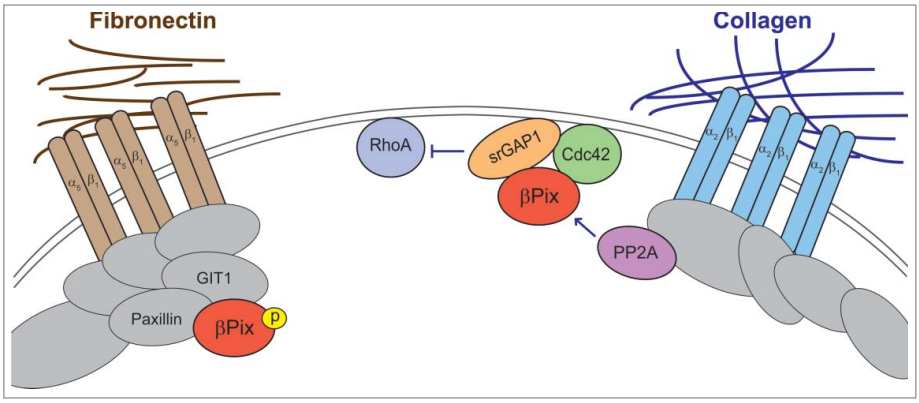

Figure 1. Summary model of the extracellular matrix-specific regulation and function of βPix during cell migration. During migration in fibronectin-rich environments, βPix is phosphorylated at threonine 526 and localizes to focal adhesions through assocations with GIT1 and paxillin. Conversely, binding of $\alpha_2\beta_1$ to fibrillar collagen leads, through PP2A, to loss of phosphorylation at T526 on βPix, βPix localization to the plasma membrane, and associations with srGAP1 and Cdc42. βPix/Cdc42/srGAP1 act to locally suppress RhoA activity at the leading edge of the cell, establishing inversely polarized gradients of Cdc42 and RhoA activity that are required for efficient cell migration in fibrillar collagen environments.

and promotes association with srGAP1. Taken together, we defined a conserved migratory signaling cascade involving PP2A/βPix/srGAP1 that coordinates suppressive crosstalk between Cdc42 and RhoA and is critical for cell migration in fibrillar collagen environments.

The existence of an extracellular matrix-specific pathway directing cell migration may at first seem puzzling physiologically, considering the heterogeneous nature of most environments found in vivo.[9-11] However, this mechanism provides an additional tier of control over the plasticity of cell migratory behavior in response to cues from each cell's extracellular environment. It provides the capacity for local changes in extracellular matrix composition and organization to elicit precise spatial control over the movement of cells during tightly regulated processes such as epithelial morphogenesis, angiogenesis, and wound healing. For example, gradients of type I collagen fibers have been reported to be sufficient to drive cytokine-independent endothelial morphogenesis and migration.[12] In fact, the increased angiogenic migratory response downstream of endothelial cell adhesion to fibrillar collagen was attributed to suppression of PKA activity, a reported kinase for T526 on βPix.[12,13] In our recent study, we observed that the collagen-specific function of βPix was indeed conserved in human umbilical vein endothelial cells (HUVECs). This suggests a potential anti-angiogenic application for the inhibition of βPix that is dependent on the ECM context.

Another exciting potential application can be surmised from recent concepts concerning the role of fibrillar type I collagen during tumor progression and metastasis. The linearization and perpendicular reorganization of fibrillar type I collagen at the tumor front is a marker of malignant transformation and metastatic potential.[14-16] In certain settings, these fibrillar collagen "tracks" serve as metastatic highways for transformed cells, facilitating dissemination away from the primary tumor.[17,18] We observed that loss of βPix in the metastatic breast adenocarcinoma MDA-MB-231 line blocks nearly all motility of these normally motile cells in 3D collagen, with no perturbation of their migration in fibronectin-rich 3D cell-derived matrices. Therefore, inhibition of βPix may have potential as an anti-metastatic therapeutic, particularly in collagen-dense tumor environments, such as the skin, breast, and pancreas,[19,20] by preventing primary tumor dissemination along collagen fibrils.

One intriguing element of this study is the identification of a pathway of active RhoA suppression, a Rho GTPase classically associated with controlling the contractile or mechanotransductive response of the cell to the microenvironment.[21,22] Traditionally, when we consider cell mechanotransduction, tension-sensitive proteins are thought to be activated through conformational changes in response to increased rigidity or other physical inputs from the surrounding microenvironment.[23,24] The newly identified collagen-specific βPix pathway of Cdc42-RhoA crosstalk surprisingly contrasts with this canonical view, in that the activity of RhoA is inherently high, yet is actively suppressed by βPix/Cdc42/srGAP1 in response to fibrillar collagen. It is not yet clear which environmental factors may be influencing RhoA activity, whether it is induced by growth-factor signaling from serum, cell-matrix adhesion between $\alpha_2\beta_1$ integrin and fibrillar collagen, or a physical aspect of the fibrillar collagen matrix itself. However, this pathway potentially presents an alternative to the classical view of mechanotransduction, i.e., a tunable RhoA responsive mechanism that adapts to changes in collagen matrix rigidity. In essence, increases in matrix rigidity could modulate the phosphorylation of βPix at T526, which would decrease the activity of Cdc42/srGAP1, remove the suppressive check on RhoA, and tailor appropriate local levels of contractile force required for efficient migration. Preliminary observations from our laboratory support this hypothesis: increasing the rigidity of 2D fibrillar collagen matrices through crosslinking is able to partially rescue the morphology and migratory defects of βPix knockdown. This observation not only delineates an alternative to traditional mechanotransduction views, but akin to the concept of distinct migratory signaling mechanisms, highlights the importance of elucidating mechanotransduction pathways that are specific to different cell-surface receptors and ECM environments.

It is surprising that no single GEF was identified as specifically active in response to fibronectin. Live-cell timelapse imaging and immunohistochemistry revealed that fibroblasts in 3D collagen and 3D cell-derived matrices migrate with no obvious differences in cell morphology and

polarization, leading edge/adhesive dynamics, or cytoskeletal assembly[7] (**Fig. 2**). Additionally, we observed through Cdc42 and RhoA live-cell FRET imaging that fibronectin is able to promote migration through similar mechanisms as fibrillar collagen, yet loss of βPix is not detrimental to migration in this condition. This finding highlights the importance of identifying matrix-specific migratory pathways and may indicate that the migratory machinery activated in response to fibronectin is driven by a distinct GEF/GAP mechanism involving Cdc42, RhoA, non-canonical GTPases such as RhoC/E/G and Rac2/3, or through some complex concerted action of multiple GTPases. This view is further supported by the observation that while srGAP1 directs GAP activity toward RhoA specifically during fibrillar collagen migration, we observed that loss of srGAP1 leads to increased Rac1 activity during migration on fibronectin. We speculate that this and other potential extracellular matrix-specific GEF/GAP mechanisms will emerge that provide local contextual regulation of cell migration in different microenvironments.

How the cell is able to regulate signaling spatially and temporally across many aspects of cell physiology is currently a major area of investigation. When one considers that the approximate 80 Rho GEFs and 70 Rho GAPs in the human genome outnumber their target GTPases by 3- to 4-fold and that many GEF/GAPs control the same processes,[25-27] it becomes clear that this apparent redundancy raises questions about the larger role served by these proteins. An emerging paradigm suggests the explanation that Rho GEFs and GAPs serve to specifically transduce the diverse number of extracellular matrix signals to the cell by restricting their exchange/hydrolysis activity to appropriate subcellular locations and the associated downstream targets of the extracellular stimuli.[25,28] Along with our recent discovery of the collagen-specific roles of βPix/srGAP1 during cell migration, other examples of contextual GEF activity dictating migratory responses include the role of the RhoA GEF PDZ-RhoGEF during breast cancer motility in response to the chemokine CXCL12[29] and the Rac1 GEF Tiam1 during keratinocyte spreading and migration downstream of $\alpha_3\beta_1$ adhesion to laminin-5.[30]

Evidence also exists for ECM regulation of GEFs/GAPs in other, non-migration related processes. The GEF Sos regulates cell cycle progression through Rac1 in endothelial cells through $\alpha_5\beta_1$ attachment to fibronectin, but not laminin or charge-mediated attachments.[31,32] Application of force on different integrin subtypes has revealed that the RhoA GEFs GEF-H1 and LARG are critical for mediating cell-matrix mechanotransduction and the cell stiffening response in collagen and fibronectin environments, respectively.[33,34] Considering the diverse range of roles played by the Rho GTPases in regulating cell physiology, the complexities of the ECM-responsive GEF/GAP system could be quite vast. Therefore, it is clear that it will be important not only to delineate the molecular functions of the many uncharacterized GEFs and GAPs, but also to elucidate the precise contexts in which they are physiologically relevant.

There are many complex interactions and forms of signaling crosstalk that occur at the leading edge of cells during migration, and the βPix/srGAP1 complex provides an elegant mechanism for restricting RhoA and concentrating Cdc42 activity toward the leading edge in collagen microenvironments. It will be of interest to expand this approach to GEFs and GAPs toward other Rho family GTPases and different cellular processes to determine whether modulation of GEF/GAP activity directs cell physiology in response to specific chemical and physical properties of the extracellular matrix. By doing so, we will gain a greater understanding of how cells respond to, and navigate within, the complex extracellular matrix environment

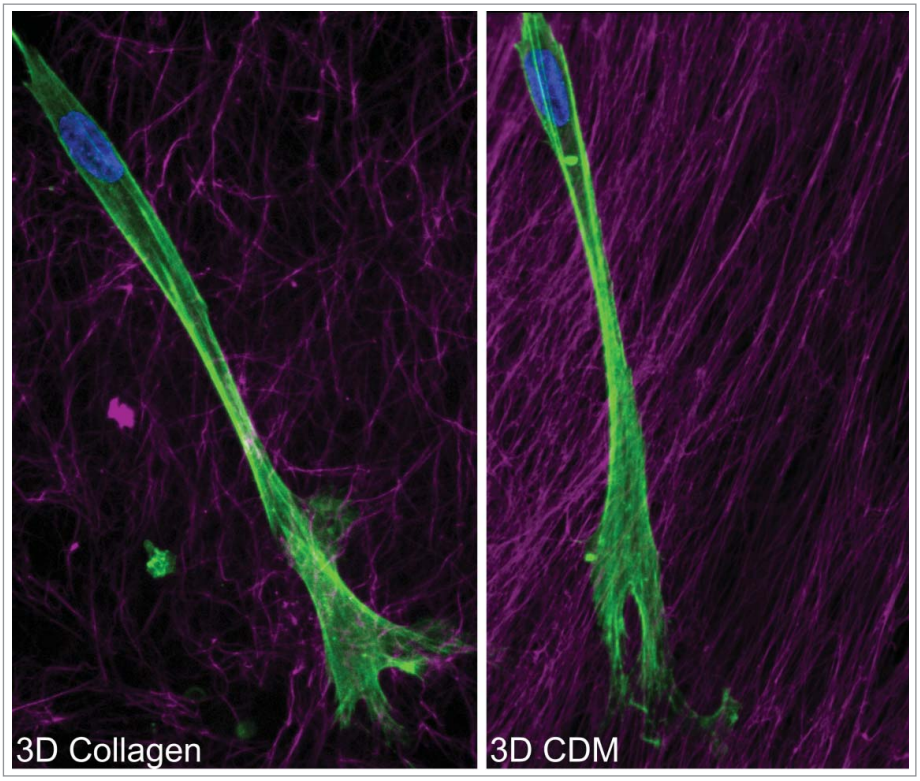

Figure 2. Primary human foreskin fibroblasts immunostained for actin (green, phalloidin) during migration in 3D collagen matrix (left, magneta collagen fibers) and 3D cell-derived matrix (right, magneta fibronectin fibers). Cells in both 3D matrix environments can migrate with similar morphologies and dynamics, yet a requirement for βPix/Cdc42/srGAP1 signaling does not exist in the fibronectin-rich cell-derived matrix. This highlights the importance of understanding how cells respond to diverse ECM environments and appropriately regulate their migratory signaling. Nuclei (blue) visualized by DAPI.

found in vivo and potentially how to intervene when these processes become deregulated in disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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