INDUCING ECTOPIC THY-1 CELL-SURFACE EXPRESSION DICTATES FIBROBLAST MIGRATION IN-VIVO

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INTRODUCTION

Fibroblasts are a critical cell in the process of wound healing. It is the normal wound healing functions of fibroblasts, including migration into and proliferation within provisional matrices as well as matrix deposition and resolution, that are likely dysregulated in fibrotic disorders leading to loss of function of the involved organ system. The goal of biomedical engineering is to improve the quality of life through the development of medical technologies, which are capable of restoring lost functionality. For this purpose fibroblasts are an ideal target of study. They hold tremendous potential in the design of tissue engineered constructs for implantation and their functions many times lead to premature loss of function of synthetic implants. Fibroblasts are heterogeneous throughout the body and thus behave differently to various stimuli. Despite this fact, scientists and engineers often neglect the heterogeneity in the design of experiments and implants. Thy-1 surface expression represents the best-defined model of fibroblast heterogeneity and despite the early characterization of their morphological differences, the mechanism directing the different morphologies has yet to be identified. In these studies the role of Thy-1 in directing fibroblast adhesion and migration is determined. Using a molecular biology approach we developed a model system and determined the role of Thy-1 in regulating the cytoskeleton, including actin stress fibers and cellular adhesions, as well as fibroblast migration. We additionally provide evidence for a possible molecular mechanism for the observed effects of Thy-1 surface expression on fibroblasts.

RESULTS AND DISCUSSION

Lung fibroblasts from Lewis rats were isolated, cultured and sorted based on Thy-1 surface expression as previously described [1]. Primary fibroblasts sorted based on the presence or absence of Thy-1 were characterized and found to differ in their focal adhesion structures and cytoskeletal arrangement (Data not shown). Fibroblasts expressing cell surface Thy-1 (Thy-1(+)) display well-developed focal adhesions (as seen by immunoflourescent staining against vinculin) distributed along the entire basal surface of the cell whereas, fibroblasts lacking Thy-1 surface expression (Thy-1(-)) exhibit a more rounded contour and contain smaller, more peripherally-distributed focal complexes. Thy-1(+) fibroblasts also demonstrate large, well-organized bundled actin stress fibers extending from the large focal adhesion structures; whereas Thy-1(-) fibroblasts display fewer stress fibers (visualized by staining with TexasRED-X-Phalloidin) and a more peripheral actin distribution. Adhesion complexes, which link integrins to the actin cytoskeleton, exert significant control on the migratory potential of cells, with loosely- or tightly-adherent cells migrating less efficiently than cells that are in a state of intermediate adhesion. Fibroblast Thy-1 subpopulations differ significantly with respect to cell migration, Thy-1(-) fibroblasts migrating more efficiently into in-vitro wounds (Fig. 1a). Thy-1(+) fibroblasts maintain continuity with the surrounding monolayer and appear to spread into the wound region rather than actively migrating (Fig. 1b).

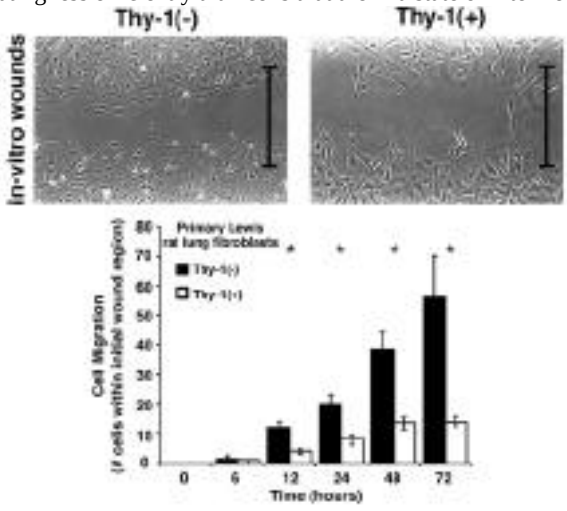

Figure 1. Primary fibroblasts expressing Thy-1 demonstrate decreased migration.

To identify the role of Thy-1 in the observed phenotypes, we examined the effects of ectopic Thy-1 expression in a Thy-1(-) fibroblast cell line. RFL6 fibroblasts transfected with murine Thy-1 cDNA (a generous gift from Dr. Hiromitsu Nakauchi, The Institute of Physical and Chemical Research, Japan) in a mammalian expression vector display a greater number of aligned focal adhesions compared to RFL6 fibroblasts transfected with the empty vector alone, recapitulating the phenotypes observed in naturally-occurring Thy-1(+) cells (Data not shown). Focal adhesions in Thy-1 transfected RFL6 fibroblasts were distributed across the entire basal cell surface, as observed in primary Thy-1(+) rat lung fibroblasts. Focal adhesions in the empty-vector transfected RFL6 fibroblasts retained a mostly peripherally distribution. Larger, more organized stress fibers were seen in the Thy-1 transfected RFL6 fibroblasts compared to cells transfected with empty vector. Thy-1 transfected cells were unable to migrate efficiently into wound spaces compared with empty-vector-transfected cells (Fig 2a, b). These findings demonstrate that Thy-1

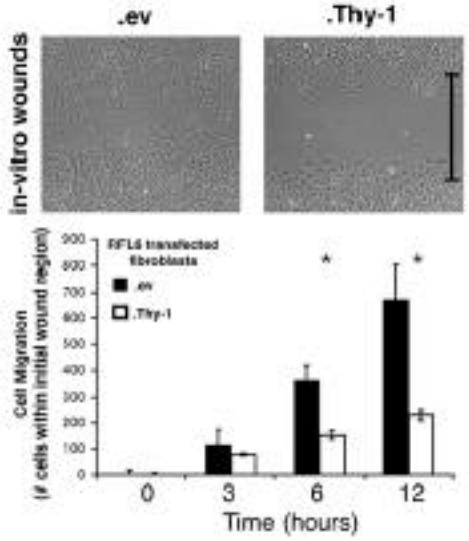

Figure 2. Ectopic expression of Thy-1 inhibits fibroblast migration.

surface expression promotes fibroblast adhesion and cytoskeletal organization, and inhibits cell migration. These findings are consistent with adhesive and anti-migratory effects in other cells expressing Thy-1. Thy-1 inhibits neurite outgrowth on astrocytes, and mediates adhesion of thymocytes to thymic epithelium. Interestingly, loss of Thy-1 surface expression has been associated with fibroblast oncogenic transformation and anchorage-independent growth.

Because Thy-1 is a glycophosphatidyl-inositol-linked protein found on the outer leaflet of the cell membrane, it is unclear how it modulates signalling. Nevertheless, crosslinking of Thy-1 in lymphocytes results in the activation of src-family protein tyrosine kinases (SFKs). Since Thy-1-mediated signaling in T-cells requires SFKs and because SFKs have been implicated in the regulation of cell-substrate attachment and stress fiber dynamics, we explored whether SFKs were affected following Thy-1 transfection of RFL6 fibroblasts. Immunoblots of unstimulated Thy-1-transfected RFL6 cells demonstrate decreased basal SFK activity when compared to empty-vector transfected cells (Fig. 3a, $p = 0.007$). This finding is consistent with an observed inhibitory effect of Thy-1 on SFK activity in other lymphocytes.

Based on the morphological data the most likely downstream signal of Thy-1 inhibition of src is the GTPase rho. Activation of Rho GTPase, promotes stress fiber assembly and focal adhesion formation which can significantly impact cell migration. Concomitant with Thy-1-induced decreases in SFK activity, we observed a 393% increase in the basal activation level of Rho GTPase in Thy-1-transfected RFL-6 fibroblasts, compared to empty-vector transfected cells (Fig. 3c, $p = 0.028$). Active Rho was determined by a Rhotekin-GST pull-down assay following previously published protocols [5]. Recently, it has been demonstrated that p190 RhoGAP, a GTPase-activating protein (GAP), is critical to SFK-mediated regulation of Rho GTPase activity and in Thy-1 transfected RFL6 cells we observed a significant decrease in phosphorylation of p190 RhoGAP to a similar degree of that observed with addition of SFK inhibitor PP2 to mock-transfected cells (Fig. 3b, $p = 0.009$), indicating a mechanism whereby Thy-1 modulates Rho activity by inhibiting SFK phosphorylation of p190 (Fig. 3d), leading to Rho acitivation and promotion of focal adhesions and stress fibers.

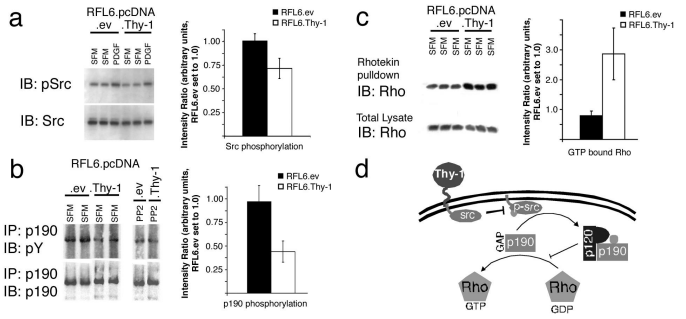

Figure 3. Thy-1 inhibits src activation leading to increased rho GTPase activity and the observed phenotypes.

Fibroblast adhesion and migration are of critical importance to tissue homeostasis, wound healing, fibroproliferation and tumor stroma formation, and are therefore tightly controlled. Rho GTPases have emerged as prominent regulators of these processes, coordinating intracellular and extracellular signals to link adhesion to cytoskeletal organization critical to multiple cellular processes. Here we demonstrate an additional level of control based on the surface expression of Thy-1, which correlates with decreased SFK activity resulting in net increased activation of Rho, culminating in increased adhesion and cytoskeletal organization and decreased motility. Conversely, fibroblasts lacking Thy-1 may migrate more robustly into a wound environment. This represents a newly-identified function for Thy-1 in fibroblasts, and indicates a likely mechanism for the effects of Thy-1 on other adhesive and migratory events, such as neurite extension. The striking effects of Thy-1 on the fibroblast phenotype underscore the importance of considering cellular heterogeneity in characterizing biological processes.

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