CONTRIBUTIONS OF FOCAL ADHESION KINASE TO CELL ADHESION STRENGTH

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INTRODUCTION

Cell adhesion to the extracellular matrix plays a critical role in numerous physiological and pathological processes as well as biotechnological applications. During adhesion, matrix-bound cellular receptors, integrins, associate with the cytoskeleton and cluster together to form focal adhesions [1]. Focal adhesions are discrete complexes of structural and signaling molecules that function to mechanically link the cytoskeleton and the extracellular matrix and trigger signaling pathways that direct cellular responses [2]. Focal adhesion kinase (FAK) is a widely expressed protein central to the regulation of focal adhesion assembly [3]. Upon integrin ligation, FAK interacts with integrins and becomes activated through tyrosine phosphorylation. Activated FAK, in turn, phosphorylates and interacts with multiple targets including talin, paxillin, src, and PI-3 kinase. FAK is essential to development as illustrated by early lethality in FAK null embryos [4]. Using FAK -/- embryo fibroblasts genetically engineered to express FAK under a tetracycline-inducible promoter, Hanks and colleagues demonstrated that FAK enhances the rate and extent of spreading as well as cell migration [5]. In the present study, we analyzed the contributions of FAK to cell adhesion strengthening using these FAK inducible cells with our spinning disk assay and micropatterning surfaces.

MATERIALS AND METHODS

Micropatterned Surfaces

Micropatterned surfaces consisting of cell adhesive circular islands (5 μm diameter) in a non-adhesive background were engineered using microcontact printing of alkanethiol self-assembled monolayers as previously described [6]. Using standard photolithography techniques, a template of an array of islands on a Si wafer was created, and a PDMS stamp having the desired features was cast from the Si molds. The stamp was inked with CH_3-terminated alkanethiols and pressed onto Au-coated surfaces to create the adhesive islands. The remaining area was coated with tri-ethylene glycol-terminated alkanethiols, which resist protein adsorption and cell adhesion. Unpatterned surfaces consisted of uniform CH_3-terminated monolayers. Substrates were coated with fibronectin (FN) (20μg/mL) for 1 hour, blocked with 1% heat-denatured bovine serum albumin, and soaked in PBS for 1 hour prior to cell seeding.

Cells with Inducible FAK

Tet-FAK cells, isolated from FAK-/- embryos and engineered to express FAK under a tetracycline-inducible promoter, have been previously characterized [5]. In the presence of tetracycline, these cells do not express endogenous FAK, whereas in the absence of tetracycline, high FAK levels are induced. For this study, cells were cultured in the absence or presence of tetracycline (1 μg/ml) for two days prior to cell seeding. Cells were then seeded under the proper culture conditions onto FN-coated micropatterned surfaces, unpatterned surfaces, or tissue culture plastic for 16 hours prior to subsequent assays. The levels of FAK expression in induced and non-induced cells after 16 hours of seeding on tissue culture plastic were analyzed by Western blotting.

Immunofluorescence Staining for Focal Adhesion Proteins

Immunofluorescence (IF) staining was used to examine focal adhesion assembly on micropatterned and unpatterned surfaces. Cells were permeablized with Triton X-100/protease inhibitor buffer and fixed with 10% formalin. After blocking in 5% fetal bovine serum, samples were incubated in primary antibodies against focal adhesion components followed by fluorochrome-labeled secondary antibodies.

Cell Adhesion Assay

Cell adhesion strength was quantified using our spinning disk assay [7]. This system applies a linear range of hydrodynamic shear forces to adherent cells and provides sensitive measurements of adhesion strength. Samples with adherent cells were spun for 5 min, fixed, and stained with ethidium homodimer. Cell numbers at different radial positions were quantified using a motorized microscope stage and image analysis system.

RESULTS

FAK Expression

High expression levels of FAK were induced by removing tetracycline from the culture medium, while culturing cells in the presence of tetracycline repressed FAK expression (Figure 1).

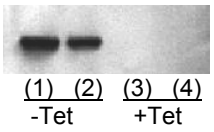
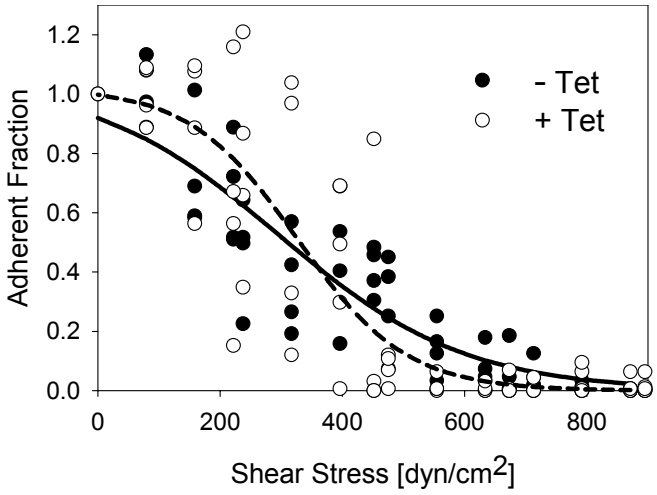


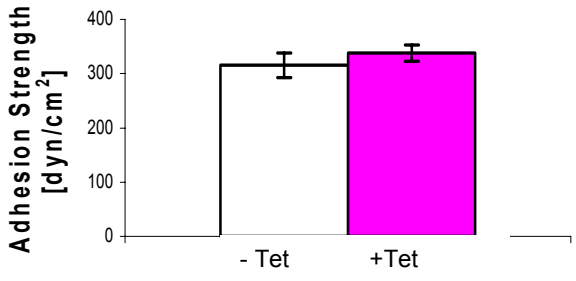
Figure 1. FAK expression in Tet-FAK cells with or without presence of tetracycline quantified by Western blotting.

Adhesion Strength

In our adhesion assay, adhesion strength is characterized as the shear stress that produces 50% of the cell population to detach from the substrate. The number of adherent cells, normalized to the number of cells at the center of the disk (no applied force), is plotted as a function of surface shear stress and a sigmoid curve is curve fit to this adhesion profile to obtain estimates of adhesion strength (Figure 2a). No significant differences in adhesion strength were detected between cells cultured in the presence or absence of tetracycline (Figure 2b). These results indicate that FAK expression does not modulate long-term adhesion strength.



(a)



(b)

Figure 2. Cell adhesion strength (16 hr) for Tet-FAK cells cultured in the presence or absence of tetracycline. (a) Adherent cell fraction profile (b) Summary of adhesion strength values.

Focal Adhesion Assembly

Focal adhesion assembly was investigated via immunofluorescence staining against focal adhesion proteins such as talin and vinculin as well as localization with the actin cytoskeleton and integrin subunit α_5. No gross differences were observed in focal adhesion size, composition or distribution in the presence or absence of FAK (Figure 3).

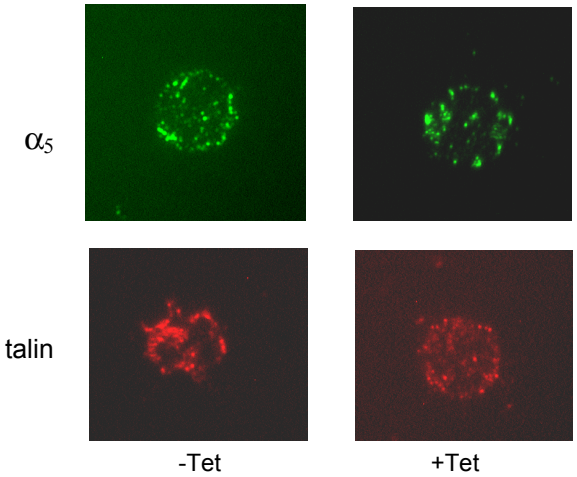


Figure 3. Focal adhesion structure with or without tetracycline stained for α_5 integrin and talin.

DISCUSSION

We used a tetracycline-responsive promoter to express FAK in FAK-null fibroblasts in order to analyze the contributions of FAK to cell adhesion strengthening. Surprisingly, no differences in steady state (16 hr) adhesion strength were observed between FAK-induced and non-induced cells. We attribute these similarities in adhesion strength to similarities in focal adhesion assembly. Although FAK plays a central role in focal adhesion assembly, other proteins, such as PYK2, have been shown to be overexpressed and compensate for the absence of FAK in FAK-null cells [8]. Previous studies have demonstrated an important role for FAK in cell migration [3-5]. Our results suggest a complex relationship between adhesion strength and cell migration. We are currently analyzing the role of FAK in adhesion strengthening kinetics and spreading. FAK has been implicated in the turnover of focal adhesions and may be important in regulating the strengthening process.

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