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Opposing effects of collagen I and vitronectin on fibronectin fibril structure and function

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ABSTRACT

Extracellular matrix fibronectin fibrils serve as passive structural supports for the organization of cells into tissues, yet can also actively stimulate a variety of cell and tissue functions, including cell proliferation. Factors that control and coordinate the functional activities of fibronectin fibrils are not known. Here, we compared effects of cell adhesion to vitronectin versus type I collagen on the assembly of and response to, extracellular matrix fibronectin fibrils. The amount of insoluble fibronectin matrix fibrils assembled by fibronectin-null mouse embryonic fibroblasts adherent to collagen- or vitronectin-coated substrates was not significantly different 20 h after fibronectin addition. However, the fibronectin matrix produced by vitronectin-adherent cells was ~10-fold less effective at enhancing cell proliferation than that of collagen-adherent cells. Increasing insoluble fibronectin levels with the fibronectin fragment, anastellin did not increase cell proliferation. Rather, native fibronectin fibrils polymerized by collagen- and vitronectin-adherent cells exhibited conformational differences in the growth-promoting, III-1 region of fibronectin, with collagen-adherent cells producing fibronectin fibrils in a more extended conformation. Fibronectin matrix assembly on either substrate was mediated by α5β1 integrins. However, on vitronectin-adherent cells, α5β1 integrins functioned in a lower activation state, characterized by reduced 9EG7 binding and decreased talin association. The inhibitory effect of vitronectin on fibronectin-mediated cell proliferation was localized to the cell-binding domain, but was not a general property of αvβ3 integrin-binding substrates. These data suggest that adhesion to vitronectin allows for the uncoupling of fibronectin fibril formation from downstream signaling events by reducing α5β1 integrin activation and fibronectin fibril extension.

1. Introduction

Extracellular matrices (ECM) are intricate networks of collagens, glycoproteins, and proteoglycans that maintain tissue cohesion, provide physical support to tissues and organs, and determine the mechanical properties of tissues (Hynes, 2009). In addition to these structural functions, ligation of ECM proteins by various cell surface receptors can initiate biochemical signaling pathways that control cell behaviors important for tissue formation and function (Kleinman et al., 2003). In order to maintain tissue homeostasis, yet allow for changes in tissue function, ECM proteins must shift between these two functional states. Mechanisms that permit ECMs to locally transition from 'inert' structural elements to 'active' signaling complexes are largely unknown but may include changes in protein composition, organization, degradation, turnover, and/or conformation (Beacham and Cukierman, 2005; Nelson and Bissell, 2006; Sottile et al., 2007; Clause and Barker, 2013). Imbalances in the relative expression of the passive structural and active signaling states of ECM proteins within the tissue microenvironment may underlie a variety of pathological conditions, including atherosclerosis, tumor progression, chronic wounds, and fibrosis.

The glycoprotein fibronectin is a principal component of interstitial ECMs. Fibronectin is ubiquitously expressed in a variety of tissues in the body, and is abundant in regions surrounding blood vessels, in lymphatic tissue stroma, and in loose connective tissue (Stenman and Vaheri, 1978). Fibronectin circulates in the plasma and other body fluids in a soluble form, and is continually assembled into insoluble ECM fibrils by a cell-dependent process (Singh et al., 2010). During the matrix assembly process, soluble cell-bound fibronectin molecules undergo a series of conformational changes that result in exposure of self-interactive sites and the subsequent formation of fibronectin fibrils (Singh et al., 2010). Tractional forces produced by cell or tissue movement can induce further conformational changes to expose neoepitopes, or "matricryptic sites" (Ohashi et al., 1999; Baneyx et al., 2001; Hocking et al., 2008) that mediate effects of ECM fibronectin on cell and tissue function (Davis et al., 2000; Gui et al., 2006; Hocking et al., 2008). ECM fibronectin fibrils have the capacity to stimulate a variety of cellular activities, including cell spreading, growth, migration,

Abbreviations: ECM, extracellular matrix; FNIII, fibronectin type III repeat; αSMA, alpha smooth muscle actin; FN-null MEFs, fibronectin-null mouse embryonic fibroblasts; DOC, deoxycholate; GST, glutathione-S-transferase.

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and contractility (Morla et al., 1994; Mercurius and Morla, 1998; Sottile et al., 1998; Hocking et al., 2000; Sottile et al., 2000; Hocking and Chang, 2003). In vivo, matricryptic sites in interstitial fibronectin fibrils contribute to resting vascular tone, and mediate transient, local vascular responses to skeletal muscle contraction via a nitric oxide-dependent pathway (Hocking et al., 2008). The formation of fibronectin fibrils in the body is a continuous process, with as much as 50% of ECM fibronectin turnover occurring every 24 h (Rebres et al., 1995). Thus, fibronectin fibril structure must be tightly regulated in order to prevent inappropriate or unregulated expression of the signaling activity of ECM fibronectin fibrils.

Vitronectin is another multifunctional glycoprotein that is present in various body fluids, including plasma and amniotic fluid, and is also found in limited quantities in the ECM (Hayman et al., 1983; Dahlback et al., 1986). In normal tissue, vitronectin is predominately associated with loose connective tissue (Reilly and Nash, 1988). Similar to fibronectin, increased tissue deposition of vitronectin has been observed in a number of disease states, including tissue fibrosis (Reilly and Nash, 1988), atherosclerosis (Stenman et al., 1980; Dufourcq et al., 1998), tumor progression (Loridon-Rosa et al., 1988; Kaplan et al., 2005) and sepsis (Brien et al., 1998; Singh et al., 2005). Similar tissue distribution patterns of vitronectin and fibronectin have been observed in fibrotic tissues, within the reactive stroma of tumors, and in the provisional matrix during wound repair (Reilly and Nash, 1988; Singer and Clark, 1999). In vitro, vitronectin can inhibit the deposition of fibronectin into the ECM (Hocking et al., 1999; Zhang et al., 1999b; Bae et al., 2004; Vial et al., 2006; Vial and McKeown-Longo, 2008), suggesting that vitronectin can regulate ECM fibronectin function by reducing the extent of fibronectin matrix deposition. Other studies indicate that vitronectin can enhance fibronectin fibrillogenesis, as ligation of the vitronectin integrin receptor plays a key role in mediating the centripetal movement of fibronectin into fibrillar adhesions (Pankov et al., 2000).

In the present study, we examined how adhesion of cells to either vitronectin or collagen I affects fibronectin fibril assembly, structure, and function. Fibronectin-null mouse embryonic fibroblasts (FN-null MEFs), cultured in defined serum- and fibronectin-free growth media, were used to directly compare the mechanisms and cellular effects of vitronectin-mediated fibronectin matrix assembly with that of collagen-mediated assembly. FN-null MEFs polymerize exogenously-added fibronectin into ECM fibrils via a process indistinguishable from that of fibronectin-expressing cells (Sottile et al., 1998), and do not express detectable levels of vitronectin (Wojciechowski et al., 2004). Thus, this model permits tight control over the composition of the adhesive substrate, the amount of fibronectin to which the cells are exposed, and the initiation and timing of the matrix assembly process. We identify similarities and differences in the assembly process utilized to produce these two fibronectin matrices and show that fibronectin fibrils produced by vitronectin-adherent cells are significantly less effective at supporting integrin activation and stimulating cell proliferation than those produced by collagen-adherent cells. These studies suggest opposing roles for vitronectin and collagen in regulating the structural and functional properties of ECM fibronectin.

2. Results

2.1. Effects of vitronectin and collagen on fibronectin-induced cell proliferation

Several previous studies have established that the active assembly of a fibronectin matrix can enhance cell proliferation (Mercurius and Morla, 1998; Sechler and Schwarzbauer, 1998; Sottile et al., 1998). Thus, cell proliferation assays were used in the present study to assess the functional state of fibronectin fibrils. To examine the effect of substrate adhesion on the proliferative response to ECM fibronectin, FN-null MEFs were seeded onto wells pre-coated with saturating concentrations of either collagen or vitronectin (Sottile et al., 1998; Wojciechowski et al., 2004), in the absence or presence of increasing concentrations of fibronectin. Cell number was determined after 4 days (Sottile et al., 1998). Addition of high concentrations of fibronectin (600 nM; ~ plasma concentration (Mosher, 1984)) to either collagen- or vitronectin-adherent cells resulted in a similar 2-fold increase in cell number on day 4, compared to cells cultured in the absence of fibronectin (not shown). The growth response of collagen- and vitronectin-adherent cells to various fibronectin concentrations was then calculated as a percentage of the growth response to 600 nM fibronectin and sigmoidal curves were generated for both substrates (Fig. 1A). Adhesion of cells to vitronectin resulted in a rightward shift of the fibronectin dose response curve compared to that of collagen-adherent cells (Fig. 1A), indicating that vitronectin-adherent cells required more fibronectin to achieve a similar increase in cell number as collagen-adherent cells. The concentration of fibronectin required to produce 50% of the maximal fibronectin-induced growth was 41.1 ± 1.17 nM for vitronectin-adherent cells and 3.7 ± 1.2 nM for collagen-adherent cells. In parallel studies, the amount of fibronectin bound to collagen- or vitronectin-adherent cells was quantified 20 h after fibronectin addition, prior to changes in cell number (Sottile et al., 1998). At this time point, the amount of fibronectin bound by vitronectin-adherent cells was not significantly different from that of collagen-adherent cells for all fibronectin concentrations tested (Fig. 1B).

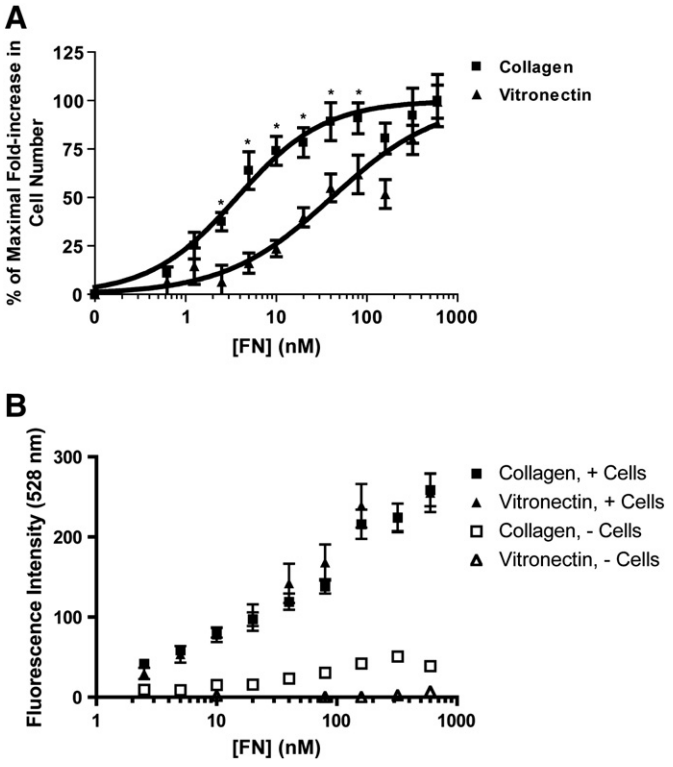

Fig. 1. Cell adhesion to vitronectin reduces the proliferative response to fibronectin. FN-null MEFs were seeded (A, 2.6×10^3 cells/cm^2; B, 3×10^4 cells/cm^2) onto collagen- or vitronectin-coated wells and allowed to adhere for 4 h. (A) Fibronectin (0.625–600 nM) or an equal volume of the vehicle control, PBS ('0' FN), was added to wells. Cell number was determined 4 days after seeding. Data are presented as a percentage of the maximal-fold increase in cell number relative to PBS-treated controls. GraphPad Prism was used to fit a sigmoidal curve to the data. *Significantly different from vitronectin-adherent cells at the given fibronectin concentration by Student's *t*-test, n > 3 for all time points. The average absorbance values for control (PBS)-treated groups were 0.28 ± 0.02 (collagen) and 0.34 ± 0.02 (vitronectin). (B) Alexa^{488}-labeled fibronectin (FN^{488}, 2.50–600 nM) was added to wells and cells were incubated for an additional 20 h. FN^{488} accumulation was determined as described in "Experimental Methods". Data are presented as mean fluorescence ± SEM and are compiled from 3 separate experiments performed in triplicate.