Engineered Matrix Mimetics Support Assembly of Two Distinct Forms of Fibronectin Matrix

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Statement of Purpose: Extracellular matrix (ECM) fibronectin (FN) promotes cell behaviors critical to wound repair and is essential for collagen deposition and ECM remodeling (1-4). We have developed a series of recombinant FN matrix mimetics that promote cell growth, migration and contractility as adhesive substrates by coupling the heparin-binding, FNIII1 fragment (FNIII1H) to the integrin-binding domains (FNIII8-10). The goal of this study was to determine whether FN matrix mimetics support cell-mediated FN matrix assembly as adhesive substrates, thus making them candidates as bioactive coatings for tissue engineered scaffolds to promote wound repair.

Methods: FN-null mouse embryonic myofibroblasts were used in this study. FN-null cells are grown under serum-free conditions and assemble exogenously-added FN into ECM fibrils via mechanisms utilized by FN-expressing cells (1). This allows us to analyze the effects of FN matrix mimetics on cell behavior in the absence and presence of FN. FN-null cells were seeded on tissue culture plates coated with saturating densities of FN matrix mimetics. After adhering to the mimetics, cells were treated with exogenous FN and allowed to polymerize it into a fibrillar matrix for 20 hours. FN matrix assembly was assessed by immunofluorescence microscopy and deoxycholate (DOC) extraction. To determine differences in cell adhesion to the FN matrix mimetic substrates, focal contact formation and binding avidity studies were conducted. Focal contacts were visualized before and after FN treatment by immunofluorescence microscopy using antibodies directed against vinculin and α5 integrin. Binding avidity was determined using a centrifugation assay that forces cell attachment to the FN matrix mimetic substrate at 70 x g force for 4 minutes before washing away unbound cells.

Results: To determine if FN matrix mimetics support matrix assembly, FN-null cells adherent to FN matrix mimetics were treated with exogenous FN and allowed to assemble a fibrillar matrix for 20 hours. At saturating densities, constructs that contained the FNIII9 module (GST/III1H,8-10) did not support FN matrix assembly (Figure 1A) and α5β1 integrins remained in central and peripheral focal contacts throughout the cell, indicating integrin ligation without translocation. Cells adherent to constructs lacking the α5β1 integrin-binding module, FNIII9 (GST/III1H,8,10 and GST/III1H,8^{RGD}), organized exogenous FN into a fibrillar matrix (Figure 1A) and supported α5β1 integrin translocation into fibrillar adhesions. DOC-extractions were performed to quantify the amount of FN deposited into the matrix by cells adherent to FN matrix mimetics. Cells adherent to GST/III1H,8,10 deposited a predominately DOC-insoluble matrix (Figure 1B). Cells adherent to GST/III1H,8^{RGD}, a construct in which the RGD sequence from FNIII10 is inserted into an analogous location within FNIII8, assembled a DOC-soluble FN matrix (Figure 1B). This suggests that FN matrix mimetics can be used as adhesive substrates to direct assembly of two distinct forms of FN matrix, one that is soluble and one that is detergent-insoluble. Cell adhesion assays were performed to assess differences in initial cell attachment to the matrix-supporting FN constructs. There were no differences cell adhesion to GST/III1H,8-10, GST/III1H,8,10 and GST/III1H,8^{RGD} when cells were allowed to attach for 30 minutes. When cells were forced into contact with the substrate for 4 minutes at 70 x g, adhesion was reduced on GST/III1H,8^{RGD} compared to GST/III1H,8-10 and GST/III1H,8,10. Taken together, these data suggest that FN matrix assembly and α5β1 integrin translocation can be supported by mimetic substrates that lack FNIII9. Further modifying the FN construct by inserting the RGD sequence into FNIII8 reduces the binding avidity for the construct and directs assembly of a DOC-soluble FN matrix.

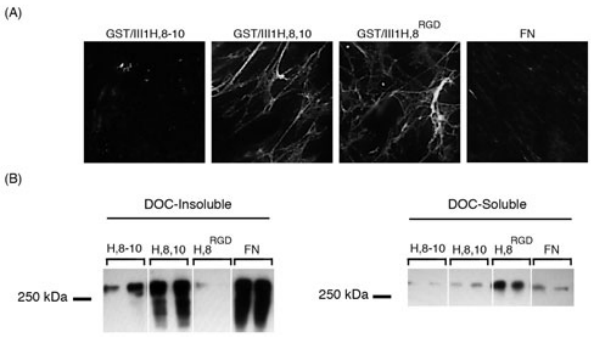

Figure 1. FN-null cells were seeded on tissue culture plates coated with either GST/III1H,8-10 (H,8-10), GST/III1H,8,10 (H,8,10), GST/III1H,8^{RGD} (H,8^{RGD}) or full-length FN. After 4 h, FN was added to each well and cells were incubated an additional 20 h. (A) Cells were fixed and processed for immunofluorescence microscopy using a polyclonal anti-FN antibody. (B) Cells were washed to remove unbound FN and incubated with 1% DOC buffer. The entire DOC-insoluble pool and 5μg of total protein from the DOC-soluble pool were analyzed by immunoblotting with an anti-FN antibody.

Conclusions: FN matrix mimetics promote cell growth, spreading, migration and contractility. Here we show that growth-promoting matrix mimetics lacking FNIII9 support cell-mediated assembly of either a DOC-insoluble or DOC-soluble matrix. The ease of production of our small FN matrix mimetics, coupled with their ability to regulate the assembly of ECM FN, make them promising candidates for incorporation into tissue scaffolds to promote wound repair.

References: 1. (Sottile J. J Cell Sci. 1998:111:2933-43) 2. (Hocking DC. J Biol Chem. 2000:275:10673-82) 3. (Hocking DC. Am J Physiol Lung Cell Mol Physiol. 2003:285:L169-179) 4. (Chiang H. Artherioscler Thromb Vasc Biol. 2009:29:1074-9)