Identification of novel Fibronectin strain-specific binding peptides

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Statement of Purpose:

Cell-biomaterial mechanical interactions are becoming increasingly important in biomaterial design. Recent studies have demonstrated that substrate stiffness is capable of instructing cell behavior [1]. One prominent example of which is compliance matching - whereby cells use their contractile machinery to match their internal stresses to extracellular matrix properties. The relevance of compliance matching in biomaterial fate is not fully understood, however, one possible consequence is aberrant cellular forces on the extracellular matrix of the foreign body capsule. Recently it has been established that cells, through their contraction are capable of unfolding the ECM protein fibronectin (FN), both during and after fiber assembly possibly exposing "cryptic sites" within the FN molecule. [2] Indeed, we have observed that a cell's capacity to unfold and assemble FN is highly sensitive to substrate compliance. This substrate compliance-mediated FN unfolding phenomena may reveal novel binding motifs for cell surface receptors, thus altering their behavior at the biomaterial interface. This cell/biomaterial dynamic interplay is dependent on hypothetical cell surface "strain sensors" that recognize fibronectin experiencing different amounts of unfolding. However, to the best of our knowledge, specific binding motifs that differentiate between unfolded and folded FN have not been reported.

Our hypothesis is that mechanically unfolding FN type-III domains by straining fibronectin fibers results in strain-sensitive binding motifs [4]. Here, we present evidence of binding partners to FN that are strain-specific. Our findings may shed light on recent studies exploring the mechanomodulation of cell behavior by biomaterials.

Methods:

Substrates (10um wide ridges separated by 50um spacing) for FN fiber deposition were prepared on PDMS via standard soft lithography. Briefly, photomasks for lithography were designed in CAD software and printed at 10000dpi (CAD/Art services). Silicon masters were fabricated using standard photolithography techniques. The PDMS surface was treated by sonication for 15minutes, followed by 0.1N NaOH for 30minutes, 3% aminopropyltriethyoxysilane (Thermo Scientific) for 20minutes, and 1% glutaraldehye (VWR) for 30 minutes. PDMS sheets were rinsed in DI and dried.

Human plasma fibronectin(VWR) was labeled with AlexaFluor 488 (Invitrogen), mixed into a 95:5 unlabeled/labeled ratio and diluted to 1mg/mL. Frozen aliquots were thawed and used immediately for fiber deposition. Fibronectin fibers were deposited manually across the patterned features to create freely suspended fibers. 20 fibers were deposited per sample. Following deposition, uniaxial strain was applied to the fibers on the PDMS surface using a home-made straining device. Strain applied to the PDMS sheet was transferred to the immobilized fibronectin fibers, and increasing strains result in type III domain unfolding. Fibers were dried after deposition, and the PDMS surfaces passivated with 1% BSA for 30 minutes. Phage display panning was then performed using with fuse5 6mer random peptide library.

Results:

Fibers were successfully deposited and phage screens up to round 4 show enrichment with respect to both compressed and strained fibers (fig 1). As expected, subsequent rounds of phage screens with increasing stringency for binding (increasing amounts of tween-20) enriches the population of strong binders, as seen by more phage recovered in the bound fractions. The decrease in the phages recovered in wash fractions by round 3 suggests reduction of weakly bound peptides. The increase in phages recovered in wash fractions in round 4 may be binding site limited.

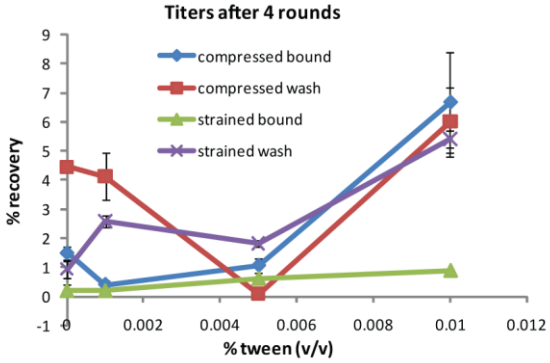

Figure 1. Peptide library screens on compressed and strained FN fibers.

Sequencing of phage clones after Round 1 of screens have identified two unique peptides as possible binding motifs that specifically bind compressed and strained fibronectin fibers, respectively. Validation of these peptides is currently underway.

Conclusions:

The implications of finding peptides that target the cryptic sites in fibronectin could provide insight into the mechanisms of how cells respond to mechanical changes in their substrate and inform biomaterial design. A better understanding of fibronectin unfolding also has applications to diseases associated with protein aggregation. Ingham and coworkers have described that self-association of unfolded type III domains can form amyloid-like fibrils [5], which is implicated in diseases progression in Alzheimer's. Whether these unique strain-specific binders have utility as imaging/detection elements also remains to be investigated.

References:

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