Integrin Activation as a Tool to Modulate Vascular Patterning

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Statement of Purpose:

Our laboratory investigates the design and engineering of scaffolds for therapeutic angiogenesis. In my work I am particularly interested in modulating integrin activation within these scaffolds to guide the angiogenic process. Integrin-mediated interactions have been known to regulate endothelial cell proliferation, migration and survival. The availability of integrin ligands also regulates cell behavior during angiogenesis. Thus, hypothesized that endothelial branching network would be affected by integrin activation and that integrin activation could be used as another angiogenic signal to regulate the formation of a mature and functional vasculature within biomaterial scaffolds..

Methods:

Two different recombinant fibronectin fragments, Fn9*10 and Fn9(4G)10 (provided by Barker Lab, Georgia Tech) , were utilized to modulate integrin specificity. Fn9*10 and Fn9(4G)10 are designed to bind α5β1 integrin and αvβ3 integrin respectively, through engineering the linker region between the 9 and 10 repeats of fibronectin[1,2]. To study how the 9*10 and 9(4G)10 fibronectin fragments affected EC activation *in vitro* we immobilized the fragments to a SAM surface through thiol chemistry and used ELISA for quantification. EC activation by these surfaces was assessed by western blot analysis of p-p38 and p-AKT and also immunofluorescence. A HUVEC sprouting assay in fibrin [3] was utilized for studying endothelial cell sprouting. 2μM of Fn9*10 or Fn9(4G)10 was added to 2 mg/ml of fibrin. Bead assays were monitored for 7 days with 2 ng per ml VEGF replenished every two days.

Results:

Surface density of ~45 ng/cm^2 was used for both Fn9*10 and Fn9(4G)10 to study EC activation in 2D (Fig. 1 a, b). Cells were either exposed to the fragments by sandwiching two surfaces (one with cells and the other with the fragments) or cells were seeded directly on the surfaces. Both fragments showed enhanced activation for p-p38 and p-AKT when co-immobilized with covalently-linked VEGF. (Fig. 1 c, d). ECs also displayed different morphologies and different integrin activation on different surfaces (Fig. 1 e). In 3D EC bead branching assay, the activation of α5β1 via the Fn9*10 induced the most tip cell filopodia. Specifically, 5/6 filopodia branches per tip cell only occurred under ECs activated through α5β1 (Fig. 1, j). Compared to matrices that did not contain Fn fragments, the inclusion of fragments resulted in significantly shorter branches but significantly more branching points (Fig. g, h). Interestingly, 9(4G)10 resulted in significantly more EC proliferation around the bead than no FN variant or 9*10 fragment(Fig1. i).

Conclusions:

Modulated integrin activation results in distinct vascular patterns and molecular-level EC activation in response to VEGF.

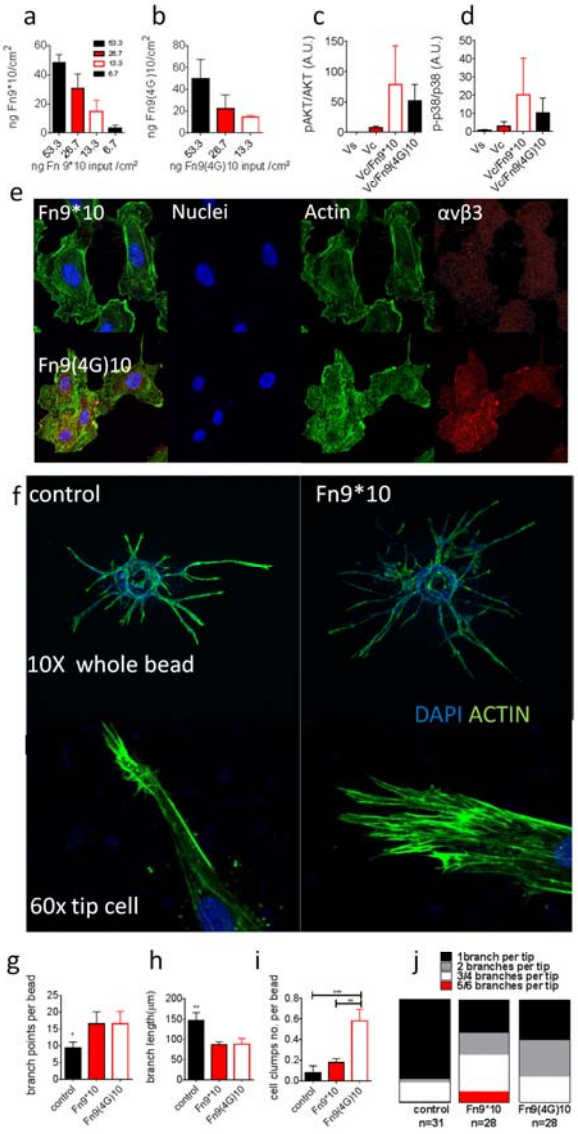

Figure 1 (a,b) ELISA result of controllable covalently linkage of Fn9*10 and Fn9(4G)10 on customized gold surface. (c,d) HUVECs showed enhanced p-p38 and p-AKT activation after 30 min in presence of Fn9*10 or Fn9(4G)10 with VEGF. (e) Different actin structure and integrin activation were observed on 2D staining after 24 hrs. (f) Representative sprouting images (10x) and tip cell images (60x). (g-i) Quantitative analysis of sprouting images (n=5 per well, 3 wells/condition).*, $p < .05$; **, $p < .01$, one way ANOVA. Error bars represent SD. (j) Quantitative distribution analysis of tip cell filopodia.

References:

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