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Fibronectin conformation regulates the proangiogenic capability of tumor-associated adipogenic stromal cells ☆

Alwin M.D. Wan [a,b,1], Emily M. Chandler [b,1], Maya Madhavan [b], David W. Infanger [b], Christopher K. Ober [a], Delphine Gourdon [a], George G. Malliaras [c], Claudia Fischbach [b,d,*]

[a] Department of Materials Science and Engineering, Cornell University, Ithaca, NY 14853, USA
[b] Department of Biomedical Engineering, Cornell University, Ithaca, NY 14853, USA
[c] Department of Bioelectronics, Ecole Nationale Supérieure des Mines, CMP-EMSE, MOC, 880 Route de Mimet, 13541 Gardanne, France
[d] Kavli Institute at Cornell for Nanoscale Science, Cornell University, Ithaca, NY 14853, USA

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ABSTRACT

Background: Changes in fibronectin (Fn) matrix remodeling contribute to mammary tumor angiogenesis and are related to altered behavior of adipogenic stromal cells; yet, the underlying mechanisms remain unclear due in part to a lack of reductionist model systems that allow the inherent complexity of cell-derived extracellular matrices (ECMs) to be deciphered. In particular, breast cancer-associated adipogenic stromal cells not only enhance the composition, quantity, and rigidity of deposited Fn, but also partially unfold these matrices. However, the specific effect of Fn conformation on tumor angiogenesis is undefined.
Methods: Decellularized matrices and a conducting polymer device consisting of poly(3,4-ethylenedioxythiophene) doped with poly(styrenesulfonate) (PEDOT:PSS) were used to examine the effect of Fn conformation on the behavior of 3T3-L1 preadipocytes. Changes in cell adhesion and proangiogenic capability were tested *via* cell counting and by quantification of vascular endothelial growth factor (VEGF) secretion, respectively. Integrin-blocking antibodies were utilized to examine varied integrin specificity as a potential mechanism.
Results: Our findings suggest that tumor-associated partial unfolding of Fn decreases adhesion while enhancing VEGF secretion by breast cancer-associated adipogenic precursor cells, and that altered integrin specificity may underlie these changes.
Conclusions and general significance: These results not only have important implications for our understanding of tumorigenesis, but also enhance knowledge of cell-ECM interactions that may be harnessed for other applications including advanced tissue engineering approaches. This article is part of a Special Issue entitled Organic Bioelectronics — Novel Applications in Biomedicine.

1. Introduction

Sustained angiogenesis represents a hallmark of cancer that is characterized by dynamic changes in extracellular matrix (ECM) remodeling [1]. However, the underlying mechanisms remain unclear due in part to a lack of reductionist model systems that allow the inherent complexity of tumor-associated ECMs to be deciphered. Tumor-associated ECMs develop by aberrant stroma remodeling (*i.e.*, desmoplasia), during which recruited mesenchymal cells (*e.g.*, fibroblasts and adipogenic precursors) deposit increased amounts of a highly fibrillar, crosslinked, and stiff ECM [2,3]. This matrix can directly promote tumor angiogenesis by enhancing transcriptional activity in endothelial cells [4]. Nevertheless, indirect mechanisms may be similarly important, including the stimulated secretion of key proangiogenic molecules (*e.g.*, vascular endothelial growth factor [VEGF]) from the recruited mesenchymal stromal cells [3]. While the characteristic physicochemical properties of the tumor-ECM are commonly attributed to modified collagen synthesis and crosslinking [5], local alterations in the fibronectin (Fn) matrix may be equally involved. In fact, Fn is critical to the formation and turnover of collagen I-based ECMs [6–8] and serves as an indicator for increased tumor aggressiveness [9,10]. Yet, the specific mechanisms by which Fn modulates the proangiogenic capability of tumor-associated stromal cells remain largely undefined.

Fn is a large glycoprotein that contains surface-exposed binding sites for engagement by cell surface receptors (in particular, the RGD loop binding site for multiple integrins), and we have previously shown that in the presence of tumor-derived soluble factors, fibroblasts

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* Corresponding author at: Department of Biomedical Engineering, Cornell University, 157 Weill Hall, Ithaca, NY 14853, USA. Tel.: +1 607 255 4547; fax: +1 607 255 7730.
E-mail address: cf99@cornell.edu (C. Fischbach).

[1] These co-authors contributed equally to this work.

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deposit Fn matrices that are characterized by stretched Fn fibrils and partial molecular unfolding [11]. Cell force-mediated partial unfolding of Fn, in turn, may modulate integrin specificity and thus cell behavior. More specifically, stretching of Fn enhances the distance between RGD-binding and PHSRN synergy sites. This decreases binding specificity of $\alpha_5\beta_1$ integrins while enhancing engagement of non-synergy-dependent integrins (such as $\alpha_v\beta_3$ integrins), with potential implications for cellular signaling [12]. Yet, tumor-associated conformational changes of Fn never occur in an isolated manner, but are typically accompanied by simultaneous alterations of Fn matrix quantity and rigidity (Fig. 1) [11,13]. These, in turn, can independently regulate cell behavior, for example, by varying the global density of cell binding motifs and activating cell contractility-dependent signaling cascades, respectively [14,15]. A variety of experimental platforms have been developed to study cell behavior as a function of adhesion peptide density and ECM mechanical properties, including RGD-modified hydrogel systems and polyacrylamide gels of varying stiffness, respectively [14,16,17]. Additionally, biomaterial systems with engineered integrin-specific ECM fragments permit interrogating the role of integrin binding specificity on cellular outcomes [18–20]. Nevertheless, culture models to independently investigate the role of full length Fn conformation on cell behaviors have been limited.

Here, we utilized a previously developed conducting polymer device to study the role of Fn conformation on the proangiogenic capability of tumor-associated stromal cells, independent of varied ECM quantity, composition, and stiffness (Fig. 1) [21–23]. This device consists of thin films of poly(3,4-ethylenedioxythiophene) doped with poly(styrenesulfonate) (PEDOT:PSS) and permits electrical control of adsorbed Fn molecular conformation in cell culture [23]. Our findings suggest that Fn conformation regulates proangiogenic factor secretion in tumors *via* altered integrin specificity, and stresses the suitability of conducting polymers in evaluating the underlying molecular mechanisms.

2. Materials and methods

2.1. Cell culture

3T3-L1 murine preadipocytes and MDA-MB231 human breast cancer cells (both from ATCC) were routinely cultured in MEM (α-modification [α-MEM], Sigma-Aldrich) containing 10% fetal bovine serum (FBS, Tissue Culture Biologicals) and 1% penicillin/streptomycin (pen/strep) (Invitrogen); 3T3-L1s were used up to passage ten. Human bone marrow-derived mesenchymal stem cells (MSCs) were acquired from Lonza and maintained in MSC-GM (Lonza). To induce tumor-associated stromal cell behavior, 3T3-L1s were cultured in tumor conditioned media (TCM) as previously described [3,11]. Briefly, TCM was collected from MDA-MB231 cells 24 h after addition of α-MEM containing 1% FBS and pen/strep, normalized to cell number, concentrated 10-fold, and reconstituted with fresh α-MEM/1% FBS/pen/strep to a final 2-fold concentration. Control media was incubated and concentrated in a manner similar to TCM. The pUR4 peptide inhibiting Fn polymerization and the Del29 control peptide were kindly provided by J. Sottile [24] and supplemented at 500 nM at the time of seeding and all subsequent media changes.

2.2. Generation and characterization of decellularized matrices and VEGF analysis

To generate control and tumor-mimetic ECMs (hereby referred to as tumor ECMs), previously published methods were used [3,25]. Briefly, 3T3-L1 cells were seeded onto gelatin (Fisher Scientific)-coated 12-well dishes at a density of 3000 cells/cm^2 and cultured in control media or TCM (both supplemented with 50 μg/mL ascorbic acid [Sigma-Aldrich]) that was changed every other day. On day 8, decellularized matrices were generated by extracting cells through digestion in a solution of 20 mM NH_4OH and 0.5% Triton-X (both from

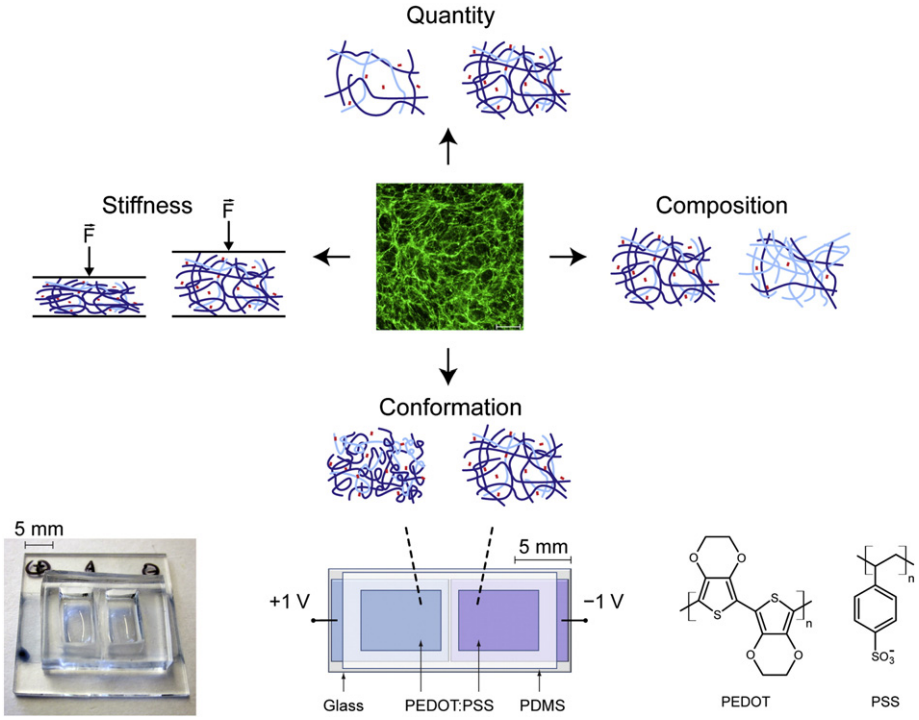

Fig. 1. Conducting polymers enable control over Fn conformation. In the presence of tumor-derived soluble factors, adipogenic stromal cells deposit ECMs that vary with regard to quantity, composition, mechanical properties, and conformation of component proteins including Fn. While a variety of approaches have been developed to study the isolated effects of ECM quantity, composition, and stiffness, there is a lack of culture models for investigating cell behavior in response to specific ECM conformations. Conducting polymer devices made from thin film pixels of PEDOT:PSS overcome this shortcoming and permit control over Fn conformation in isolation [23], enabling studies of how changes in Fn conformation direct cell responses.