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Collaboration of fibronectin matrix with other extracellular signals in morphogenesis and differentiation

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Tissue formation and cell differentiation depend on a properly assembled extracellular matrix (ECM). Fibronectin is a key constituent of the pericellular ECM, forming essential connections between cell surface integrin receptors and structural components of the ECM. Recent studies using vertebrate models, conditional gene knockouts, tissue explants, and cell culture systems have identified developmental processes that depend on fibronectin and its receptor α5β1 integrin. We describe requirements for fibronectin matrix in the cardiovascular system, somite and precartilage development, and epithelial–mesenchymal transition. Information about molecular mechanisms shows the importance of fibronectin and integrins during tissue morphogenesis and cell differentiation, as well as their cooperation with growth factors to mediate changes in cell behaviors.

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Introduction

The extracellular matrix (ECM) is a critical regulator of cell behaviors [1,2]. Cell–ECM binding provides chemical signals, structural support, and local cues organized to promote integration of environmental information by intracellular pathways. Cell interactions with the ECM are dynamic, usually involving new ECM protein polymerization and matrix remodeling. Cell-mediated assembly of ECM proteins into multimeric structures directs cell and tissue organization and controls cell differentiation, making the assembly process a key player in tissue morphogenesis. Assembly of the ubiquitous ECM protein fibronectin (FN) into fibrils requires coordination of FN expression levels with integrin receptor activity and connections to cytoskeletal components [3,4]. FN fibrils then form the foundation upon which collagens and many other ECM proteins are deposited. This review summarizes recent data on the critical role of FN matrix during tissue development with emphasis on how the ECM cooperates with other molecules to promote morphogenetic processes.

Fibronectin matrix in cardiovascular development

FN dimers are assembled into fibrils primarily by the integrin receptor α5β1 and both ligand and receptor play central roles in cardiovascular development. FN-null mice die as embryos with defects in formation of the heart and early embryonic and extraembryonic blood vessels [5,6]. A closer examination of heart development in the absence of FN showed defects in the cardiac outflow tract and right ventricle that were linked to decreased proliferation of cardiac precursor cells [7]. Similar cardiac defects were observed in conditional mouse knockouts of FN or α5 targeted to the pharyngeal region [8] as well as in fibroblast growth factor 8 (Fgf8) hypomorphs [9]. Transcriptional targets of Fgf8 signaling were down-regulated in FN-null or α5-null embryos implicating FN matrix in the potentiation of Fgf8 signaling at least for this stage of heart development [7].

FN and α5 have also been linked to the migration of cardiac precursor cells. In zebrafish between 18 and 24 hpf, myocardial precursors migrate to the midline and then fuse to form a single heart tube [10]. Knockdown of the transcription factor Snail1b causes a reduction in α5 expression and discontinuous FN matrix in the region of cell migration [11]. Both FN matrix assembly and cardiac fusion were rescued by injection of α5 mRNA indicating that precursor cell migration depends on Snail1b-mediated regulation of α5 expression and subsequent FN matrix assembly. Perhaps Snail1b is acting downstream of sphingosine-1-phosphate (S1P). Mutation of the S1P transporter Spns2, which is required for S1P secretion, combined with knockdown of FN caused a severe, two-heart phenotype in zebrafish [12]. Cardiac progenitor cell differentiation appeared normal but cell movements needed for cardiac fusion did not occur.

Analysis of epicardial cell migration *in vitro* has uncovered a novel mechanism that is executed by two proteins, Bves and NDRG4, previously implicated in vesicular trafficking

[13••,14,15]. Knockdown of the Bves/NDRG4 complex increased FN accumulation in cytoplasmic vesicles and decreased deposition of vesicular FN onto the substrate [13••]. These changes in FN subcellular localization accompanied loss of directional persistence in cell migration suggesting an interesting model in which autocrine FN recycling supports *de novo* matrix assembly to direct epicardial cell migration.

Conditional knockout of α5 in the anterior mesoderm, the source of cardiac tissue and the anterior vasculature, identified a surprising connection between mesoderm and neural crest development. Differentiation of cardiac neural crest into vascular smooth muscle cells (VSMCs) was affected by the absence of this integrin in mesodermal cells which caused morphogenetic defects in aortic arch arteries [16••]. These results suggest a model in which the ECM assembled by α5β1 on mesodermal cells influences neural crest-derived cells to develop into VSMCs. FN-binding integrins are also needed in the neural-crest derived VSMCs. Double knockout of α5 and αv integrins in VSMCs, both of which bind to the RGD domain of FN, caused embryonic lethality with cardiovascular defects similar to the anterior mesoderm α5 knockout mice [17•]; single integrin knockout showed no defects in cardiac development. Both VSMCs and the vessel wall ECM were abnormal in α5/αv-null mice, which corresponded with severe defects in FN matrix assembly in cultured double-null VSMCs [17•]. Matrix incorporation of a number of other ECM proteins was deficient. Of particular note is latent transforming growth factor β (TGFβ) binding protein (Ltbp1); its deficiency disrupted Smad signaling downstream of TGFβ, a likely contributor to the observed cardiovascular defects [17•]. Together these analyses of cardiac neural crest differentiation demonstrate a central role for integrins and their FN matrix in controlling cell differentiation by providing pericellular signals from the ECM itself as well as by presenting exogenous factors like TGFβ. Other FN-dependent signals may participate in development of a VSMC-phenotype [18,19•]. Interestingly, activation of Notch was limited to those neural crest cells that express FN and α5β1 and Notch activation was needed for VSMC differentiation by neural crest. These findings suggest the novel idea that FN induces an autocrine signaling response by cells [19•]. Future interpretations of FN's effects should consider potentially distinct roles of paracrine and autocrine mechanisms of FN action.

Synergy between FN-integrin and homophilic cadherin interactions

Matrix assembly and integrin activity determine somite border formation and recent genetic studies in zebrafish identified Rap1 GTPase as an inside-out activator of α5 integrin in this process. Whereas loss of α5 has an effect on anterior somite formation with a modest reduction in FN matrix [20], knockdown of both Rap1 and α5 caused loss of all somite borders and complete disruption of FN matrix [21]. The regulation of integrin activation and FN matrix assembly during somitogenesis occurs by a unique mechanism. Homophilic cell–cell interactions between cadherin 2 molecules in the presomitic mesoderm stabilize the association of α5 integrins on adjacent cells to maintain them in an inactive state [22••]. The unusual integrin–integrin association was suggested when cells lacking α5 were introduced into a mutant host zebrafish that lacks proper segmental patterning and these cells rescued α5 activation and FN matrix formation. Cadherin 2-null zebrafish have ectopic FN matrix assembly and α5 clustering, supporting its role as an integrin regulator [22••]. Eph/ephrin signaling can activate integrins at somite boundaries [23] suggesting that a pathway from ephrin through Rap1 activates α5 and promotes FN matrix assembly during morphogenesis of somite boundaries.

Cadherins have also been linked to FN matrix assembly in chondrocyte differentiation and salivary gland cleft formation. The early events in chondrogenic differentiation such as cell condensation and changes in gene expression are recapitulated in a micromass culture system using mesenchymal stem cells [24]. We showed that cell aggregation depends on FN matrix assembly [25]. As mesenchymal stem cells condense, FN and N-cadherin levels are up-regulated and matrix assembly increases. Once cells have condensed into an aggregate, N-cadherin is down-regulated but FN matrix assembly continues. During this period, cells proliferate and initiate the differentiation program, which requires induction of the Sox9 transcription factor, which is essential for cartilage-specific gene expression. Either the blockade of FN polymerization [25] or the knockdown of N-cadherin [26] in micromass cultures prevents cell condensation. Incomplete condensation prevents up-regulation of Sox9 expression and cell differentiation into chondrocytes. These data and the role of cadherins in somite morphogenesis suggest that cadherin interactions bring or keep cells juxtaposed during the initial stages of morphogenesis but then FN matrix assembly takes over to maintain cell connections while allowing cell rearrangements needed for subsequent steps in tissue development.

Differential regulation of cadherins and FN matrix assembly may be a common mechanism for cell aggregation as it has been connected to differences in cohesion of glioblastoma cells that vary in invasiveness [27]. Cell clustering, as shown on a collagen matrix substrate, depends not only on FN and α5β1 but also on development of a pro-contractile phenotype. LPA treatment stimulates contractility and clustering concomitant with FN matrix assembly [28]. In fact, the clustering appears to depend on a balance between pro-contractile signals that promote matrix formation and pro-migratory signals such as PDGF and the matrix metalloprotease MMP-2 that