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ECM-modulated cellular dynamics as a driving force for tissue morphogenesis

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Abstract

The extracellular matrix (ECM) plays diverse regulatory roles throughout development. Coordinate interactions between cells within a tissue and the ECM result in the dynamic remodeling of ECM structure. Both chemical signals and physical forces that result from such microenvironmental remodeling regulate cell behavior that sculpts tissue structure. Here, we review recent discoveries illustrating different ways in which ECM remodeling promotes dynamic cell behavior during tissue morphogenesis. We focus first on new insights that identify localized ECM signaling as a regulator of cell migration, shape, and adhesion during branching morphogenesis. We also review mechanisms by which the ECM and basement membrane can both sculpt and stabilize epithelial tissue structure, using as examples *Drosophila* egg chamber development and cleft formation in epithelial organs. Finally, we end with an overview of the dynamic mechanisms by which the ECM can regulate stem cell differentiation to contribute to proper tissue morphogenesis.

Keywords

fibronectin; basement membrane; cell dynamics; development; morphogenesis

Introduction

Numerous studies in diverse developmental systems have revealed that dynamic changes in cell motility, shape, and adhesion are major driving forces sculpting tissue-specific form and function. Over the last decade, it has become increasingly apparent that many of these processes can be modulated by chemical, physical, and topographical cues present in the cellular microenvironment [1-3]. Since the extracellular matrix (ECM) surrounding cells *in vivo* is a major component of this microenvironment, it comes as no surprise that the ECM is a critical regulator of developmental dynamics [4-6].

The ECM, composed of a fibrous mesh of glycoproteins and proteoglycans [7], is more than a static structure supporting tissue architecture. The binding of ECM proteins to cell surface integrins and other receptors promotes a variety of cellular responses including survival, proliferation, adhesion, and migration [1,2,8]. Furthermore, the ECM is dynamically

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remodeled during development and disease states, as cells constantly degrade and resynthesize the ECM to promote rapid changes in the microenvironment [5,6]. In this review, we describe particularly insightful recent examples highlighting ways in which ECM remodeling can regulate cell dynamics during tissue morphogenesis. We focus on specific concepts, including ECM effects on cell motility and adhesion, basement membrane-mediated sculpting of tissue shape, and ECM regulation of tissue differentiation, which provide clear examples of the reciprocity between ECM and cellular dynamics governing epithelial tissue morphogenesis. For recent comprehensive reviews on the role of ECM in development, please see references [5,6,9-12].

ECM promotes local changes in cell dynamics during tissue morphogenesis

An evolving theme in developmental biology is that signals from the ECM promote localized (rather than global) changes in cell behavior. For example, localized deposition of a specific matrix protein can trigger integrin signals that alter patterns of cell motility and adhesion. Recent work has delineated a fibronectin (FN)-mediated signaling cascade that promotes local cell dynamics during branching morphogenesis [13,14], a conserved developmental mechanism by which a primary epithelial bud or tube undergoes dynamic, coordinated cellular rearrangements to give rise to the complex branched epithelial architecture of many mammalian organs [15,16]. Cleft formation is a major mode of branching, which subdivides an epithelial bud into two new buds. Local FN deposition rapidly induces Btbd7 [BTB (POZ) domain containing 7] in a focal region at the base of progressing clefts, which in turn up-regulates the transcription factor Snail2 and down-regulates the adhesion molecule E-cadherin (Figure 1). These focal changes in cell signaling promote localized changes in cell behavior at the base of progressing clefts associated with altered cell shape, a more motile phenotype, and decreased cell adhesion leading to the formation of transient intercellular gaps [13] (Figure 1). Thus, cooperative interactions between FN and local cell dynamics appear to drive cleft progression.

Since Snail2 is a well-known promoter of epithelial-to-mesenchymal transition (EMT) [17], it is possible that branch formation involves FN-induced partial EMT at focal locations at the epithelial periphery. Indeed, EMT scatter factors such as Snail2 are transiently expressed at mammary gland branch sites *in vitro*, where they promote dynamic cell migration [18]. Furthermore, mammary terminal end buds migrate collectively as multilayered epithelia exhibiting reductions in apico-basal polarity and cell-cell adhesion [19,20], which are features of epithelial cells undergoing partial EMT. Although a role for ECM molecules such as FN in this process has not been investigated, dynamic signaling from the ECM to the nucleus may be a general mechanism to promote such EMT-related dynamic cell behavior during branching morphogenesis.

Recent work has also elucidated the upstream mechanisms by which FN is assembled into the basement membrane in branching organs. FN assembly is a cell-mediated process involving cytoskeletal forces applied to FN-bound integrin receptors to unfold dimeric FN molecules for fibril assembly [21-23]. Rho kinase (ROCK)-mediated actomyosin contraction and focal adhesion kinase (FAK) activation promote FN fibril assembly in branching organs [24,25]. Because FN accumulation itself also appears to induce activation of integrins and FAK in salivary epithelial cells [14,25], it is interesting to speculate that a dynamic FN-mediated feedforward regulatory loop could promote ongoing FN assembly to drive continued cleft propagation once this process is initiated.

Basement membrane sculpts and stabilizes tissue structure

As discussed above, localized ECM-regulated signaling causes motile cells to undergo dynamic changes in shape, protrusive activity, and adhesion during morphogenesis. Such plasticity provides a morphogenetic substrate for the forces that guide and stabilize changes in global tissue structure. Indeed, structural stabilization by the ECM itself, especially via the basement membrane, can contribute to sculpting of overall epithelial tissue structure by providing regional force anisotropies in the microenvironment to permit tissue expansion in only certain directions [26,27]. We will consider two recent examples that illustrate this role of the ECM during development: *Drosophila* egg chamber elongation and branching morphogenesis.

Egg elongation requires an ECM “molecular corset”

The *Drosophila* egg follicle consists of a cyst that develops into an oocyte surrounded by a simple follicular epithelium; as the oocyte matures, this initially rounded structure elongates along the anterior/posterior axis to produce an oval-shaped egg. Recent investigations into the mechanisms of this shape change have provided surprising insight into a new morphogenetic behavior.

Using live imaging, Haigo and Bilder recently demonstrated that as it elongates, the entire egg chamber rotates around its circumferential axis [28]. Interestingly, *Drosophila* mutants lacking either integrin PS or collagen IV fail to rotate and elongate, suggesting that coordinate interactions between the follicular epithelium and basement membrane are required for this behavior. Individual cell motility is also required: Misshapen (Msn) kinase promotes cell motility in this system by decreasing integrin levels at the rear of migrating cells to facilitate tail retraction as the cells migrate [29]. What is the purpose of this novel morphogenetic behavior? Further analyses revealed that as the follicle rotates, it creates a planar polarized basement membrane around its anterior/posterior axis by rearranging randomly oriented fibers existing prior to these rotational motions (Figure 2). Moreover, round egg mutants that fail to elongate lack this polarized basement membrane, while experimental treatment of elongated chambers with collagenase results in a return to a symmetrical rounded morphology [28]. Taken together, these results suggest a model in which epithelial rotation is required to produce a planar polarized basement membrane around the circumferential axis of the egg chamber, which may in turn serve as a molecular corset that acts to physically restrict the direction of tissue expansion, thereby stabilizing an elongated tissue structure [28].

Two recent studies have identified similar rotational motions during mammary epithelial acinar development in an *in vitro* system, which cease upon acinar maturation [30,31]. In the first study, these rotations were correlated with the presence of planar polarized actin microfilaments at the basal surface aligned in the same direction as rotational migration. Furthermore, rotational motility was disrupted by inhibitors of actin and myosin II activity, implicating actomyosin contraction as a critical regulator of this process [30]. The second of these studies identified a direct relationship between rotational motion and the assembly of endogenous basement membrane; when structures failed to rotate, they could not assemble a laminin matrix, and conversely, when basement membrane was disrupted in mature non-rotating acinar structures, they began to rotate again to reassemble the matrix [31]. These studies suggest that rotational cell motility can provide an evolutionarily conserved mechanism for basement membrane reorganization necessary for morphogenesis.

Regional accumulation of ECM proteins stabilizes tissue structure during branching morphogenesis

Early studies of salivary gland development established that collagen accumulates preferentially at branch points, and removal of collagen not only prevents subsequent branching morphogenesis, but also triggers the regression of pre-existing clefts [32,33]. Similar observations in the mammary gland and lung have also identified heterogeneous ECM expression patterns, with thick accumulations of basement membrane around bud flanks, ductal structures, and in cleft regions. In contrast, ECM is thinner at end bud tips at which epithelial expansion occurs (Figure 3) [34,35]. Taken together, these findings suggest that local density of collagen and basement membrane can modulate expansion or stabilization, somewhat analogous to the molecular corset theory during *Drosophila* egg development.

More dynamically, however, ECM proteins can also actively translocate. For example, ECM appears to serve as an inward-directed wedge to promote cleft formation during branching. Pulse-chase experiments using salivary epithelial rudiments in three-dimensional ECM reveal that FN moves to the base of progressing clefts [36]. Moreover, live imaging with fluorescent FN during epiblast cell migration in *Xenopus* development shows that these cells appear to carry the ECM with them as they migrate [37,38]. Whether these cases of ECM translocation are mechanistically related to the production and alignment of ECM by rotational cell motility remains to be determined, but both processes show how ECM remodeling can regulate morphogenesis by physically stabilizing or promoting tissue shape change.

ECM cues in tissue differentiation

Thus far, we have focused on ECM roles in tissue morphogenesis. Another important component of organogenesis, however, is the subsequent acquisition of cell type-specific function, particularly the regulation of stem cell differentiation. Although finding diverse ECM receptors on stem cells suggested a role for ECM [39], recent progress has firmly established crucial roles for ECM signaling in both maintenance of stem cells and regulation of their subsequent differentiation.

ECM regulation of the stem cell niche

The stem cell niche consists of unique supporting cell types and microenvironmental factors that provide instructional cues to maintain a stable stem cell population [40,41]. A general role of ECM within the niche is to promote stem cell interactions with various niche cells and the surrounding microenvironment (Figure 4). One example of such ECM interactions in the niche involves neural stem cell binding via integrin $\alpha 6 \beta 1$ to laminin in the vascular basement membrane (which comprises the niche) in the subventricular zone of the mouse brain [42]. Inhibition of this integrin receptor reduces numbers of neural stem cells. In the *Drosophila* testes, a distinct population of hub cells comprises the germline stem cell (GSC) niche, and the hub cells must bind to the ECM through integrin $\beta 5$ to support GSC function [43].

Besides promoting stem cell binding to a niche, specific ECM proteins can also directly influence stem cell behavior. For example, deposition of endogenous FN by mouse ES cells is required for their self-renewal [44]. In addition, muscle satellite cells (progenitor cells) transiently up-regulate FN upon skeletal muscle injury. In this system, FN binding to Syndecan 4 stimulates it to form a co-receptor with Frizzled 7 that supports downstream signaling via Wnt7a to increase satellite cell expansion within the niche [45]. Thus, stem cell binding to a specific ECM protein such as FN can repopulate the satellite cell niche as stem cells undergo differentiation for muscle regeneration. Other ECM proteins that either

positively or negatively regulate stem cell number include osteopontin in bone marrow (the hematopoietic niche) [46], nephronectin in hair follicles [47], and biglycan in tendons [48].

The ECM can also directly influence stem cell function by modulating signaling by growth factors, many of which are required for stem cell maintenance and/or differentiation [49]. For example, the ECM protein anosmin is required for avian cranial neural crest formation [50]; stem cell-like neural crest cells migrate and differentiate to diverse tissues. Local anosmin deposition was recently shown to enhance FGF8 signaling in these cells while at the same time dampening BMP5 and Wnt3a, thereby positively regulating cranial neural crest formation. These results illustrate how a single ECM protein can coordinate multiple growth factor activities to affect the cell biology of a stem cell-like population.

Mechanical regulation of stem cell differentiation

Besides promoting stem cell retention and maintenance within the niche, ECM physical properties can have a profound effect on stem cell differentiation. A recent, recurring theme is that ECM mechanical signals influence stem cell lineage commitment. When mesenchymal stem cells (MSCs) are cultured on gels of varying stiffness, their fate depends on the mechanical compliance of their underlying substrate; specifically, MSCs are directed to neuronal, muscle, or osteogenic lineages by cell culture on surfaces that approximate the mechanical stiffness of the respective *in vivo* tissue [51]. Adding yet another level of complexity, other studies suggest that ECM can also indirectly affect stem cell differentiation by modulating cell shape. When micropatterned ECM substrates were used to control the amount of cell spreading in stem cell cultures, round cells became adipocytes while flattened cells underwent osteogenesis [52]. A common theme appears to be the level of Rho-stimulated actomyosin contractility in these cells, which is regulated by the mechanical properties of the underlying substrate; that is, both stiff matrices and micropatterns that support cell spreading trigger increased actomyosin contraction, while more compliant matrices and micropatterns that only allow cell rounding trigger a reduction [51,52]. Thus, ECM topography, as well as biomechanical rigidity, is clearly capable of influencing stem cell fate decisions. Recent progress in this area has further revealed that MSCs will first undergo durotaxis, migrating to a region of “preferred” substrate rigidity. When MSCs are cultured on hydrogels containing physiological gradients of matrix stiffness, some cells migrate to the stiffest regions and commit to an osteogenic lineage, while others prefer more compliant regions and undergo alternate lineage commitment [53]. Although all of these studies involved 2D culture conditions, these findings can be reproduced in a 3D environment. Just as on a 2D substrate [51], cells in 3D express neurogenic, myogenic, and osteogenic markers as a function of increasing substrate rigidity [54].

Conclusions

Recent research has established that dynamic ECM-modulated signaling plays important roles in the remodeling and morphogenesis of epithelial tissues. The dynamic mechanisms of cell-ECM interactions and the ways in which they regulate complex developmental processes such as epithelial morphogenesis constitute an exciting, rapidly expanding area of research. In this review, we have considered a number of emerging biological principles by which cell interactions with the ECM and basement membrane coordinately shape mammalian epithelial tissues; these include the highly localized regulation of cell dynamics by ECM-mediated signaling, ECM-induced forces that guide alterations in tissue structure, and the regulation of tissue differentiation by chemical and physical properties of the ECM. A major future challenge will be to integrate these multiple roles of cell-ECM interactions with our knowledge about the many critical soluble regulators of morphogenesis to provide a more complete systems biology understanding of tissue development.

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Figure 1. Focal ECM deposition regulates dynamic cell behavior during branching morphogenesis

Fibronectin (FN) is focally assembled to promote cleft progression during epithelial morphogenesis. FN induces Btbd7 at the base of an initiated cleft, which in turn up-regulates Snail2 and down-regulates E-cadherin. This increases local cell dynamics at the cleft base (black wavy arrows) and opens up transient intercellular gaps (between dark gray cells) to advance the cleft (yellow arrow). The FN assembly requires intracellular Rho kinase (ROCK)-mediated actomyosin contraction and focal adhesion kinase (FAK) activation to unfold dimeric globular FN for fibril assembly (left panel).

Figure 2. Directional cell migration orients ECM to drive tissue shape change during egg chamber morphogenesis

The *Drosophila* egg chamber is an initially rounded structure that elongates along the anterior/posterior axis to produce an oval-shaped egg. This requires the complete 360° rotation of the entire egg chamber (red arrows), which polarizes the alignment of basement membrane fibrils (green dashed lines) to act as a molecular corset restricting the direction of tissue expansion. At the cellular level, the mechanism of this ECM alignment involves the directional rotational migration (gray arrows) of individual follicular epithelial cells.

Figure 3. Differential regional accumulation of ECM proteins stabilizes tissue structure during branching morphogenesis

ECM is deposited heterogeneously in the basement membrane and stroma surrounding epithelial branched organs. This produces regional force anisotropies that either stabilize or guide the direction of epithelial tissue expansion. For example, thick accumulations of basement membrane (heavy green lines) are present around bud flanks and ducts, and in cleft regions. In contrast, ECM is thinner at end bud tips where epithelia expand (thin green lines).

Figure 4. ECM regulates stem cell retention within the niche and determines lineage commitment

Binding to ECM and basement membrane components is required for both stem and niche cell localization within the niche (1). Niche-associated ECM and soluble factors secreted by cells present within the niche help regulate the decision between stem cell self-renewal and differentiation (2). ECM mechanical properties also determine mesenchymal stem cell lineage commitment along osteogenic, myogenic, and neurogenic differentiation pathways (3).