Extracellular Matrix Signaling in Morphogenesis and Repair

Kelly C. Clause, Ph.D.[a] and **Thomas H. Barker, Ph.D.**[a,b]

Kelly C. Clause: kelly.clause@bme.gatech.edu

[a]The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, 313 Ferst Drive NW, Atlanta, GA 30332, USA

[b]The Parker H. Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology, 315 Ferst Dr. NW, Atlanta, GA 30332, USA

Abstract

The extracellular matrix (ECM) is critically important for many cellular processes including growth, differentiation, survival, and morphogenesis. Cells remodel and reshape the ECM by degrading and reassembling it, playing an active role in sculpting their surrounding environment and directing their own phenotypes. Both mechanical and biochemical molecules influence ECM dynamics in multiple ways; by releasing small bioactive signaling molecules, releasing growth factors stored within the ECM, eliciting structural changes to matrix proteins which expose cryptic sites and by degrading matrix proteins directly. The dynamic reciprocal communication between cells and the ECM plays a fundamental roll in tissue development, homeostasis, and wound healing.

Introduction

The extracellular matrix (ECM) is critically important for many cellular processes including growth, differentiation, survival, and morphogenesis. The ECM consists of a complex assembly of many proteins and polysaccharides whose precise composition varies from tissue to tissue. The primary components include insoluble fibrous structural proteins (i.e. collagens, laminins, fibronectin, vitronectin, and elastin), proteoglycans, and specialized proteins (i.e. growth factors, small matricellular proteins and small integrin-binding glycoproteins). Recent reviews discuss the unique properties of these individual proteins in detail [1,2]. The ECM not only provides support, tensile strength, and scaffolding for tissues and cells, but also provides biochemical signals (i.e. growth factors, chemokines, and cytokines), both of which affect cell morphogenesis and differentiation. Cells remodel and reshape the ECM by degrading and reassembling it, thus playing an active role in sculpting their surrounding environment and directing their own phenotypes. Thus, the dynamic reciprocal communication between cells and the ECM plays a fundamental roll in tissue development, homeostasis, and wound healing. This review will focus on a relatively few examples of ECM signaling in morphogenesis and tissue repair with particular emphasis on mechanically liberated or exposed biologically active sites as a result of proteolysis or conformation modifications.

Corresponding Author: Thomas H. Barker, Ph.D., Address: The Wallace H. Coulter Department of Biomedical Engineering at Georgia Tech and Emory University, 313 Ferst Drive NW, Suite 2108, Atlanta, GA 30332-0535, USA, (404) 385-5039 – phone, (404) 894-4243 – fax, thomas.barker@bme.gatech.edu.

ECM signaling via cellular receptors

The ECM can signal via the cellular receptors it interacts with by mediating both physical linkages with the cytoskeleton and the bidirectional flow of information between the extracellular and intracellular compartments [3]. Many different receptors have been identified that transduce signals to the cytoskeleton and nucleus including integrins, receptor tyrosine kinases and phosphatases, immunoglobulin superfamily receptors, dystroglycan and cell-surface proteoglycans [4]. Integrins, a large family of heterodimeric transmembrane glycoproteins comprised of many different combinations of and subunit pairs, have emerged as a critical component in signal transduction because of their role in both bidirectionally transmitting mechanical forces and regulating a number of intracellular signaling pathways. Integrins contribute to most, if not all, of the morphogenetic events that shape a developing, complex, multicellular organism [5,6], as knockout mice for most integrin subunits are embryonic (or perinatal) lethal [7], with many early integrin functions appearing to contribute to cell rearrangement and migration [5,8]. During vasculogenesis endothelial cells migrate, proliferate, and form 3D tubular structures. This tubular morphogenesis requires integrin receptor signaling to regulate cell shape through changes in the cytoskeleton and cell-cell interactions that control the shape of the tubules [9,10]. Similarly, morphogenesis of branching organs, such as the salivary gland, lung, breast, and kidney as well as prostate and pancreas, is dependent on the multiple downstream activities related to ECM integrin receptor interactions [11,12]. Integrins not only contribute to development and tissue morphogenesis, but also play a key role in tissue homeostasis and tissue repair. The effect of integrins in wound repair has been most extensively studied in squamous epithelia, such as the skin, or in airway epithelia. In response to injury, cells undergo fundamental changes in spreading, migration, and proliferation, with each of these processes requiring the participation of integrins, as well as dramatic changes in the spatial distribution and level of expression of integrins [13]. A number of recent reviews have illustrated further in depth the role of integrins in ECM signaling [5,14].

ECM signaling via proteolytic cleavage

The ECM can also signal via the proteolytic cleavage of active components. As stated previously, the ECM is constantly being remodeled as cells degrade and reassemble it, with remodeling rates being particularly high during development and wound repair. Mechanical remodeling of the ECM is often the result of cell-matrix crosstalk. Mechanical stiffening of the ECM itself can lead to Rho activation and results in mechanical remodeling of the matrix through focal adhesion maturation and increased force generation by the cell [15]. These processes ultimately lead to increased matrix traction and compaction, further elevating ECM stiffness [16]. This positive feedback is balanced by negative regulation in the form of mechanically-induced biochemical matrix remodeling via a complex proteolytic response [17]. The proteolytic cleavage of ECM and subsequent release of active ECM components is one mechanism by which cells and the ECM signal. Proteases, including those in the matrix metalloproteinase (MMP), serine protease (i.e. plasmin, plasminogen activator and uPAR) and cysteine protease (i.e. cathepsins) families, influence matrix dynamics at multiple levels; they can convert structural molecules to signaling molecules by releasing small bioactive peptides, release growth factors stored within the ECM (i.e. TGF), elicit structural changes to matrix proteins, and degrade matrix proteins directly [1,18,19].

Proteolytic cleavage of ECM substrates generates fragments that have different biological activities from their precursors and many of these play a distinct role in development and tissue repair. Leukocytes, such as neutrophils and macrophages, that are recruited to an injury site cause ECM denaturation by the action of secreted proteases like elastase, collagenases and gelatinases [20,21]. Increased blood flow to injured tissue is a well-known

consequence to injury and recent work has shown that this increased flow may in part be controlled by induction of arteriolar vasodilation via proteolytic fragments of denatured collagen type I [22]. ECM proteins fibrinogen and fibronectin are major components of the provisional ECM that forms within injured tissues after increases in vascular permeability. Fibronectin has the ability to selectively adsorb to denatured as opposed to native collagen. Because collagen denaturation occurs at areas of tissue injury [23,24] this affinity may allow fibronectin to preferentially adsorb to injured areas. Cleavage of laminin-5 or collagen IV results in the exposure of cryptic sites that promote cell migration [25,26], a response that is also necessary in wound healing. Proteolytic degradation of ECM can also result in conformational changes in ECM secondary to denaturation after proteolysis [27,28]. Type I collagen degradation that is mediated by MMP1 is necessary for epithelial cell migration and wound healing in culture models [29].

Development of bone is also affected by proteolytic cleavage of ECM components. The appendicular and axial skeleton, including the long bones, develops by endochondral ossification, whereby a cartilage template forms first and is then resorbed and replaced by mineralized bone. Cartilage cells, or chondrocytes, differentiate and are replaced at the growth plate, where they follow a stereotyped progression of proliferation, differentiation, hypertrophy, angiogenic invasion and apoptosis. Galectin-3, a lectin with anti-apoptotic activity that is localized to the ECM, is proteolytically cleaved and inactivated. Blocking this cleavage results in a failure of chrondrogenic apoptosis and a defect in bone formation [30]. Chondrocytes unable to cleave collagen II and aggrecan also show an expansion of the zone of hypertrophic chondrocytes and a delay in apoptosis [31]. In branched organ development, like lung, kidney, and salivary and mammary glands, rapid and dynamic ECM remodeling and turnover via proteolytic degradation is necessary. Proper branching requires local ECM degradation at the tips of buds via enhanced local MMP expression [32,33], with overexpression of MMPs resulting in excessive matrix remodeling in the form of fibrosis [34]. Proteolytic cleavage of ECM proteins can also release ECM-bound growth factors, including insulin growth factors and fibroblast growth factors [35,36]. Cleavage of laminin during mammary gland involution releases a fragment that binds to the epidermal growth factor (EGF) receptor and increases cell migration [4]. Cleavage of collagen produces biologically active fragments such as tumstatin and endostatin that regulate migration, proliferation, and cell survival [37] as well as releases important signaling molecules such as Wnts, TGF and FGF, which can regulate branching [38]. Thus, the regulated release of bioactive ECM fragments via proteolytic cleavage during development or tissue repair following injury provides important signals to control these events.

ECM signaling via exposure of cryptic sites

In addition to the proteolytic cleavage of ECM and subsequent release of active ECM components, the ECM can signal via the mechanical exposure of cryptic sites as well. Activation of cryptic ECM sites requires structural modification of the ECM macromolecule which can occur by conformational changes elicited via cell-generated tension or binding to other ECM molecules or cell-surface receptors. Any of these processes exposes the cryptic site which then becomes available for recognition and exertion of its function. Contractile force generated by cells is sufficient to partially unfold the ECM protein fibronectin [39]. Cells bind to secreted fibronectin and exert tension on them. This tension exposes cryptic sites by separating the intramolecular contacts of repeats which promotes fibronectin-fibronectin binding and assembly of a fibronectin matrix [40,41]. The protein-disulfide isomerase activity of fibronectin is also partially masked to prevent possible spontaneous crosslinking of fibronectin molecules into a stable matrix within the ECM [42]. Many studies suggest the RGD amino acid sequence in many ECM proteins to by cryptic. Murine 8 1 integrin, for example, binds to the RGD site of tenascin-C fragments but not to native

tenascin [43]. In collagen, antibodies specific for integrin and RGD peptides, which block RGD-binding integrin function, do not interfere with cell binding to native collagen, but cell attachment to denatured collagen is RGD-dependent. v 3 integrin has been shown to bind strongly to denatured collagen and minimally to native collagen [1]. The RGD integrin- and cell-binding site of vitronectin has recently been shown to be cryptic, in that it is not exposed in plasma vitronectin unless it adsorbs to surfaces or multimerizes [44]. This property may allow the RGD site of vitronectin to be exposed only when it is needed (i.e. after increases in vascular permeability and binding of vitronectin into the ECM of injured tissues). Fibrinogen, after binding to the platelet integrin IIb 3, exposed new epitopes that facilitate platelet aggregation and fibrinogen polymerization to stabilize developing fibrin-platelet clots at a tissue injury site [45]. Plasmin-derived fibrin fragments have also been shown to increase vascular permeability. These studies support the concept that cell-mediated mechanical forces can generate and perhaps regulate the exposure of cryptic sites in ECM to effect ECM assembly and subsequent cellular responses [16].

Conclusion

This review has highlighted a few ways the ECM signals and its enormous contribution to the regulation of key processes in morphogenesis and tissue repair. Both mechanical and biochemical molecules influence ECM dynamics in multiple ways; by releasing small bioactive signaling molecules, releasing growth factors stored within the ECM (i.e. TGF), eliciting structural changes to matrix proteins which expose cryptic sites, and by degrading matrix proteins directly [1,18,19]. This complex and dynamic ECM environment is even more complex, as ECM signals can act alone or in concert with other signaling molecules. For example, studies have shown molecular and mechanical synergy crosstalk between integrins and numerous receptor types; including growth factors and growth factor receptors, dystroglycan, syndecans, receptor tyrosine kinases, and cytokine receptors [14,46,47], which can precisely regulate cell behavior. In addition to the multiple mechanical and chemical signals from the ECM which can act alone or synergistically, the ECM is constantly being moved and remodeled, making the environment extremely dynamic both over time and space.

Future Opportunities

In recent years the field of regenerative medicine has instituted the used of biomaterials as platforms for the presentation of mechanics and bioactive molecules to direct cell behavior. This has led to only relative success, partly because of the lack of knowledge of the complex biologic environment that controls these responses. Enhanced understanding of the importance of the mechanics and signals within the ECM in regulating many aspects of cell fate in both development and tissue repair has led to the development of increasingly sophisticated biomaterials. Biomaterials have incorporated many categories of bioactive signals including; (1) insoluble molecules (i.e. fibronectin, vitronectin, laminin, etc.), either as whole molecules or as recombinant protein fragments [48], (2) soluble molecules (growth factors, cytokines, chemokines, etc.) [49], and (3) proteins via cell-cell contact (cadherins, CAMs, etc.) [50]. Development of novel strategies that improve the ability to manipulate 3D biomaterials scaffold architecture into tissue or organ structures as well as generation of structures necessary for vascularization of these scaffolds is essential for success of these materials. Biology has indicated a complex need of biomaterials with precisely controlled scaffold architecture that regulate the spatio-temporal release of growth factors and morphogens, and respond dynamically to both environmental and cellular cues. It has become clear that biomaterials are needed to provide the signals necessary to govern cell fate in regenerative medicine applications, however the enormously complex environment the cell experiences *in vivo* most likely cannot be recapitulated *in vitro*. Investigations in

developmental biology, matrix biology, mechanobiology, and stem cell biology will provide insight into design rules guiding better development of material specifications for a functional biomaterial of optimal complexity.

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Highlights

ECM signaling in morphogenesis and tissue repair.

ECM signals with mechanically liberated or exposed biologically active sites.

ECM signals via proteolysis or conformational modifications.

Enhanced understanding of ECM mechanics and signals for biomaterials development.