Pediatr Nephrol (1993) 7: 96–104

Pediatric Nephrology

Invited review

Regulation of cell function by extracellular matrix

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Received September 29, 1992; accepted October 6, 1992

Abstract. The extracellular matrix (ECM) provides structural support and adhesive substrates for the body tissues. Recent advances in our understanding of the biology of matrix indicate that the ECM also plays a significant role in regulating the behavior of cells. Matrix proteins engender changes in cell shape and movement, bind growth factors, and facilitate cell-cell and cell-matrix interactions. Matrix-induced differentiation results from multiple stimuli that include: tensile forces on the cell, cytokine- or growth factor-mediated stimulation, and interaction with bioactive domains of matrix glycoproteins. Because these signals are important determinants of cell behavior, pharmacological manipulation of cell-matrix interactions may offer a valuable new approach to disease treatment.

Key words: Basement membrane – Cell-matrix interactions – Extracellular matrix – Laminin

Introduction

Extracellular matrix (ECM) components have long been recognized as adhesive and support structures for the body tissues. Nephrologists view the basement membrane with particular interest because this specialized form of ECM also has important sieving properties in glomerular filtration. The basement membrane matrix compartmentalizes tissues, separating epithelium from mesenchyme. It has become increasingly clear that the ECM is not simply a passive structure, but also is an active biological entity that helps determine both the shape and function of cells. For example, segregation of cell types within an organ by the basement membrane may involve not only the physical barrier imposed by the matrix, but also regulation of cell movement and activity. By interacting with cell surfaces, matrix proteins serve as regulatory mediators of cell-cell and cell-substrate interactions. Matrix components also bind and concentrate soluble messengers (growth factors, cytokines, etc.), and induce cell growth and/or differentiation. These biological properties are important in determining the function of cells in diverse tissues, and abnormal expression of these properties may underlie many pathogenetic processes. Insight into the nature of cell-matrix interactions may therefore be essential to understanding diseases as disparate as glomerulosclerosis and metastatic carcinoma.

Three potential mechanisms have been implicated in regulation of cell activity by the ECM. First, mechanico-chemical stimulation occurs as the cell binds to the matrix, undergoes shape changes, and is subjected to tensile stress. A second means by which ECM affects cells is through its ability to modulate the actions of cytokines and other growth factors. A third mechanism has been suggested by studies showing that cell binding to specific domains of ECM proteins modulates cell function. In this article, after reviewing the composition of the ECM, we will discuss tensile forces and cytokine effects, and then consider how they act in experimental models. The role of specific matrix adhesion proteins will be reviewed. Finally, we will address the therapeutic implications of cell-matrix interactions.

Composition of the ECM

The ECM is a meshwork of interacting protein components that, with a substantial amount of water, form a gel. The exact nature of the molecules comprising this structure varies with the location and function of the matrix, but each matrix generally contains at least one collagen, a proteoglycan, and a glycoprotein (Table 1). For example, the ECM of the interstitial stroma contains type I collagen, dermatan sulfate, and fibronectin, whereas the basement

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Table 1. Proteins in the extracellular matrix (ECM)[a]

COLLAGENS

Type	*Tissue locations*	*Form*
Type I, III	Interstitial	Fibrils
Type II, XI	Cartilage	Fibrils
Type IV	Basement membrane	Sheets
Type V	Basement membrane	Fibrils
Type VI	Vessels, skin, vertebral disks	Beaded filaments
Type VII	Dermoepidermal junction	Anchoring filaments
Type VIII, X	Descemet's membrane, growth plate	Sheets
Type IX, XII, XIV	Cartilage, fetal tendon	Non-fibrillar
Type XIII	Endothelial, epithelial cells	?

PROTEOGLYCANS

Protein	*Tissue locations*	*Size*
HSPG (Perlecan)	Basement membrane	~800 kDa
CSPG (Aggrecan)	Cartilage	~225 kDa core; >1,000 kDa total
Decorin	Cornea, mesangium	~40 kDa core protein
Biglycan	Differentiating tissue	~40 kDa core protein

ECM GLYCOPROTEINS

Protein	*Tissue locations*	*Size*	*Structural motifs*
Fibronectin	Multiple tissues, serum	Dimers and higher multimers; monomer ~ kDa	Cell-, collagen-, fibrin-, and heparin-binding regions; matrix assembly region, growth factor-binding regions
Laminin	Basement membranes, specialized ECMs	A chain – 400 kDa B1 chain – 210 kDa B2 chain – 200 kDa	Cruciform structure, ligand-binding domains similar to fibronectin; plus EGF-like domains, specialized sequences (see text)
Thrombospondin	Platelets, growing cells	3 chain; 140 kDa each	Heparin-, fibronectin-, and calcium-binding domains
Tenascin	Sites of tissue remodeling	6 chains; 2 each of 190 kDa, 200 kDa, 220 kDa	Cell-binding, anti-adhesive regions
Entactin	Basement membrane	150 kDa	Self-aggregating region, laminin- and fibronectin-binding region

Other proteins in the ECM

Growth factors: bFGF, TGF-β, interleukin-1, other interleukins, interferons
Proteases: plasminogen, plasminogen activators, collagenases

HSPG, Heparan sulphate proteoglycan; CSPG, chondroitin sulfate proteoglycan; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor; TGF-β, transforming growth factor-β
[a] Data from [1, 3, 5–7, 45, 48, 74]

membrane contains collagen IV, heparan sulfate proteoglycan (HSPG), and laminin. Some matrices are highly specialized. The mesangial matrix is similar to vascular basement membrane but it contains the proteoglycan biglycan, and greater amounts of fibronectin. The glomerular basement membrane is richer in proteoglycans than most basement membranes, accounting for its special sieving properties.

Collagens

The major structural protein, collagen, is the most abundant ECM protein and is the only protein in the body that contains the amino acid hydroxyproline. Collagen chains have both globular and helical domains; three chains, each approximately 150 kDa, generally comprise a collagen molecule. There are multiple, chemically distinct collagens. Each type, identified by a roman numeral, is the product of a distinct gene and has a tissue-specific localization. For example, type I collagen is usually found in the interstitium, type II in cartilage, and type IV in basement membrane. At least 14 different collagen types have now been identified (for more extensive review see [1] and [2]). Collagens may form fibrils or sheets (a network of interconnecting molecules), with the former providing strength and the latter elasticity.

Proteoglycans

Proteoglycans have a protein core with covalently attached glycosaminoglycans, and in some cases, glycoprotein-like oligosaccharides [3]. The resulting complexity provides

multiple opportunities for pre- and posttranslational modification of both the structure and function of these components. The proteoglycan most well-known to nephrologists is HSPG, the moiety that accounts for the fixed negative charge in the glomerular filter. Other ECM proteoglycans include decorin, biglycan, and chondroitin sulfate proteoglycan. Some proteoglycans such as HSPG are also found on the surface of certain cells and can function as matrix receptors.

Glycoproteins

A third group of ECM proteins is the adhesion glycoproteins. The most abundant of these in basement membrane is laminin [4], a cruciform molecule that consists of three chains: A, B1, and B2. In some tissues, alternative chains have been identified, such as the A2 chain (merosin) or B3 (S-laminin). Considerable work on the structure of laminin has identified and localized biologically active sites that will be discussed below. Another ECM glycoprotein, fibronectin, is found in serum, fibrous connective tissue, and certain basement membranes. Fibronectin also has multiple biological activities. A third glycoprotein, entactin (nidogen), is found in basement membrane but in lesser amounts than laminin. It may serve to link laminin and collagen molecules within the gel matrix.

Other proteins in the ECM

In addition to the structural proteins listed above, many other proteins are present in the ECM. Growth factors that have been detected in matrix preparations include: transforming growth factor (TGF)-β, basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), interferons, and interleukin [5]. Other hormones may also be present. Finally, multiple enzymes are present, including proteases that modulate the composition of the matrix itself [6, 7]. These non-structural proteins may be at least as important as the matrix proteins themselves in determining the biological properties of matrix.

Interaction of cells with matrix substrata

The field of matrix biology began in the 1970s with the identification of matrix glycoproteins and initial efforts to determine their function. Prior to this, investigators had recognized the ECM as an important part of organs and tissues, and had begun to characterize its components. It had been apparent that cells grown in vitro appear to dedifferentiate, losing many of the morphological and functional attributes that they show in the body, and that tissue matrices such as lens or collagen could help to partly maintain the differentiated phenotype. However, the characteristics of the environment that were important for maintaining "normal" physiology were not known. In vitro studies demonstrated that specific ECM proteins provide signals that affect cell morphology and function. These findings suggested that the ECM might play an important role in maintaining the physiological status of the cell in vivo.

In an effort to better define the physiological relevance of substrata for cell cultures, researchers employed simple gels of gelatin (denatured type I collagen), native type I collagen, or fibrin, or more complex gels containing mixtures of ECM components. Even on simple substrata, cells were observed to alter their shape and exhibit other phenotypic changes compared with their usual behavior when grown on glass or plastic culture ware [8]. The first important activity of cells on a substratum, also commonly observed on plastic, is *adhesion.* A second activity is exhibited when cells partly detach and then re-attach to another portion of the substratum, using their ability to adhere to move across the surface in the process of *migration.* Especially after exposure to soluble agents (cytokines) that stimulate cell activities, some cells are able to enter the gel matrix, exhibiting *invasion.* Many of the cells are able to attach to each other and form secondary structures with *organization.* Finally, all of these activities contribute to phenotypic changes that occur in the cell, accompanied by alterations in the expression of specific genes. This is the process of *differentiation.* While proliferation of cells is often reported as an index of cell activity, it is readily apparent that the ability of cells to replicate is a relatively non-specific measure of function. To accomplish physiologically useful activity as a tissue, some of the cellular machinery must be diverted away from cell division to these other activities observed on matrix substrata.

The observation of behavioral changes when cells bind to a matrix substratum suggested the existence of more than one signal for cell differentiation [9]. It was already established that soluble agents such as cytokines, prostaglandins, and lipid mediators modulate cell function. The changes in cell behavior on matrix preparations suggested that a second signal is provided by the solid-phase matrix. How might a solid-phase signal induce changes in a cell? One means is to generate cell polarity [10]. For example, as the fertilized ovum divides, it remains a collection of multipotential cells during the two-cell to the eight-cell stage. Thereafter, synthesis of the laminin A chain begins. Previously, the morula was an undifferentiated ball of cells floating in a fluid phase. With the synthesis and secretion of this new protein, a point of orientation is formed that allows the cells to become compacted and form a polarized structure that then begins to differentiate [11]. Similarly, the carboxy-terminal end of the laminin A chain appears to be important in the polarization that mediates renal tubular morphogenesis during development [12].

Tensile forces and cell activation

An alternative mechanism for cell activation may involve tensile forces on the cytoskeleton. When cells migrating across a matrix substratum in vitro receive an appropriate stimulus (e.g., achieving contact with another cell or binding to a specific amino acid sequence on an ECM protein), they stop migrating and differentiate into a more functional form. This phenotypic change may be induced by the simple process of alteration in cell shape. In fact, shape

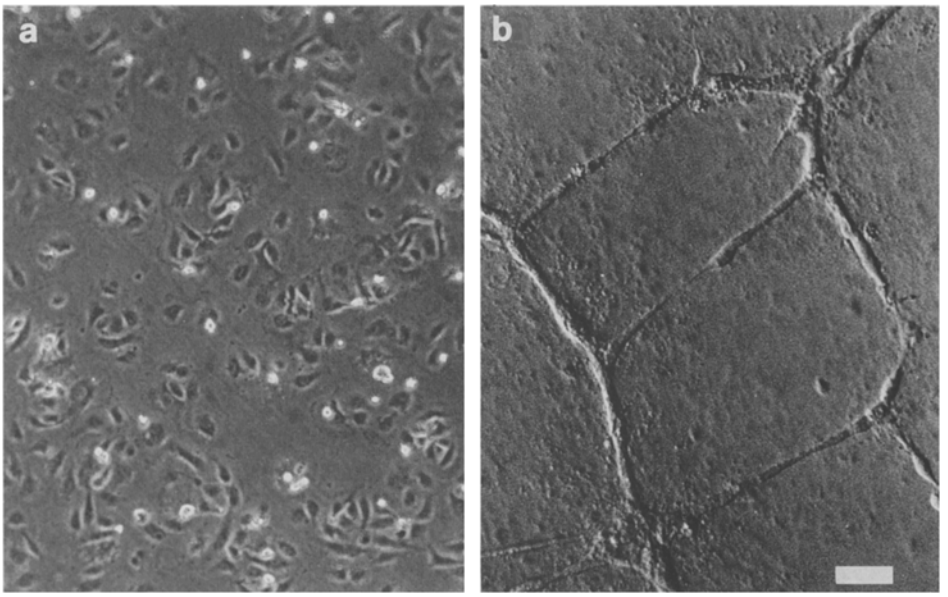

Fig. 1. Morphogenesis of endothelial cells on Matrigel. **A** Human umbilical vein endothelial cells cultured on plastic. **B** Identical cells cultured on Matrigel. After 18 h, the cells have migrated and aligned into tubular structures with tight cell-cell junctions and apical orientation towards a lumen. The *bar* in the lower right hand corner is 50 μm in length.

changes alone have been shown to influence cell proliferation [13] or induce genes involved in differentiation [14]. Cells bind to matrix through a variety of receptors [15, 16] that recognize collagen [17], HSPG [18], laminin [4], or other matrix proteins. These receptors are linked to the cytoskeleton. By adhering to matrix proteins, cells thus establish a connection between external structures and the internal cytoskeleton [19]. Abnormality of the linkage between ECM proteins and the cytoskeleton could affect the formation of, for example, capillary tubes in angiogenesis [9], and such an abnormality is likely to underlie the clinical findings in Duchenne muscular dystrophy [20].

The ECM is itself subject to deformation and tensile stress. A dynamic interaction between tensile forces in the cell and those in the matrix is likely to result from the traction produced by cells that have bound to the matrix and to each other during organization [21]. Stretch-activated channels may be opened in the cell surface. Polymerization of actin and tubulin, mediated by protein kinases [22] that regulate phosphorylation of integrin receptors on the cell surfaces or other proteins [23, 24], may result. Since changes in cell shape may turn off cell growth and alter cell behavior [13, 14], the degree to which the substratum resists tensile forces generated by the cell could be central to inducing differentiative changes in that cell.

Just as cells are activated by tensile forces, modulation of these forces may account for much of the organizational behavior shown by cells on a matrix substratum. Lines of tension generated as cells adhere to the substratum may help direct the migratory patterns shown by the cells [25]. Once a network of cells has been organized, mechanical tension or local secretion of substances known as antiadhesins, such as tenascin [26], might disrupt binding in focal areas and cause the edges of the cell to lift up. This would generate further changes in tensile forces and in shape that could give rise to three-dimensional structures across the surface of the substratum.

It is important to recognize that, while the studies cited here strongly support an association between changes in the cytoskeleton and cell differentiation, it is not clear whether cytoskeletal reorganization is a primary cause, a contributing factor, or an epiphenomenon of differentiated behavior by the cell. According to the theory advanced above, when a cell binds to a substratum, changes in cell shape may result from adhesion of the cell to matrix binding proteins; subsequent organizational modifications communicated through the cell membrane to the cytoskeleton might then stimulate protein kinases. However, it is equally possible that binding of the cell to ECM proteins triggers receptor-mediated signal transduction mechanisms, inducing changes in cell shape along with other manifestations of altered cell activity. The order in which these events occur has not been resolved experimentally, and likely differs depending on the cell type.

Modulation of cytokine effects by the ECM

Thus, it is likely that other signals play an equally important role in cell differentiation [27]. We have already alluded to the role of cytokines in directing cell behavior. Often, cytokines or growth factors do not act independently of the surrounding matrix, but rather function synergistically with the ECM [9]. For example, when adherent to laminin, fibronectin, or vitronectin, neutrophils produce H_2O_2 in response to tumor necrosis factor-α, whereas the same cells in suspension do not [28]. This could relate back to the issue of tensile forces, since changes in shape may alter responsiveness to hormones or growth factors.

Cytokine effects also may be enhanced by the ability of ECM to bind soluble mediators. For example, collagen IV binds TGF-β [29]. HSPGs bind bFGF and TGF-β, and the chondroitin sulfate proteoglycan, decorin, also binds TGF-β [30]. Binding to ECM could significantly affect the bioavailability of these cytokines. Growth factors and other intercellular mediators are probably secreted in a quick

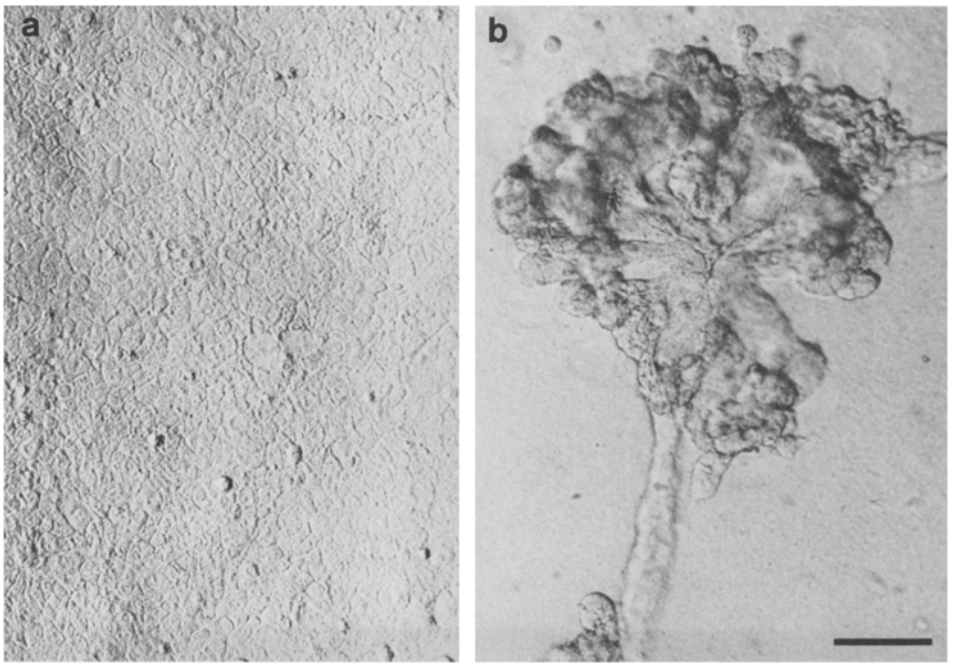

Fig. 2. Organization of cultured salivary gland epithelium on Matrigel. Salivary gland epithelium has been plated on **A** plastic or **B** Matrigel 3 days before these photomicrographs were taken. *Bar* in the lower right hand corner is 100 μm in length. Courtesy of Dr. Maura Kibbey, Laboratory of Developmental Biology, National Institute of Dental Research, NIH.

burst after a given stimulus. Such a burst should cause a sharp, local increase in concentration of the mediator, followed by an equally rapid disappearance as the substance is diluted in the tissue fluids or carried away by the flow of blood through the area. However, binding of the factor to the ECM will retain some of the mediator locally. At the same time, the concentration in the extracellular fluid surrounding the ECM will be slightly lower than that in the ECM itself, encouraging diffusion out of the matrix. In order for the ECM to serve as a "reservoir" for growth factors, the binding affinity of the growth factor for the ECM must be lower than the binding affinity of the growth factor for the cell receptor. Indeed, the dissociation constant for most high-affinity bFGF receptors is 10^{-9}–10^{-12} M, whereas the K_d for bFGF binding to HSPG is 10^{-8}–10^{-9} M [31]. Thus, bFGF will bind more tightly to the cell receptor than to the ECM. The net result will be slow release of the bFGF from the matrix. The factor may then either diffuse into the body fluids or bind to a nearby cell. Growth factors bound to matrix may also be relatively resistant to proteolytic degradation [32]. Together, these interactions would produce a lower peak concentration of secreted growth factors, but a prolonged local duration of bioavailability. In addition to bFGF, similar mechanisms have been identified for TGF-β [30], interleukin-3, and granulocyte-macrophage colony stimulating factor [33], and likely exist for other mediators.

Beside maintaining some degree of local concentration, ECM may broaden the area over which locally released growth factors are effective. Heparan sulfate increases the radius of diffusion of bFGF through agarose, fibrin, or a monolayer of bovine aortic endothelial cells. Morphological changes are induced in endothelial cells with heparan sulfate at a radius 10 times greater than that seen with endothelial cells without heparan sulfate [34]. This may simply reflect increased diffusion of the factor, but it may also indicate facilitated binding of growth factors to receptors because of HSPG present in or near the cell membrane. Such enhanced binding could represent a non-specific effect of proteoglycans, since it results from either endogenous production or addition of HSPG or heparin to cell cultures [35].

Cell differentiation on a basement membrane substratum in vitro

The effects of tensile forces and growth factors have been evaluated in multiple models of cell culture on matrix substrata. A commonly used substratum is basement membrane. Epithelial and endothelial cells, which are normally in contact with this matrix in vivo, differentiate when plated on basement membrane preparations in vitro. For example, endothelial cells cultured on plastic usually have a flat, polyhedral shape. When they are cultured on Matrigel, a basement membrane preparation extracted from the murine Engelbreth-Holm-Swarm sarcoma [36], they form a network of tubular structures within 12–16 h (Fig. 1). The cells in these networks are connected via tight and gap junctions and are polarized with the apex toward the lumen [37]. In contrast, endothelial cells cultured at confluence on plastic for up to 6 weeks eventually form tubular structures that resemble capillaries but appear to be inside out [38, 39]. This phenotypic change may result from alterations in the growth and differentiation factors present in the medium [38, 39]. Alternatively, the cells may differentiate because they have had sufficient time in culture to produce a matrix substratum. Matrigel contains laminin, type IV collagen, entactin, and HSPGs [36], as well as a variety of growth factors and other biologically active molecules [5]. Thus, an ECM substratum that is similar to naturally occurring basement membrane causes the cells to behave more like they do in vivo, with

decreased rates of cell division and formation of a network of structures that resemble capillaries.

Matrigel is of particular interest as an experimental substratum because of its constituents: rather than representing one component of the ECM, it approximates the in vivo milieu. It should be noted, however, that studies performed with Matrigel have the drawback that it is difficult to determine what specific component or group of components mediate a particular cellular event. Many cells undergo morphological differentiation on Matrigel, including smooth muscle cells [25] and osteoblasts [40]. A variety of cells cultured on Matrigel tend to clump or organize into roughly linear structures, although many do not form the fine network of tubules seen with endothelial cells (H. W. Schnaper, unpublished work). A submandibular gland epithelial cell line cultured on Matrigel forms structures that resemble salivary gland organization in vivo (Fig. 2) [41]. Mouse mammary epithelial cells differentiate on Matrigel into globular clusters with polarization of the cells into basal (external) and apical (internal) regions, accompanied by secretion of casein (milk protein) into a central lumen [42]. Thus, morphological changes are accompanied by function (secretory) differentiation.

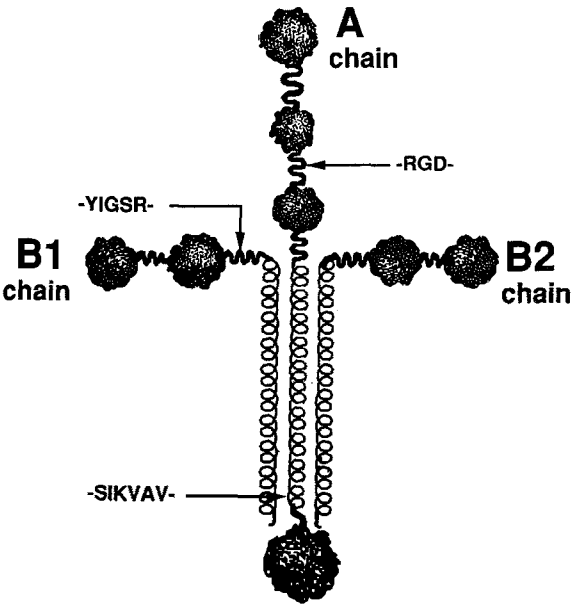

Fig. 3. Structure of laminin. The locations of specific biologically active amino acid sequences described in the text are shown.

Modulation of differentiation by specific matrix proteins

Studies of cell differentiation on Matrigel and other complex matrix substrata suggest that, in addition to tensile forces and cytokines or growth factors, a third signal is provided by specific interactions between the cell and functional domains of matrix molecules. In theory, all of the ECM proteins could provide direct signals to alter cell activity. Binding of cells to collagen gels regulates transcriptional activity in a specific manner [43]. This probably represents the effect of tensile forces, although down-regulation of collagen synthesis could also occur through a specific feedback mechanism. Some proteoglycan molecules could have direct biological activities, as suggested by a structure that includes large amounts of carbohydrate and amino acid sequence motifs that are homologous with growth factors. The complex proteoglycan structure is subject to extensive modification during expression [44], allowing for modulation of binding affinity and even of specificity that could affect functional properties. However, the biological activities identified to date for proteoglycans involve binding of growth factor to the matrix and facilitation of binding between cell receptors and other molecules, rather than recognition of specific signals.

In contrast, considerable data support a role for ECM glycoproteins such as fibronectin, laminin, chondronectin, and thrombospondin in receptor-mediated direction of cell function. The ECM glycoproteins share a general structure that includes a heparin-binding domain, a cell-binding domain, and a collagen-binding domain [45]. These proteins can thus bind to both collagens and HSPG, as well as to the cell. In addition, most of the matrix glycoproteins also have subunits that are homologous with EGF. This structural pattern is ideal for regulating cell function, since it would combine close approximation of matrix and cell components with the presence of structural motifs that have a high probability of showing biological activity.

The two most abundant ECM glycoproteins are fibronectin and laminin. Fibronectin is secreted locally into basement membranes and fibrous connective tissues and also is found circulating in the blood. It is not known whether the "cellular" fibronectins have more significant bioactivity than the fibronectin found in the circulation, but the presence of multiple splice sites in the cell-associated protein suggests that its regulatory effects are more pronounced [45]. Adhesion and migration of fibroblasts and other cells across matrices are mediated in part by distinct and widely separated portions of the fibronectin protein [46, 47]. These findings indicate that fibronectin could modulate such processes as cell migration during embryogenesis [45].

Laminin binds to all other major basement membrane components (collagen IV, HSPG, entactin), as well as to cell surface glycoproteins, sulfatides, gangliosides, and the C1q component of complement [48]. It serves a structural purpose, but numerous studies have demonstrated that it also has biological effects. For example, laminin stimulates neurite outgrowth from cultured human fetal ganglia [49], probably through a mechanism involving the action of protein phosphatases [50]. Laminin may also mediate cell adhesion to matrix [51] and to other cells [52], increase cell proliferation [53], function as a chemoattractant [54], and promote metastatic activity [55].

Laminin is a large (M_r = 800,000), complex trimeric molecule (Fig. 3) with several biologically active sequences that have been identified using analysis of sequence data and duplication of activities of the intact molecule with small, synthetic peptides. It has structural

sequences similar to EGF in the short "cross arms" of the B chains that have been shown to have EGF-like activity, although these sequences do not compete with EGF for binding of the EGF receptor [56]. Other biologically active segments have been identified. Remarkably short sequences within these segments, 3–6 amino acids in length, have been found to be essential for activity. One such sequence on the A chain, RGD (arginine-glycine-aspartate), is a common sequence found in some 200 proteins and is known to mediate binding of proteins to cells. A sequence on the B1 chain, YIGSR (tyrosine-isoleucine-glycine-serine-arginine), mediates cell attachment [57] and migration, also affecting morphological differentiation in several experimental systems and inhibiting angiogenesis and tumor growth in vivo [58]. Other binding sites in laminin mediate additonal interactions with cells or with other ECM proteins. More recently, a sequence near the carboxy-terminal end of the A chain, SIKVAV (serine-isoleucine-lysine-valine-alanine-valine), has been found to induce neurite outgrowth [59] and enhance formation of a tube network on Matrigel by endothelial cells [60]. A synthetic peptide corresponding to this sequence enhances tumor metastasis [61] and growth [62], and angiogenesis in vivo [62] and plasminogen activation in vitro [63]. Other segments have had additional activities described; for example, the carboxy-terminal portion of the A chain is critical for generating the epithelial cell polarity that leads to formation of the renal tubule [12].

These biologically active sequences act by binding to the cell. In some cases, receptors for the sequences have been identified on the cell surface. The presence of an RGD sequence in the ECM protein suggests that a cell surface integrin receptor [64] binds to the protein at that site. A 67-kDa, non-integrin, binding protein has been identified for the YIGSR sequence on laminin [57], and a 110-kDa, non-integrin, binding protein from neural cell membranes has been found to bind the SIKVAV sequence of laminin [65]. The mechanism by which cell activity is modulated by interaction of surface receptors with these specific binding sites probably varies. Binding may create tensile stresses. The specific sequence may interact with the cell in concert with cytokines, or, in some cases, binding may directly activate receptor-mediated signal transduction pathways. As the cell becomes activated, it differentiates and new proteins are synthesized. Some of the new proteins could be growth factors that induce further phenotypic changes. Matrix proteins may be newly synthesized and, directly or by facilitating binding between growth factors and their receptors, affect the cell through amplifying or inhibiting feedback mechanisms. Proteases that are activated could expose potential internal recognition sites in the ECM proteins, with these having additional effects on cell behavior. All of these complex interactions may ultimately affect the differentiated status, and hence the function, of the cell.

Clinical relevance of cell-matrix interactions

In summary, at least three different mechanisms appear to mediate ECM-directed cell differentiation: tensile forces, modulation of cytokine/growth factor effects, and specific signals provided by sequences of the ECM glycoproteins. The relationship of these observations to clinical findings remains to be considered. The ability of a matrix substratum to induce differentiation of mammary carcinomas into rudimentary, milk-producing organelles is intriguing, and offers insight into several normal and pathogenetic processes. Assumption of a more differentiated phenotype by cells plated on Matrigel suggests that the matrix provides a series of signals that serve to maintain the cells in a differentiated state. Alterations in matrix composition might allow cells to "de-differentiate" and become more receptive to abnormal stimuli. In this regard, it is generally accepted that more differentiated tumors are less malignant. Basement membrane may thus limit both the degree of anaplasia of a tumor and its ability to spread. By producing proteases [66], neoplastic cells may degrade existing basement membrane, allowing further neoplastic transformation and the process of metastasis.

The ECM is also important in a variety of physiological events not related to neoplastic behavior. For example, matrix proteins play a critical role in development and organogenesis, including nephrogenesis. Changes in the ECM that remove inhibitory signals or directly stimulate cells could also stimulate abnormal processes that lead to scarring, fibrosis, or neovascularization, such as occurs in the eye in diabetes mellitus. Many pathological processes in the kidney and elsewhere are mediated by cellular immunity; cell-matrix interactions regulate trafficking of immunocytes to lymph nodes and to areas of immune injury [67]. In addition, a stable vascular basement membrane serves as a barrier to the passage of immunocytes into the tissues. The ability of immune cells to react to the presence of this barrier and degrade it [68] is critical for the cellular immune response. Cell-matrix interactions also have direct relevance to the kidney, since models of mesangial cell binding to matrix and of mesangial cell deformation have been developed and indicate that the observations cited in this review are generally applicable to mesangial cell biology [69].

In addition to providing insight regarding pathophysiology, the findings described here may, in the near future, have more direct clinical applications. For example, an RGD sequence in thrombin promotes endothelial cell adhesion [70]. Thus, matrix may play an important role in endothelialization during vascular repair [71], and matrix preparations could be useful agents for coating the lining of vascular grafts. Control of thrombin formation may prove to be important in approaching cell-matrix interactions in renal disease, since abnormal coagulation system activity has been associated with progressive glomerulosclerosis, and thrombin affects mesangial cell adhesion and shape [72]. Regulation of cell-matrix interactions could be employed to inhibit metastasis or prevent tumor growth via regulation of vascular supply [73]. Perhaps short sequences of ECM binding sites could be used therapeutically to compete with matrix for binding sites in vivo and decrease undesirable neovascularization. Alternatively, matrix preparations (including growth factors) might be employed to promote vascularization and healing of extensive wounds such as burns, or to decrease or prevent scarring. The

potential benefits of therapeutic manipulation of cell-matrix interactions offer an exciting challenge to biomedical science.

Acknowledgements. Supported in part by NRSA Senior Fellowship DK08712 from the NIDDK, NIH, a grant from the Children's Research Institute, Children's National Medical Center, and the ESHE Fund (HWS). We appreciate careful review of the manuscript by Drs. Benjamin Weeks and Steven Akiyama.

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