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Extracellular matrix signaling: integration of form and function in normal and malignant cells

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Abstract

A growing number of studies have established reciprocal linkages between extracellular matrix (ECM)-integrins, growth factor signaling and cell–cell adhesion molecules. ECM-dependent tissue-specific gene expression has also been linked to chromatin remodeling. With respect to tissue morphogenesis and differentiation, crosstalk has been established between the ECM and the homeobox morphoregulatory genes. Each of these linkages is profoundly influenced by the cell's microenvironment and the resulting tissue form. Thus for a cell to achieve a differentiated phenotype, the ECM molecules and their receptors must integrate both form and function. In contrast, mutated genes and aberrant interactions with the microenvironment conspire to undermine this integration, often resulting in malignant transformation.

Introduction

Over the past 10 years, the field of extracellular matrix (ECM) biology has overcome the initial challenge of providing evidence that this ground substance does in fact provide instructive information to the cell and modulate gene expression. Research in this area has moved, and is now largely investigating how ECM molecules signal by interacting with their respective transmembrane receptors. Since many of the players have been identified, studies have begun to address how the ECM might coordinate morphological cues and direct signaling pathways to generate tissue-specific gene expression and phenotypes. This review will summarize some of the major advances made which link signaling mediators and cell morphology as well as aspects of crosstalk between integrins and other cell surface molecules.

Extracellular matrix and tumorigenesis

Although a critical role for the ECM in establishing a differentiated phenotype in many tissues has been well documented, it is rather ironic that some of the strongest evidence supporting the ECM's role comes from observations that most, if not all, transformed cells have abnormal interactions with their extracellular environment. In some breast tumors, epithelial cells are incapable of producing an organized basement membrane (BM) which would normally induce growth arrest, while in other breast cancers, the malignant cells fail to recognize their ECM because of expression of inappropriate or non-functional integrins [1••]. The underlying causes for altered cell–ECM communication in tumors remain to be established and are likely to be heterogeneous in nature. Nonetheless, an altered interaction between tumor cells and the surrounding ECM is one of the few common features, aside from a deregulated cell cycle, shared by a wide variety of tumors. In fact by correcting cell–ECM interactions, it is possible to restore normal differentiated function to a breast tumor cell, regardless of the underlying genotypic abnormalities. For example, malignant breast epithelial cells could be returned to their quiescent, differentiated state by simply restoring the level of signaling from the

appropriate integrins [1••]. In normal breast epithelial cells, interactions with BM laminin via the α6β4 integrin induces a differentiated polarized acinar morphology and growth arrest [2, 3]. In malignant cells, however, signaling through α6β4 is impaired and cells instead express high levels of β1 integrin, which continues to generate growth promoting signals [4]. Upon blocking β1 integrins, these epithelial tumor cells formed normal, polarized acinar structures, established proper cell–cell junctions and withdrew from the cell cycle [1••].

The observation that signaling through α6β4 integrin is impaired in breast tumors correlates well with studies in which α6β4 was shown to be required for epithelial cells to form hemidesmosomes, establish polarity and undergo growth arrest via expression of the cyclin-dependent kinase inhibitor p21/waf1 [3]. Whereas tumorigenic colon cells lack the α6β4 integrin, malignant breast epithelium cannot properly target α6β4 to the plasma membrane, and therefore cannot respond to growth arrest signals. Recently, a more sinister and somewhat contradictory role has been assigned to the α6β4 integrin in both breast and colon epithelial carcinoma. Signaling via α6β4, but not β1 integrin, was found to contribute to tumor cell invasiveness and motility associated with metastasis by the localized activation and targeting of phosphatidylinositol 3-kinase (PI3-K) [5•]. Invasion and α6β4-dependent activation of PI3-K was also linked to subsequent activation of Rac, a member of the family of small GTPases which have previously been shown to modulate the actin cytoskeleton dynamics [6]. Together these results provide a mechanistic basis for the induction of increased motility by α6β4 integrin signaling [6]. Moreover, in breast epithelial cells, direct activation of Rac disrupts cell polarity and induces migration of tumor cells through collagen matrices [7•]. It remains to be established whether binding of α6β4 to its ligand in ‘normal’ cells also activates P13-K and Rac or whether normal cells possess mechanisms which limit or suppress the activity of these enzymes. Such comparisons may help clarify the apparently contradictory roles for α6β4 integrin signaling in epithelial cells. Furthermore, although integrin-dependent activation of small GTPases, including Rac and Rho, has generally been linked to increased cell motility and tumor invasiveness, the increasing focus on these proteins will probably reveal a significant role for them in ECM-dependent morphogenesis and differentiation. Indeed, Rho activity has been shown to be essential for morphological changes which accompany gastrulation in *Drosophila* [8].

It is not surprising that signals arising from ligand binding to the α6β4 integrin can influence the cytoskeleton as the unusually long cytoplasmic tail of the β4 subunit has been suspected to interact directly with cytoskeletal components and to allow epithelial cells to establish proper polarity. Two recent studies have identified cytoskeletal molecules which appear to directly associate with the β4 cytoplasmic tail. In one case, β4 was found to be linked to the intermediate filament cytoskeleton via plectin [9], while studies employing a yeast two-hybrid screen uncovered a novel β4 integrin binding protein called p27(BBP) which associated with the insoluble intermediate filament fraction of the cell [10]. It will be of interest to determine whether interaction between α6β4 and plectin or p27(BBP) is altered in transformed cells.

Integrins, growth factor receptors and cell–cell adhesion molecules

In earlier work that focused on identifying intracellular proteins which interact with integrin receptors, Dedhar and colleagues [11] identified a serine-threonine kinase, integrin-linked kinase (ILK). ILK was found in association with both the β1 and β3 integrin cytoplasmic subunits and when overexpressed led to transformation of rat intestinal epithelial cells. More recent work has shown that ILK promotes motility of both mammary and intestinal epithelial cells through downregulation of E-cadherin expression [12]. Similarly, blocking excessive β1 integrin mediated signals in malignant mammary epithelial cells resulted in growth arrest and re-establishment of E-cadherin–catenin junctional complexes [1••]. These findings complement the earlier observations that in terminally differentiated keratinocytes, cadherins

could downregulate expression of integrins, establishing a precedent for crosstalk between these two classes of adhesion molecules [13]. Crosstalk between cadherins and integrins has also been observed in muscle and neural crest cells where they co-operate to regulate cellular migration [14•,15]. In the case of neural crest cells, β1 or β3 integrin dependent signals were required to prevent N-cadherin mediated clustering [15]. In contrast, α5 or β1 dependent signals not only upregulated expression of N-cadherin in muscle cells, but also acted in a coordinate manner with N-cadherin to suppress motile activity.

Earlier work also demonstrated crosstalk between integrins and membrane-bound growth factor receptors. ECM-induced clustering of integrin receptors was accompanied by recruitment of growth factor receptors to focal adhesion complexes, resulting in synergistic activation of intracellular signaling cascades [16]. These observations have recently been extended to *in vivo* studies in angiogenic endothelial cells. Following an initial burst of mitogen-activated protein kinase (MAPK) expression induced by exposure to basic fibroblast growth factor (bFGF), adhesion of endothelial cells to stomatal matrices via αvβ3 integrin was necessary to sustain MAPK signaling necessary for migration and proliferation of endothelial cells [17]. Like β1 integrin, αvβ3 has also been found to associate with activated insulin and platelet-derived growth factor β (PDGFβ) receptors to potentiate PDGFβ-dependent mitogenicity and chemotaxis [18]. Binding of αvβ3 by tenascin also promotes recruitment of the epidermal growth factor (EGF) receptor to focal adhesions, which in turn leads to increased proliferation of vascular smooth muscle cells [19].

Interestingly, in normal breast epithelial cells, the EGF receptor signaling pathway is coupled to the β1 integrin pathway in a bi-directional manner. Ligation of β1 integrin influences both the expression of EGF receptor and the magnitude of signaling, while EGF-dependent signals modulate β1 integrin expression (Wang *et al.*, unpublished data). In addition to modulating integrin expression, signaling through growth factor receptors has been shown to modulate integrin localization. For example, within 10 minutes following treatment of neurites with nerve growth factor (NGF), dense aggregates of β1 integrin were observed in the tips of the extending filapodia [20]. In a related study, it was observed that surface levels of α6β1 integrin in neurons could be increased by reducing its turnover, a process which could be brought about by plating cells on low rather than high levels of laminin [21].

Morphology and signaling

In order to establish an appropriate differentiated phenotype, it is essential that the interactions between growth factor receptors, cadherins, integrins and the ECM be coordinated. The morphological changes which arise through cells interacting with their ECM appear to serve this purpose. For example, in smooth muscle cells, β3-integrin-dependent recruitment of EGF receptor to focal adhesions and enhanced signaling are dependent upon cells adopting an elongated morphology in response to tenascin [19]. Similarly, in cultured breast epithelium cells, crosstalk between the EGF receptor and β1 integrins occurs only when cells adopt a distinctive morphology which arises when they are cultured in three dimensions but not on two-dimensional tissue culture plastic (Wang *et al.*, unpublished data).

The critical role of cell morphology in mediating ECM–integrin-dependent responses have been emphasized in studies by Ingber and colleagues [22•]. They demonstrated that, despite maintaining similar ECM and integrin concentrations, cells could be directed to either proliferate or undergo programmed cell death by altering cell shape alone. By maintaining equal concentrations of ECM ligand and integrin, it might be assumed that signaling would be similar in both round and spread cells and that shape affects the outcome or interpretation of these signals. Recent studies in synovial fibroblasts, however, indicate that shape in fact influences the qualitative nature of signals generated by integrin interactions with ECM.

Specifically, collagenase expression induced by ligation of the α5β1 integrin was found to be dependent upon the small GTPase Rac1 when cells adopted a rounded morphology in response to soluble α5β1 antibodies but not when cells were allowed to spread on substrate-bound antibodies against α5β1 [23••]. ECM–integrin interactions are capable of simultaneously modulating morphology as well as activating signaling cascades; thus, it might be expected that both these components are essential and must be intimately linked in order to establish and maintain the differentiated phenotype.

A role for proteolytically processed extracellular matrix molecules?

It might follow then that the loss of a particular ECM component would result in the loss of appropriate signaling and morphology and ultimately the loss of the differentiated phenotype. Our previous work has shown that proteolytic degradation of BM by matrix metalloproteinase (MMP)-3 results in apoptosis of mammary epithelial cells [24]. Proteolysis of laminin by the serine proteinase plasmin also results in apoptosis of hippocampal neurons *in vivo* [25]. Recent studies, however, have suggested that proteolytically processed laminin or other BM components may directly generate signals which are distinct from those generated by intact ECM components. For example, compared to intact laminin, plasmin-digested laminin-5 was shown to impede the motility of epithelial cells, possibly by promoting assembly of hemidesmosomes [26•]. In contrast, MMP-2 treatment of laminin-5 exposes a putative migratory signal and as a result promotes migration of breast epithelial cells [27•]. Interestingly, both the intact and MMP-2-treated laminin-5 could still interact with the α3β1 laminin receptor, yet only MMP-2-treated laminin was capable of inducing migration. It will be of interest to see whether native and proteolytically processed laminin generate different signals through the same receptor or preferentially interact with distinct laminin receptors to evoke their specific functions.

In other instances proteolytic processing and exposure of cryptic sites in ECM molecules results in a change in the specificity of integrins which can bind to them. For example, MMP-2-dependent processing of type I collagen exposes a site which allows cells to utilize integrin αvβ3 rather than α2β1. In melanoma cells, this change allows cells to receive survival signals via αvβ3 while in vascular smooth muscle cells the result is increased cell proliferation [19, 28]. Cryptic sites within ECM molecules can also be exposed by applying mechanical tension to cells. It was noted that, in fibroblasts, overexpression of Rho and increased contractility enhanced assembly of a fibronectin matrix by exposing a cryptic site in the fibronectin molecule. This process could be mimicked by stretching fibronectin covalently linked to rubber culture dishes [29]. Tension-induced matrix assembly would be beneficial for wound healing or alternatively could contribute to vascular wall thickening associated with hypertensive vascular disease.

Although studies continue to elucidate and define a role for distinct domains of ECM components as well as for their receptors, it is worth noting that, in cultured neurons, the activities of intact tenascin C cannot be mimicked by individual domains but rather require the concerted action of several domains [30]. This again emphasizes the need to adopt integrated approaches to study the influence of the ECM on cellular phenotype.

Towards an integrated picture of the extracellular-matrix-mediated phenotype

One of the challenges in the field of ECM biology has been to develop innovative approaches to understanding how the entire cellular program is integrated. It is perhaps not surprising that the homeobox (Hox) morphoregulatory genes have recently been linked to ECM-dependent changes in gene expression and phenotype. Although a role for Hox genes and the ECM in

organogenesis or branching morphogenesis have been established separately, work in the past few years has helped to link these two areas. Expression of particular Hox genes have now been shown to be influenced by cellular interactions with the ECM as summarized in Table 1. Although the consequences of ECM-mediated changes in Hox gene expression remain to be established, emerging evidence suggests a critical role for Hox gene expression in ECM-dependent phenotypes. For example, HOXB7 has been linked to production of bFGF in melanoma cells [31], and thus downregulation of its expression in murine mammary epithelial cells by the BM would be consistent with the need for cells to arrest growth before expressing a differentiated phenotype. Similarly, Hoxa1 has been linked to expression of laminin [32] and thus may be responsible for the high levels of laminin expression observed in undifferentiated mammary epithelial cells attempting to establish a functional BM [33].

In addition to the ECM modulation of Hox gene expression, the pioneering work by Edelman and Jones [34] demonstrated that both cell–cell adhesion molecules and ECM components such as tenascin were themselves targets of Hox gene regulation. Similar studies in F9 teratocarcinoma cells have linked retinoic-acid-induced differentiation to upregulation of Hoxa1 and a Hox-dependent upregulation of laminin promoter activity [32,35]. Several papers in the past year have extended this relationship to both epithelial and endothelial cells (also summarized in Table 1). Interestingly, the Hox and integrin gene clusters are physically linked on human chromosome 12q13 and appear to have evolved via coordinate evolution, suggesting that they may indeed be functionally linked [36].

Another means whereby Hox genes may have an impact upon the cellular relationship with the ECM is by modulating expression of matrix-degrading proteinases, including the urokinase-type plasminogen activator (u-PA) [37•]. On the other hand, the serine protease inhibitor (SPI)-3 was identified in mice as a direct target of Hoxb5 in the brain [38]. Although the precise role of SPI-3 has not been established, it could inhibit proteolytic activity and thus support neuronal survival [38]. Interestingly, Hoxb5 has also been linked to branching morphogenesis and epithelial cell fate in developing lung [39], a process which can be profoundly altered by imbalances between ECM-degrading proteinases and their inhibitors [40]. Together these studies reinforce a link between Hox genes and ECM-mediated organogenesis and differentiation.

The relationship between the ECM and Hox gene products illustrates the concept of dynamic reciprocity [41] whereby changes in the ECM result in alterations in the pattern of Hox gene expression which in turn modulates the expression of ECM molecule receptors and matrix-degrading proteases and their inhibitors. This ultimately alters the composition of the ECM and thus further alters Hox gene expression.

Not surprisingly, as with altered ECM–cell interactions, aberrant Hox gene expression has been linked to tumorigenesis. In breast tumor cells high levels of HOXA1 were observed; this was also found in growing but not differentiated normal mammary epithelial cells [42,43•]. Melanoma cells express relatively high levels of HOXB7 as well as several members of the Hoxc cluster [31,44]. Sustained retroviral expression of HOXD3 in endothelial cells results in formation of endothelial tumors related to continuous degradation of BM by high levels of u-PA [37•]. On the other hand loss of Hox genes associated with differentiation may also contribute to tumor development. For example, introduction of HOXA10 in MCF-7 breast carcinoma cells results in growth arrest [45]. Given our current understanding of the relationship between Hox genes and the ECM, it is possible that alterations in Hox gene expression associated with tumorigenicity could conceivably underlie the altered expression of integrins or the synthesis or degradation of ECM components.

Cellular architecture, chromatin and differentiation

In order for the ECM to induce a tissue-specific phenotype, it must coordinate cellular morphology, interactions with other cell adhesion or receptor molecules and the signals evoked by each of these variables. In the past year several papers have added to our understanding of mechanisms by which this coordination may occur. Studies by Chicurel *et al.* [46••] demonstrated that binding of the ECM to integrins and formation of focal adhesion complexes can induce movement of mRNA and ribosomes to focal adhesions and thus locally increase translation of existing message. Such a mechanism could be used to increase production of signaling mediators at the site of ECM–integrin interaction and thus may help cells spatially organize intracellular signaling.

Fundamental advances in the past few years have also increased our understanding of basic mechanisms controlling gene expression. Changes in the acetylation state of histone proteins wrapped around promoter regions have been shown to regulate transcriptional activity [47]. Interestingly, BM-dependent expression of the β-casein gene in mammary epithelial cells is, in fact, affected by changes in histone acetylation [48••]. Although cells in the presence or absence of BM express similar profiles of three transcription factors necessary for activation of the β-casein enhancer, transcriptional activity is dependent upon the presence of the BM. In the absence of BM, cells can be induced to activate the β-casein enhancer by modulating the acetylation state of the associated histones. How BM mediates histone acetylase activity is not currently known. One possibility is that the co-transcriptional activator p300, which possesses histone acetylase activity, may participate in BM-induced gene expression [49]. p300 has previously been identified as a critical mediator of differentiation in keratinocytes and complexes with CEBP/β, one of the transcription factors required for BM-induced expression of β-cascin [50]. Moreover, p300 was originally discovered as a target of the transforming oncogene Ela, and evidence suggests that it, like ECM, plays a key role in maintaining a differentiated phenotype [51]. Whether ECM-induced changes in chromatin organization or p300 are involved in mediating ECM-dependent expression in other tissues remains to be established.

Conclusion

In conclusion, although evidence continues to accumulate that ECM molecules or their proteolyzed counterparts may selectively activate signaling pathways, a comprehensive understanding of how ECM/integrin interactions generate the specificity required for expression of complex phenotypes is lacking. Furthermore, although cell and tissue architecture impose an additional layer of control in generating or interpreting intracellular signaling mediatiors, an understanding of how this affects cross talk between integrins, growth factor receptors and cell–cell adhesion molecules in complex tissue environments is only beginning to be addressed. Experimental approaches which investigate how signaling mediators and cellular morphology interact and are coordinately regulated should help elucidate how a relatively limited number of ECM molecules and integrin receptors give rise to a variety of complex phenotypes ranging from the fully differentiated to a malignant state.

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Abbreviations

bFGF	basic-fibroblast growth factor
BM	basement membrane
ECM	extracellular matrix
EGF	epidermal growth factor

ILK	integrin-linked kinase
MMP	matrix metalloproteinase
NGF	nerve growth factor
PI3-K	phosphatidylinositol 3-kinase
SPI	serine protease inhibitor
u-PA	urokinase-type plasminogen activator

Table 1
Relationship between Hox genes and cell–extracellular matrix interactions

Hox genes whose expression is modulated by ECM	
Hoxb9	Expression decreased in α5 integrin null mice [52]
Hoxa1, b7	Expression is suppressed by basement membrane ECM [43•]
HOXD3	Expression is suppressed by basement membrane ECM [37•]
Cdx2	Expression is induced by basement membrane [53•]
Dlx5	Expression is upregulated by mineralized ECM [54]
Hox gene expression and influence on cell–ECM interactions	
Evx-1	Causes increased expression of tenascin [34]
Hoxa1	Expression correlates with laminin expression [32,35]
Cdx2	Causes increases laminin and β4 integrin expression [53•]
Hoxd3, HOXD3	Causes upregulation of β3 integrin expression [37•,55]
Hoxc10, 11, 13	Cause decreased surface expression of α2β1, α5β1 and α6β1 [44]