Role of the extracellular matrix in regulating stem cell fate

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Abstract The field of stem cells and regenerative medicine offers considerable promise as a means of delivering new treatments for a wide range of diseases. In order to maximize the effectiveness of cell-based therapies — whether stimulating expansion of endogenous cells or transplanting cells into patients — it is essential to understand the environmental (niche) signals that regulate stem cell behaviour. One of those signals is from the extracellular matrix (ECM). New technologies have offered insights into how stem cells sense signals from the ECM and how they respond to these signals at the molecular level, which ultimately regulate their fate.

Stem cells have an extensive ability to self-renew and to produce daughter cells that undergo further differentiation. Embryonic stem (ES) cells can give rise to all of the different cell types of the body and are thus referred to as pluripotent. Stem cells in adult tissues generate differentiated cells appropriate for that tissue and may be unipotent (founding a single differentiated lineage), as in the case of the testes, or multipotent (giving rise to multiple lineages), as in the case of the blood. Somatic stem cells in adult tissues can be reprogrammed in vitro to the pluripotent state – the resulting cells are referred to as induced pluripotent stem (iPS) cells.

Regardless of whether stem cells are pluripotent cells in a culture dish or reside in adult tissue, with a more restricted repertoire of differentiation options, stem cell fate is regulated by a combination of intrinsic and extrinsic mechanisms. Intrinsic mechanisms include specific transcription factors expressed by the cells. Extrinsic mechanisms are signals provided by the local microenvironment (niche), including growth factors, the extracellular matrix (ECM) and contact with other cells. Interactions with the niche are reciprocal, as stem cells are able to remodel the niche in response to the signals they receive from it.

Virtually every cell in the body is exposed to ECM proteins (Fig. 1). In epithelia and endothelia, the ECM is organized into a basement membrane that confers polarity: cells contact the basement membrane via the basal, and not the apical, plasma membrane. In connective tissue, cells tend to be completely surrounded by the ECM, as in the case of fibroblasts in the dermis or chondrocytes in cartilage. Even cells in the blood are exposed to soluble ECM proteins, for example fibronectin. Cells adhere to the ECM via several different cell surface receptors, of which the major class is integrins. This interaction allows cells to sense mechanical cues, such as forces, from the ECM and respond in an appropriate manner (for example, changes in cell shape and size and responses such as differentiation and proliferation); this process is known as mechanotransduction.

Thus, as a key component of the stem cell niche, the ECM is not just an inert scaffold, but instead can profoundly influence cell fate choices. In this Progress article, we discuss how new tools for studying cell-ECM interactions have offered new mechanistic insights into how the ECM regulates whether stem cells divide or differentiate.

Studying stem cell-ECM interactions

The ECM varies in composition and concentration, both within and between tissues, leading to different ECM properties.

Basement membranes are typically rich in laminins and type IV (non-fibrillar) collagen, whereas soft connective tissue is dominated by the presence of fibrillar collagens, such as types I and III. The ECM of cartilage is able to absorb the pressure on the joints, a property that is conferred by hyaluronic and the large proteoglycan aggrecan. Conversely, bone rigidity is a function of calcium phosphate deposition within a fibrillar collagen matrix. Even within a single tissue there can be considerable variation in ECM composition: proteoglycan composition in the ECM of cartilage differs depending on the distance from the surface of a joint at which the ends of bones meet; and laminins show heterogeneity in different regions of the skin basement membrane.

Our understanding of stem cell-ECM interactions, and how the ECM can influence whether a stem cell differentiates or not, has been greatly increased by the use of novel techniques. Although the responses vary between different cell types, it has become apparent that factors such as adhesion to the ECM, ECM stiffness and topography of ECM components all have a role.

In vivo models A great deal of evidence for the importance of cell-ECM interactions in tissue morphogenesis and homeostasis has been obtained by generating and characterizing mice in which specific ECM and integrin genes have been knocked out individually or in combination, either in specific tissues or throughout the body. However, these studies typically lack the resolution to characterize the full nuances of how cells respond to the ECM. A stem cell expansion or depletion phenotype may be a direct result of disturbing stem cell-niche interactions or a secondary consequence of perturbing other aspects of tissue homeostasis. In the epidermis, for example, modulating β1 integrin expression in differentiating cells triggers inflammation by disrupting the skin barrier and, as a consequence, stimulates stem cell proliferation. There may be strong in vivo selection against a phenotype that results in stem cell depletion: if gene deletion is not achieved in 100% of the targeted cells, the remaining cells can rapidly restore tissue function.

A further aspect of in vivo models is that an ECM-related phenotype may be masked by compensatory mechanisms that do not operate in vitro. An example of this is the effect of expressing a constitutively
activated β1 integrin subunit in epidermal stem cells. Whereas the effect of this mutation on cell adhesion and differentiation is evident in vitro, an in vivo phenotype is only observed when the skin is exposed to chemical carcinogens, resulting in tumour formation.

Micropatterned islands: shape influences fate. To complement the in vivo analysis of stem cell–ECM interactions, several different approaches offer resolution at the single-cell level. One of these is to generate micropatterned islands that accommodate single cells and direct them to adopt specific shapes. This approach is not new but has become more widely used recently because of more sophisticated methods to generate the substrates, identify stem cell populations and analyse cell behaviour, either by characterizing their fate at the end of each experiment or by monitoring the dynamic behaviour of living cells. Typically, the substrate consists of plastic or gold-coated glass, and the pattern is imposed with protein-resistant materials such as POEGMA (polyoligo(ethylene glycol methacrylate)) polymer brushes. When the substrates are placed in a solution of ECM proteins, the proteins are selectively adsorbed onto the exposed substrate to create ECM islands.

Micropatterned islands have been used to control the degree of spreading of single human epidermal stem cells. The cells initiate terminal differentiation when spreading is restricted on circular islands. In that context, ECM concentration and composition are not important, provided that the ECM concentration is sufficient to allow the cells to attach. When the surface area of an adhesive island is kept constant while the shape is altered, epidermal stem cells also exhibit a response: a cell is less likely to initiate differentiation if it adopts an elongated shape than if it is on a circular island.

Whereas the epidermal stem cell response to different micropatterned islands is to differentiate or remain in the stem cell compartment, in the case of human mesenchymal stem cells the island area determines the differentiation programme. Specifically, cell spreading favours osteogenesis and cell rounding promotes adipogenesis. More complex island morphologies (for example, rectangles and pentagons) also direct mesenchymal stem cell differentiation.

Micropatterned islands have been used to study asymmetric cell division. This is a feature of many stem cell populations and typically results from asymmetric partitioning of cellular components such as the PAR1 (partitioning-defective 1) polarity complex and template DNA strands. Micro-patterned islands can be used to impose asymmetry on the outcome of individual cell divisions, for example by defining the position of the axis of cell division or the shape adopted by daughter cells. Such studies have shown that the spatial distribution of the ECM on a substrate has a role in determining the orientation of the division axis of epithelial cells. This occurs through remodelling of the actin cytoskeleton in response to the substrate pattern.

Substrate stiffness regulates fate. The bulk stiffness of most tissues is several orders of magnitude lower than that of tissue culture plastic or glass and can change within a given tissue as a function of age or disease. Changes in the bulk stiffness of ECM-coated hydrogels elicit different responses in stem cell populations. In the case of mesenchymal stem cells, bone differentiation is favoured by stiff substrates, whereas adipocyte differentiation is promoted by soft substrates.

Micropatterned substrates with differing thickness indicate that cells can sense the ‘hidden’ substrate at a depth of approximately 5 μm and can deform a substrate to a depth of 15 μm.

Although there is ample evidence that substrate stiffness influences stem cell fate, the specific environmental cue that cells sense can vary in different contexts. Recent data indicate that human epidermal and mesenchymal stem cells cultured on ECM-coated hydrogels sense ECM tethering, as in the presence of serum, softer gels promote neuronal differentiation, whereas stiffer gels promote differentiation to glial cells.

The influence of stiffness on stem cell differentiation has been demonstrated on a range of model substrates, including collagen and hyaluronic acid gels, polymer networks, electrospun nanofibres and polydimethylsiloxane (PDMS). Attachment of mouse ES cells to PDMS substrates is unaffected by stiffness, but cell spreading and growth are enhanced with increasing stiffness, as are mesendodermal and osteogenic differentiation. Osteogenic differentiation of rat mesenchymal stem cells grown on PDMS of varying stiffness gradients is strongly influenced both by substrate stiffness and by the ECM pre-adsorbed onto the substrates. Electrospun fibres with identical microstructures and surface chemistries but differing degrees of stiffness influence mesenchymal stem cell differentiation, with softer fibres promoting chondrogenesis and stiffer fibres favouring osteogenesis. Studies of mesenchymal stem cell responses to rigid substrates overlaid with soft hydrogels of differing thickness indicate that cells can sense the ‘hidden’ substrate at a depth of approximately 5 μm and can deform a substrate to a depth of 15 μm.

Glossary

Asymmetric cell division A cell division that results in two daughter cells adopting different fates.

Articular cartilage Cartilage that covers the ends of joints.

Elastic modulus Mathematical description of the tendency of an object or substance to be deformed non-permanently when a force is applied to it.

Formins A family of formin homology proteins that are involved in actin polymerization.

Fluorescence resonance energy transfer sensor (FRET sensor). Tool that uses FRET to visualize protein interactions by light microscopy.

Linear elasticity theory Mathematical study of how solid objects deform and become internally stressed under prescribed loading conditions.
hydrogels exhibit increasing porosity with decreasing stiffness\(^2\) (FIG. 2). By varying the concentration of the chemical crosslinker used to tether collagen fibres to the surface of hydrogels, mesenchymal and epidermal stem cell differentiation can be modulated independently of bulk stiffness\(^3\). When a low concentration of crosslinker was used to attach collagen to stiff hydrogels, epidermal stem cells were stimulated to terminally differentiate, and mesenchymal stem cells differentiated into adipocytes rather than osteoblasts. Key unanswered questions relating to these observations are the stiffness of the collagen fibres themselves and how collagen fibres are tethered within tissues. To answer these questions, new experimental tools for both in vitro and in vivo analyses are required.

The stiffness of the substrate can affect how cells respond to a specific concentration of ECM protein attached to the substrate, independently of tethering\(^4\). For example, fibroblast spreading initially increases with increasing collagen density, but above a certain concentration the relationship is reversed, such that increases in the levels of surface collagen cause a reduction in cell spreading\(^5\). This has led to a model whereby the availability of collagen-binding sites is limiting the attempt of a cell to spread maximally, as cells exert as much force as possible on the few available sites. By contrast, at high collagen density, adhesion is limited by the availability of free integrins on the cell surface, and the force per bond is very low. A recent study has measured the force that cells apply to single integrin–ligand bonds during initial adhesion to the ECM\(^6\), which opens up the possibility of determining whether substrate stiffness influences ECM interactions at this level of resolution.

Substrate topography regulates fate. Studies with both adult tissue stem cells and pluripotent stem cells have highlighted that specific topographical (physical) cues in the sub-micrometre range elicit specific cell responses. The techniques used include increasing the ‘roughness’ of the substrate or displaying specific geometrical shapes on the substrate. By chemically and geometri
cally optimizing the surface of tissue culture plastic, it is possible to promote self-renewal of human ES and iPS cells in the absence of feeder cells\(^7\). Such topographical substrates facilitate the deposition of the ECM protein vitronectin and promote integrin-mediated cell adhesion\(^8\). By varying the nanotopography of culture substrates using nanotubular-shaped titanium oxide, human mesenchymal stem cells can be induced to differentiate into osteoblasts in the absence of soluble inducing factors\(^8\).

By generating libraries comprising over 2,000 different topographies, made up of combinations of circles, triangles and rectangles, one study showed that specific topographies have different effects on mesenchymal stem cells, promoting stem cell proliferation or differentiation into osteoblasts according to the topographical cue\(^9\). For example, osteogenic differentiation was enhanced on topographies that restricted spreading and promoted elongated cell morphologies. It will be interesting to explore the effects of these substrates on other stem cell populations and to determine how different cells ‘read’ the same topographical cues: whether the degree of integrin clustering and cytoskeletal rearrangement provoked by specific topographies is equivalent to specific concentrations and combinations of ECM components.

More complex ECM environments. In vivo, the ECM is only one of the components of the stem cell niche. Cell responses to the ECM are influenced by, and influence, the presence of growth factors and intercellular adhesions\(^10\). Cell behaviour can also be affected by whether exposure to the ECM is in the context of a two-dimensional (2D) or three-dimensional (3D) environment. For example, collagen encapsulation provides a 3D environment and promotes chondrocyte differentiation and morphogenesis of mammary epithelial cells, whereas adhesion of a collagen...
**Box 1 | Measuring forces generated by individual cells**

Many of the recent insights into mechanotransduction have been gained from the application of novel techniques to produce high-resolution distribution maps of forces, stresses and strains inside and outside cells. For example, a recent study involved inserting a vinculin fluorescence resonance energy transfer sensor (FRET sensor) based on an elastic peptide repeat sequence between the head and tail domains of the protein (see the figure, part a). This calibrated biosensor enabled the measurement of time-dependent force development in focal adhesions with piconewton (pN) sensitivity and showed that the tension across vinculin in stable focal adhesions is 2.5 pN. To measure the force exerted by cells on the extracellular matrix (ECM), another group generated a fibronectin FRET sensor and showed that cells exert different forces on fibronectin according to how it is anchored to the underlying substrate.

Force development outside the cell can be tracked by culturing cells on or inside hydrogels containing small fluorescent beads (see the figure, part b). The displacement of these particles under the influence of cellular traction forces can be used to compute the distribution of traction stresses (forces per unit area) using linear elasticity theory. Forces, instead of stresses, can be measured by culturing cells on micromoulded pillars. In this method, the length of the pillars (and hence their rigidity) is altered, whereas the surface area for focal adhesion assembly is kept the same (see the figure, part c), and this allows a direct determination of force per focal adhesion. It shows that the distribution of forces across a single cell is not homogenous, with forces developed by interior focal adhesions being noticeably higher than those exerted by peripheral ones.

### Strain stiffening of the ECM

The mechanical properties of the ECM are determined by a network of collagen, fibronectin and fibrin fibrils of different length and stiffness. There is a growing awareness that both the cytoplasm and the ECM exhibit strain stiffening, which is a highly unusual material characteristic and causes the elastic modulus to increase by orders of magnitude as the applied strain increases. Thus, the more the ECM is deformed by a cell, the stiffer it becomes. This nonlinear elastic response is in sharp contrast to what occurs in response to the commonly used soft, elastic materials for cell culture such as polyacrylamide gels and crosslinked silicones. In this case, the elastic modulus of the material does not change as a function of deformation (Fig. 2). Whereas the deformation of individual tethered collagen fibres is likely to be in the nanometre range, cell deformation of an underlying soft bulk substrate is an order of magnitude greater.

Cytoskeletal and cellular adhesion proteins such as myosin and integrins exhibit increased affinity for their ligands under applied load. Thus, an increase in intracellular strain will enhance adhesion of the cell to the ECM and is likely to alter the conformation of cytoskeletal proteins. Combined, the mechanical properties of the cytoskeleton and associated proteins form an intracellular ‘strain gauge’ with multiple activation thresholds. Differences in rigidity of the extracellular environment lead to an intracellular feedback mechanism that subunits participate in more than one heterodimer. Different integrins can have different affinities for the same ECM ligands, and some integrins have broad specificity. For example, α2β1 integrin mediates cell adhesion to both fibrillar and basement membrane collagens. Integrins can exist in different conformations and transmit signals into the cell and relay signals from the cell to the ECM. They interact with various regulatory proteins, both at the plasma membrane and within the cytoplasm, and act synergistically with growth factor receptors to enhance growth factor responsiveness.

The initial integrin-mediated adhesions to the ECM mature into junctional complexes known as focal adhesions. Intracellularly, focal adhesions are connected to the actin cytoskeleton via the cytoskeletal protein vinculin in a highly dynamic manner. Tension (in the order of several piconewton) across vinculin leads to stable focal adhesions, which establish a mechanical link between the forces generated by the actin cytoskeleton and the ECM (Box 1).
regulates the corresponding levels of pulling forces generated and tunes the sensitivity of focal adhesions to applied forces.

The importance of strain stiffening in stem cell responses to the ECM is likely to vary with time, being important in initial stem cell–ECM interactions but less significant at a later stage. Furthermore, time-dependent changes in ECM stiffness can provoke changes in stem cell behaviour. In culture, differentiation of human mesenchymal stem cells into adipocytes is favoured by later ECM stiffening, whereas osteogenic differentiation is favoured by earlier ECM stiffening. Changes in tissue stiffness with time could be highly relevant in vivo. Maturation of the mesoderm into adult myocardium results in a considerable increase in tissue stiffness. When this is mimicked in hydrogels, pre-cardiac cells exhibit increased expression of mature cardiomyocyte markers and form more muscle fibres.

Signal transduction pathways. By examining stem cell–ECM interactions at single cell resolution, it has been possible to map the signalling mechanisms that mediate the different stem cell responses. In the case of human epidermal stem cells, differentiation is mediated by distinct pathways, depending on the nature of the external stimulus (Fig. 3).

When keratinocytes are stimulated to differentiate by restricted ECM adhesion on micropatterned substrates, they form fewer and smaller focal adhesions than when they are able to spread. Nevertheless, treatment with latrunculin A (a drug that sequesters free actin) or with a RHO kinase inhibitor reduces focal adhesion assembly but inhibits differentiation. This indicates that integrin clustering and focal adhesion formation are not involved in keratinocyte responses to micropatterned islands of different diameter. Instead, what seems to be important is the state of assembly of the actin cytoskeleton: a decrease in the ratio of G-actin to F-actin prevents sequestration of MAL (megakaryocytic acute leukaemia; also known as MRTF) in the cell periphery, thus allowing it to relocalize (by a process that is regulated by formins) and act as a cofactor for serum response factor (SRF). Together, these two proteins stimulate the transcription of the AP1 factors FOS and JUNB, which upregulate genes that are expressed during differentiation. Thus, growth factors present in serum act synergistically with the actin cytoskeleton to regulate the differentiation of keratinocytes that are adherent to micropatterned substrates.

By contrast, when human epidermal stem cells differentiate on ECM-coated hydrogels of low bulk stiffness, the differentiation signal is mediated by a failure of integrins to cluster in focal adhesions. This results in decreased ERK MAPK signalling and an increase in AP1 activity that is thought to be due to reduced activity of a phosphatase that acts on JUN N-terminal kinase (JNK). Thus, in both cases AP1 factors are involved, but the role of integrin clustering differs.

Other pathways are also implicated in triggering epidermal differentiation in response to the ECM, including p38 MAPK. Inhibition of p38 impairs SRF activation in response to restricted keratinocyte spreading on micropatterned islands and reduces histone H3 acetylation at the promoters of the genes encoding FOS and JUNB, thus impeding differentiation. Notably, this pathway is also involved in signal transduction in other stem cell populations, as well as fibroblasts. Specifically, restricted spreading of fibroblasts on ECM-coated micropatterned islands suppresses serum-induced proliferation, and this involves SRF, ERK and p38 MAPK signalling.

Recent evidence indicates a role for the nuclear factors YAP (Yes-associated protein) and TAZ (transcriptional co-activator with PDZ-binding motif) in regulating the differentiation of mesenchymal stem cells grown on hydrogels that differ in bulk stiffness. Differentiation into osteoblasts was inhibited by culturing cells on soft ECM-coated substrates or by knockdown of YAP and TAZ in cells grown on stiff substrates, indicating that YAP and TAZ are key mediators of the stem cell responses to mechanical cues from the ECM. YAP was found to be activated in a RHO GTPase-dependent manner in response to tension of the actomyosin cytoskeleton, independently of the canonical Hippo cascade. These findings fit well with earlier observations that endogenous RHOA activity regulates differentiation of human mesenchymal stem cells in response to changes in cell shape.

Intrinsic regulatory mechanisms. It has long been appreciated that communication between cells and the ECM is reciprocal; for example, the intrinsic state of the cell influences ECM adhesion by regulating integrin gene expression. Nevertheless, new high-throughput approaches for modifying gene expression with siRNA libraries have considerably broadened our knowledge of the interplay between cell intrinsic and extrinsic regulators. An siRNA-based screen of over 300 chromatin modifiers identified functional interactions between different proteins that negatively regulate differentiation of human epidermal stem cells. One network of genetically interacting factors was found to affect distinct but functionally
related genes, including those encoding α6 and β1 integrins and other proteins involved in mediating interactions with the ECM. Thus, chromatin modifiers that regulate differentiation act in part by influencing how stem cells interact with the ECM. This suggests that the interplay between diverse epigenetic strategies protects epithelial stem cells from differentiation by promoting ECM adhesion. This observation is consistent with the finding that the histone deacetylase inhibitor trichostatin A (TSA), which modifies the chromatin status, blocks epithelial terminal differentiation on micropatterned substrates and in suspension and maintains the expression of several stem cell markers, including β1 integrin.

In a different experimental strategy, single-cell global gene expression profiling revealed two subpopulations of cells in the basal layer of the human epidermis that differ in their expression of the Notch ligand Delta-like 1 (DLL1) and its binding partner, syntenin. The DLL1-positive population has increased levels of genes associated with endocytosis, integrin-mediated adhesion and receptor Tyro kinase signalling, and it shows enhanced adhesion to the ECM. The previously unknown heterogeneity revealed by these studies thus results in differences in the interaction of undifferentiated basal keratinocytes with their niche, including the ECM and soluble factors. The enhanced adhesiveness of DLL1-positive stem cells may protect them from detaching from the basement membrane and undergoing terminal differentiation.

Conclusions and perspectives

Although it has been known for many years that the ECM is a key component of the stem cell niche, recent advances in analysis at single-cell resolution have provided new information about the underlying mechanisms by which the ECM regulates stem cell behaviour. Different types of ECM interactions trigger cell responses via diverse sensing mechanisms and downstream signalling pathways. Nevertheless, the outcome, stem cell renewal or differentiation, may be the same. In addition, the intrinsic transcriptional or epigenetic state of a cell can influence its interaction with the ECM, potentially rendering it more or less sensitive to niche signals.

Looking ahead, it will be interesting to couple some of the individual microenvironmental cues, including topography, ECM composition and stiffness, into more complex substrates that may resemble the in vivo situation more closely. There is also a pressing need to broaden the range of stem cell types being evaluated and to define more rigorously the stem cell populations that are currently being assayed. It is important to distinguish between cells that are undergoing fate switching and selective outgrowth of subpopulations of committed progenitors. By drawing together expertise in cell biology and bioengineering, it will be possible to improve cell-based therapies in humans and to create ‘tissue chips’ to accurately model human organs for improved evaluation of drug safety.

In particular, there is a growing realization that for cell therapies to succeed, it may not be sufficient to implant cells into the body, as without an appropriate ECM scaffold they are likely to die or differentiate.


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Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

Fiona M. Watt's homepage: http://wattlab.org
Human Induced Pluripotent Stem Cell Initiative: www.hipsi.org

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