Integrins as architects of cell behavior

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ABSTRACT **Integrins are cell surface receptors that bind cells to their physical external environment, linking the extracellular matrix to cell function. They are essential in the biology of all animals. In the late 1980s, we discovered that integrins are required for the ability of breast epithelia to do what they are programmed to do, which is to differentiate and make milk. Since then, integrins have been shown to control most other aspects of phenotype: to stay alive, to divide, and to move about. Integrins also provide part of the mechanism that allows cells to form tissues. Here I discuss how we discovered that integrins control mammary gland differentiation and explore the role of integrins as central architects of other aspects of cell behavior.**

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INTEGRINS IN EPITHELIAL DIFFERENTIATION

Breast epithelial cells have a discrete function, which is to make milk. They exist in a tissue that is built reasonably simply, containing a network of ducts connected to spherical milk-making alveoli at one end and the nipple at the other (Hinck and Näthke, 2014; Sherratt *et al.*, 2016). However, when breast epithelia acquire genomic defects, they can cause one of the most prevalent cancers. An understanding of the normal development and function of mammary epithelial cells, as well as the changes that lead to tumorigenesis, would represent important paradigms for both mammalian biology and disease.

In both ducts and alveoli, the epithelial cells interact with and respond to their surrounding extracellular matrix (ECM), which consists of basement membrane proteins, via integrins. These cell surface receptors are essential for cell function. On the outside of the cell, integrins interact with ECM molecules in ways that are well defined at the atomic level, at least for fibronectin (Tamkun *et al.*, 1986; Buck and Horwitz, 1987; Sharma, 1999). On the inside, integrins coordinate the assembly of some of the largest molecular machines in biology, called adhesomes, which both link to the cytoskeleton and transmit signals to control most aspects of cell behavior (Glukhova and Streuli, 2013; Figure 1).

The integrin family was discovered ~30 years ago, and by the late 1980s, it was known that they are essential for binding cells to the ECM (Ruoslahti and Giancotti, 1989). The Bissell laboratory discovered that correct cell–ECM interactions are needed for breast epithelia to undergo differentiation and was one of the first to use the basement membrane extract Matrigel as a culture substratum (Barcellos-Hoff *et al.*, 1989). Although the ability to differentiate is not possible in mammary cells plated directly on tissue culture plastic or on stiff collagen-I gels, it was found that cells make milk on soft collagen gels and assemble into functional in vivo–like alveoli on Matrigel (Emerman and Pitelka, 1977; Lee *et al.*, 1984; Barcellos-Hoff *et al.*, 1989; Aggeler *et al.*, 1991). This showed that the type of ECM with which cells are in contact controls tissue-specific gene expression. These were new results, strongly suggesting that cell–matrix interactions have a central role in determining cellular phenotype and differentiation.

In the late 1980s/early 1990s, it was known that epithelia in vivo are complex collections of cells, which undergo cell–ECM and cell–cell interactions, and that the cells are usually polarized. However, it had not been established how these parameters affect cellular function. We therefore developed new tissue culture assays to distinguish among their roles in regulating mammary epithelial cell behavior. These involved culturing single cells in three-dimensional (3D) ECM in order to distinguish the influence of cell–cell versus cell–matrix interactions and to find out which ECM proteins—for example, basement membrane versus connective tissue—were crucial in controlling phenotype (Streuli *et al.*, 1991). Our results were some of the first indications that ECM contributes molecular signals for tissue-specific gene expression and that neither cell–cell interaction nor morphological polarity is needed. Using function-blocking antibodies, we found that β1-integrins are required for cells to express milk proteins and that these receptors bind to laminin in the basement membrane (Tomaselli *et al.*, 1987; Streuli *et al.*, 1995). These

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Abbreviations used: ECM, extracellular matrix; 3D, three dimensional.
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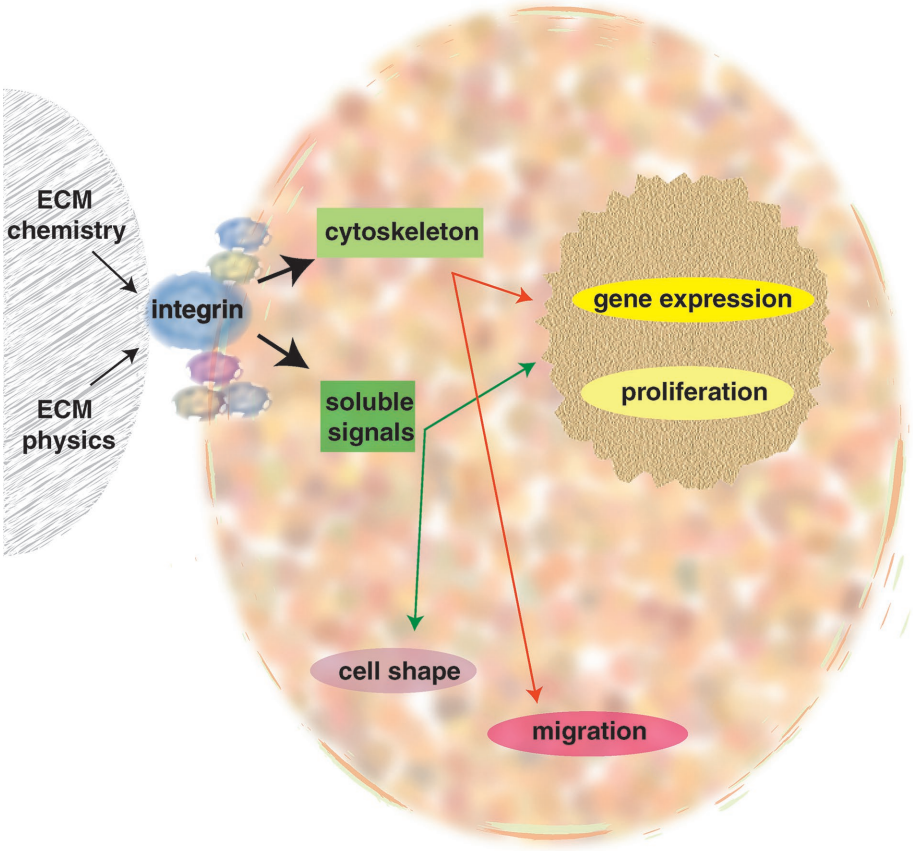

FIGURE 1: Cells interact with their ECM microenvironment via integrins, detecting both chemical and physical signals from the matrix. Integrins interpret this information and deliver it to the cell via large, multiprotein plasma membrane complexes. This becomes conveyed via cytoskeletal and signaling proteins to determine the function of both nuclei (gene expression and proliferation) and cytosol (cell shape and migration).

findings provided early evidence that, whereas cell–cell interactions can transduce signals for orienting intracellular architecture, integrins are dominant in controlling the expression of tissue-specific genes.

The β1-integrins are the major class of integrins and are expressed in most cell types. They were shown by gene deletion to be essential for embryonic development (Fässler and Meyer, 1995). At the time, it was known that integrins are important for many developmental events to occur and for cell migration and tissue growth (Bronner-Fraser, 1986; Menko and Boettiger, 1987; Reichardt *et al.*, 1989; Sorokin *et al.*, 1990). Not much was known about their role in controlling gene expression, and our study revealed that integrins are necessary for cells to carry out their normal differentiated function.

Since that time, we have confirmed the function of β1-integrins by deleting the gene in the mammary gland in vivo (Naylor *et al.*, 2005), and discovered that their role is to allow the soluble ligand prolactin to activate its own receptor and trigger Jak-Stat signaling, which then leads to the transcription of milk protein genes (Edwards *et al.*, 1998). This signaling link occurs through key integrin-regulated downstream proteins, including vinculin, integrin-linked kinase and its associated protein α-Pix, and Rac1 (Akhtar and Streuli, 2006; Akhtar *et al.*, 2009; Rooney *et al.*, 2016; Wang *et al.*, unpublished data). Other well-studied integrin signaling proteins, such as focal adhesion kinase, are not involved (Walker *et al.*, 2016).

A key aspect of normal animal biology is the ability of cells to differentiate. Given that tissues have a complex 3D architecture, it is not surprising that integrins and the consequent interaction of cells with a correct ECM determine gene expression. Our work strengthened the concept that integrins are necessary for expression of tissue-specific genes and identified some of the proteins that link ECM proteins on the outside of the cell with transcription factors on the inside.

INTEGRINS ALSO CONTROL THE OTHER KEY ASPECTS OF CELL BEHAVIOR

Integrin-mediated cell–ECM interactions are also required for cell survival and proliferation, as well as for migration. The correct balance between apoptosis and proliferation maintains tissue size throughout animal life.

Cells are constantly dying in order to eradicate damage, and being replaced by others via replication (Meredith *et al.*, 1993; Gilmore *et al.*, 2000). Cell–matrix interactions have a central role in cell survival, with integrins mediating the signals that keep proapoptotic proteins away from mitochondria, thereby allowing them to stay alive (Schellenberg *et al.*, 2013).

Cell division is also integrin dependent. It is well known that growth factors drive the cell cycle, but in normal biology, they can do so only when cells are in the appropriate ECM environment. Such controls are often evaded in cancer. The integrin adhesion complex contains numerous signaling enzymes, as well as structural and adhesion-specific proteins. Most classes of signaling enzyme, small GTPases, S/T and Y-kinases, and phosphatases are associated with integrins, as detected by highly specific proteomic analysis and immunoisolation studies (Horton *et al.*, 2015). Although Y-kinases such as FAK and classic oncogene Y-kinases such as Src are associated with integrin complexes, so too are major growth-controlling GTPases such as Rac (Paul *et al.*, 2015).

Cell migration has been known to be an integrin-dependent process since the 1980s (Bronner-Fraser, 1986). Perhaps the ability of integrins to link physically with the cytoskeleton inside the cell provides the key visual clue for this association with migration (Horwitz *et al.*, 1986). However, the sheer complexity of the cytoskeleton, its different domains close to and away from the plasma membrane, and its terminal links to intracellular organelles such as nuclei means that exactly how integrins control and orchestrate cell migration is only just beginning to be understood (Sarris and Sixt, 2015; Zhang *et al.*, 2016).

Although often overlooked, integrins are fundamental for all of animal biology and contribute to most aspects of cellular behavior, including differentiation, survival, proliferation, and movement. They assemble large signaling machines, the components of which have been termed the adhesome, from which links are made to a range of cellular endpoints, whereas other proteins trigger different responses.

NEW WAYS TO UNDERSTAND THE ARCHITECTURAL DETERMINANTS OF CELLULAR FUNCTION

In vivo, most cells exist within tissues, which provide architectural features contributing to function. The extremely hard and

non-ECM-compliant plastic culture dishes allow surviving epithelial cells to proliferate and migrate, but other cellular functions are usually lost. It is becoming recognized that tissue-compliant conditions are essential to maintain normal cell biology, and as a consequence, new systems are emerging to study how integrins respond to the architectural constraints of their environment.

In addition to adhesion per se, cellular interactions with correctly configured ECM are vital for cell function. Different tissues contain "soft" and "stiff" ECMs that are many orders of magnitude softer than artificial culture environments. Matrigel is useful as an ECM with tissue-like flexibility, and for epithelia, it provides the basement membrane proteins that are required for survival, polarity, and function. However, in vivo, basement membranes are only 30–70 nm thick, and they separate the basal surface of epithelia from collagen-rich stroma.

Soft and stiff cellular microenvironments have differential control on nuclear composition and function, determining, for example, the number of nucleoli within cells, as well as their potential to control transcription, genome activity, and the rhythmic expression of large numbers of genes that are regulated by circadian clocks (Olson and Nordheim, 2010; Dupont *et al.*, 2011; Maya-Mendoza *et al.*, 2016; Yang *et al.*, unpublished data). The mechanisms are not fully understood, but integrin signaling determines proliferation in mammary cells via Rac1, and clocks via the Rho pathway (Jeanes *et al.*, 2012). Some diseases are characterized by differing architecture of the ECM; for example, in malignant cancer, the ECM is often stiffer, which can promote proliferation, as well as metastatic lesions, via altered integrin signaling (Paszek *et al.*, 2005).

Novel models of tissue architecture are now emerging that will make a major contribution to the understanding of cell function. Moreover, 3D hydrogels that are compositionally the same but have different stress-relaxation properties reveal that the biophysics of the cell microenvironment, that is, ECM mechanics, is profoundly important for most aspects of cell behavior (Chaudhuri *et al.*, 2016). We now culture mammary epithelia in a 3D hydrogel based on alginate but containing a little Matrigel (Chaudhuri *et al.*, 2014). A new gel containing collagen-I, hyaluronan, laminin, and fibronectin was recently developed, which has made possible the 3D culture of primary breast epithelia from humans (Sokol *et al.*, 2016).

In addition to having a different stromal composition and architecture, all tissues are multicellular, providing real complexity in analyzing the function of individual cell types. This has necessitated new multi–cell type systems to be developed. For example, when breast luminal epithelia, basal epithelia, and ds-Red stromal fibroblasts are embedded in collagen-I gels, the resulting 3D structures closely resemble the design of tissue in vivo (Nash *et al.*, 2015). This provides new ways to determine how cells within tissues operate.

Finally, new systems are emerging to study the function of single cells in tissue-compliant 3D ways. For example, individual cells can be engineered into ECMs of different stiffness using 3D integrated tissue-organ printers (Kang *et al.*, 2016). Perfecting such a methodology will provide future possibilities for dissecting the way that individual cells within tissues really work. In addition, these technologies present therapeutic opportunities to reconstruct tissues containing multiple cell types, which could be used for restorative surgery.

CONCLUSION

Integrins are central architects of cell behavior in animals. They detect the local microenvironment of a cell and convey this essential information to permit tissue-specific gene expression. Integrins are also paramount for survival, proliferation, and migration, underscoring that correct communication with the ECM is needed to determine cell fate.

Adhesion systems have a greater role than just perceiving the biochemistry of the molecules contained within the ECM. They also detect its architectural qualities. Integrins therefore discern the biophysical properties of the cellular environment and transduce information about the stiffness or compliance of the ECM (Figure 1). For breast epithelia, the latter is required for accurate control of gene expression and cell survival. In addition, different ECM mechanics in humans convey contrasting risk factors for acquiring cancer (McConnell *et al.*, 2016).

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