Chapter 1
Responses of Cells to Adhesion-Mediated Signals: A Universal Mechanism

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This chapter is part of Section I: Mechanisms of Cell Adhesion and Mechanotransduction

Abstract Cells are exposed to a plethora of signals that typically coerce them to function properly, but aberrant signaling can lead to pathological conditions. In the treatment of diseases and the rational design of functioning tissues, it is vital to understand and be able to manipulate these inputs. In the past, much of the interest has been on chemical signaling but recently, there has been an explosion of research into a diverse array of mechanical signals. Mechanical signals have been shown to influence cellular growth, survival, migration, and differentiation. Despite its obvious importance, relatively little is known about the mechanism of mechanosensing. In this chapter, we describe what is currently known about potential mechanosensing molecules and then describe a model by which a wide array of mechanical signals can be interpreted by a common mechanism. By understanding this mechanism, one may be able to develop new therapeutic interventions for devastating diseases such as cancer and break through critical barriers facing the field of tissue engineering. We expect the knowledge gained from the study of basic biology to greatly impact the treatment of many patients in the clinical setting in the coming years.

Keywords Mechanical signals · Traction forces · Durotaxis · Focal adhesions · Cancer

1.1 Introduction

Cellular behavior including growth, survival, migration, and differentiation is regulated by the complex interplay between cells and their environment. While much attention has been focused on chemical factors, it is becoming increasingly

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evident that adhesion-mediated non-chemical signals such as mechanical forces and topography can play an equally important, complementary role. However, despite a large volume of phenomenological records, our understanding of the mechanisms of cellular responses to these signals remains fragmentary. While some signals such as mechanical forces may interact directly with intracellular components [1], many other signals are likely converted into intracellular chemical events near the plasma membrane where adhesion takes place.

Adhesion-mediated non-chemical signals take a variety of forms. Applied mechanical forces are able to cause behavioral responses both directly through intracellular signaling and indirectly through changes in gene expression [2, 3]. Equally important extracellular signals include rigidity [4, 5], shape [6, 7], and topography [8]. For example, fibroblasts cultured on soft substrates undergo apoptosis while those on rigid substrates show enhanced growth [9]. In addition, the differentiation of mesenchymal stems cells in vitro appears to be dictated by substrate rigidity [10]. Interestingly, shape and geometry are able to elicit similar responses as mechanical forces. Adhesive cells show active growth when allowed to spread without constraints, and undergo apoptosis when inhibited from spreading [6]. Osteogenic cell differentiation is favored only within a range of spreading areas [7, 11], while adipogenic cells fail to differentiate when allowed to spread fully [12]. The fate of mesenchymal stem cells can similarly be directed via shape constraints [13].

Cell migration and cytoskeletal structures also respond to a similar set of adhesion-mediated signals. Contact guidance was discovered decades ago as the orientation of adhesive cells and their actin cytoskeleton along micrometer sized grooves [8]. In addition, motile adhesive cells were found to orient toward tensile forces [3, 14], while migrating fibroblasts turn preferentially toward stiff substrates, a phenomenon known as durotaxis [14].

Among different adhesion-mediated signals, mechanical forces are the best understood. While it is possible that each type of sensing uses separate mechanisms, various observations suggest that there may be a universal mechanism for sensing diverse forms of adhesion-mediated signals. We will first discuss the potential mechanism of force sensing, then propose a common force-dependent sensing mechanism that, with proper positive and negative feedback loops, may function universally for sensing a wide range of signals.

1.2 Mechanisms for Sensing Mechanical Forces

Mechanical forces may induce transmembrane signals by triggering the entry of calcium ions through stretch-activated channels [15–17], and/or by inducing structural changes at adhesion sites. For adherent cells, focal adhesions have been the focus of attention as they are both the direct link connecting the cell's cytoskeleton to the extracellular matrix [18], and the site of concentration of important signal transduction enzymes such as the Src kinase and FAK [19]. Responses to

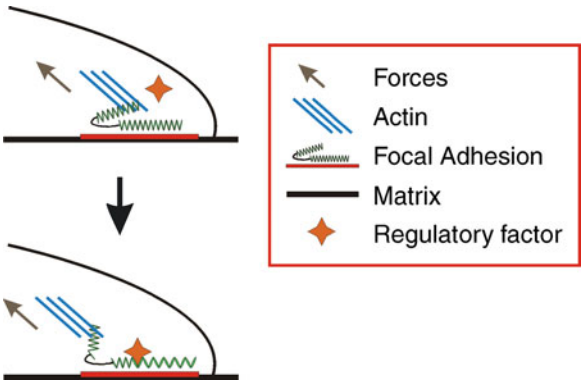

Fig. 1.1 A potential signal transduction mechanism based on force-induced conformational change. Pulling forces generated by the actin cytoskeleton cause the unfolding of associated proteins, which may expose binding sites for regulatory factors

mechanical forces include activation of the small GTPase, RhoA, coupled to an increase in the size of focal adhesions and enhancement of intracellular contractility and traction forces [20, 21].

How mechanical forces modulate the activities of signaling enzymes remains to be an area of active investigation. Integrins – the membrane spanning component of focal adhesions – were first hypothesized to be mechanosensors [22–24]. Integrin clustering, ECM-binding, and recruitment of focal adhesion proteins are known to be enhanced by mechanical forces [21, 25, 26]. Thus mechanosensing may involve the activation of integrins and the resulting concentration of focal adhesion kinase (FAK) and Src [27, 28]. A second possible mechanism involves inherent mechanosensitivities of these signaling molecules. Mechanical forces may directly induce conformational changes and expose autoregulated catalytic domains, shielded substrate domains, and/or cryptic binding sites of scaffold proteins (Fig. 1.1). Focal adhesion proteins, such as vinculin [29], may change their conformation in response to mechanical input. In addition, stretching of p130Cas induces a conformational change that enhances its phosphorylation by the Src kinase [30], which in turn activates the recruitment of binding partners including many small GTPases.

Force-induced structural changes may occur not only at the intra-molecular but also inter-molecular level [31]. Focal adhesion-associated actin filaments show force-dependent assembly and retrograde flux. Differential association of focal adhesion components to actin filaments may then lead to differential transport and relative shear movements of these components (Fig. 1.2). Thus, interactions among focal adhesion proteins may be regulated according to their relative affinity for integrins/membrane components vs. the actin cytoskeleton. While an upstream event is required to regulate actin flux, this mechanism may serve to amplify the responses.

In addition to direct responses to external forces, positive and negative feedback loops are likely to play an important role. A combination of local positive feedback and global negative feedback has long been recognized as a key component for the extreme sensitivity of chemotaxis [32–34]. A similar mechanism may function

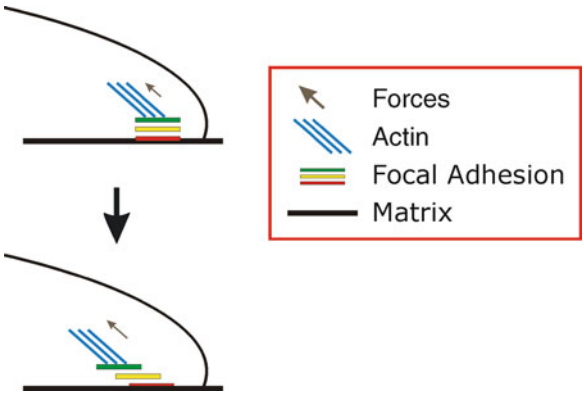

Fig. 1.2 A potential signal transduction mechanism based on force-induced protein shear movements. Pulling forces generated by the actin cytoskeleton cause lateral shear and relative movements of focal adhesion proteins, which may alter protein-protein interactions and affect enzymatic activities within focal adhesions

constitutively in mechanosensing. The so-called "inside-out" signaling may in fact represent the positive feedback mechanism. Inside-out signaling was first recognized as actin cytoskeleton and contractility-dependent enhancement of integrin-ECM interactions [35]. The process may include both transmembrane activation of integrins and force-induced conformational changes of fibronectin to expose cryptic binding sites [36, 37]. The latter then causes fibronectin molecules to change conformation and form a long, multi-molecular fibrillar structure [38], and enhances the mechanical input from the matrix. Together, these responses create a positive feedback loop that, upon the initial response to mechanical stimulations, increases the cytoskeletal contractility and further enhances mechanical stimulations and/or responses (Fig. 1.3).

1.3 A Universal Sensor for Diverse Adhesion-Mediated Signals

While passive force-sensing may be responsive only to external mechanical forces, a highly versatile sensing mechanism may be created by incorporating internal contractile forces as part of the mechanism. In addition to mediating inside-out signaling as discussed above, when transmitted to the extracellular matrix, such forces may function as probing forces for parameters such as substrate elasticity, cell shape, and size. This notion is further supported by the similarity of cellular responses to these diverse signals as discussed earlier.

Substrate elasticity is characterized by the Young's modulus, which under ideal conditions is a proportional constant between applied stress and the resulting strain (deformation). As a cell actively applies increasing forces on the substrate, both the strain and resistive counter forces increase. While the cell may use either the resistive force for a given deformation, or the deformation under a given

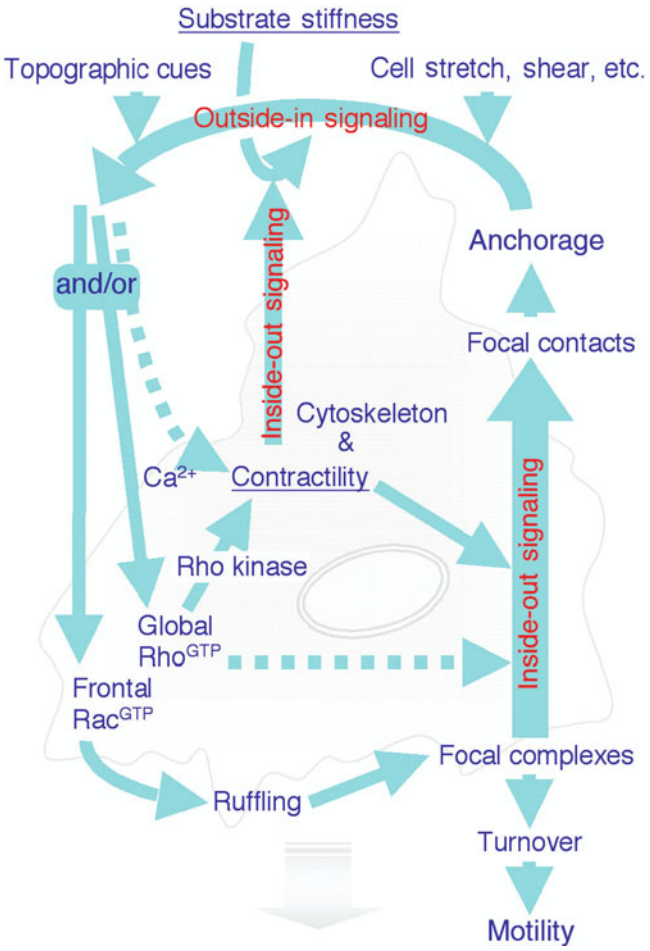

Fig. 1.3 Interplay between physical signals and chemical signaling pathways. Physical signals such as substrate stiffness, applied mechanical forces, and topographic cues activate intracellular signaling pathways and stimulate contractility (Outside-In signals). Increased contractility in turn amplifies focal adhesions and enhances signal sensitivity (Inside-Out signals). This positive feedback loop can then lead to large changes in cell motility, growth, and differentiation. From Discher et al. [4]

probing (and counter) force, for the detection of elasticity, one study supports the former mechanism by showing a relatively constant deformation of the substrate irrespective of its elasticity [39]. However this does not necessarily indicate that intracellular structures undergo a constant deformation. To the contrary, intracellular deformation is determined by the magnitude of counter forces against probing forces, and both the counter force and intracellular deformation must increase under a constant amount of substrate deformation as its rigidity increases (Fig. 1.4).

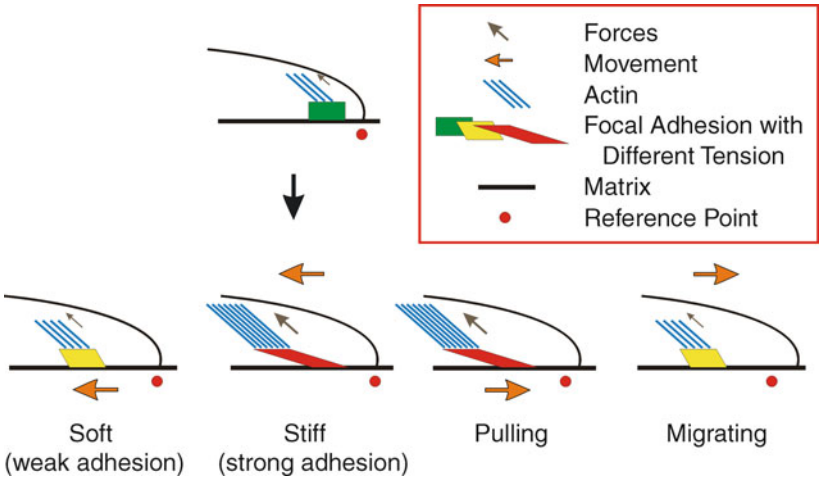

Fig. 1.4 A universal model for the detection of physical and topographical signals. Focal adhesions are represented as parallelograms of different colors and skews. Cell migration and soft substrates share a common feature of minimizing structural changes at focal adhesions, due to the forward movement of the cell body or the backward movement of the substrate that reduces tension at focal adhesions. Cell immobilization, a stiff substrate, or a pulling force transmitted through flexible substrates causes an opposite effect and generates strong tension at focal adhesions. Thus a common mechanism may be able to sense a wide range of signals. Adapted from Guo and Wang [31]

The same mechanism may also be used for the detection of a cell's own shape, spreading and migration. Translocation of the cell body cancels the deformation of a mechanically coupled substrate and cell body, thereby diminishing the signal. In addition, as an elastic self-spreading object, adhesive cells must generate increasing forces against the substrate to propel an increasing extent of spreading. Responses to the corresponding, increasing counter forces may then allow a cell to detect its extent of spreading. An adherent cell may further detect its own shape through a combination of local positive feedback to enhance activities in the extended region, where mechanical input is strong, and global negative feedback to suppress activities elsewhere.

Importantly, these explanations are consistent with experimental observations. For example, it has been determined that focal adhesion size, cell spreading area, and traction force increase as a function of substrate stiffness [40]. The model also explains the phenomenon of durotaxis. Focal adhesions are reinforced in stiffer regions causing an increase in local forces, which in turn causes the cell to migrate away from soft regions. In addition, consistent with a force-based mechanism for the detection of spreading, cells confined either by micropatterning or decreasing ligand density exert markedly reduced forces on the substrate [41, 42]. However, although this model can succinctly explain the sensing of many forms of adhesion-mediated signals, much needs to be done to gain a full understanding of the process particularly the initial mechanosensitive events and the mechanisms of feedback.

1.4 Implications in Biomedical Engineering and Disease Treatment

Despite the still limited mechanistic understanding of mechanotransduction, tissue engineers and pathologists have already realized the profound implications of cellular responses to various forms of adhesion-mediated signals in disease treatment and regenerative medicine. Mechanical sensing may add a critical dimension to the effective treatment of cancer, which generally fails due to uncontrolled cell growth and migration – both are regulated by chemicals as well as adhesion-mediated signals. Two aspects in particular contribute to the disease phenotype of cancer. First is the well known loss of anchorage dependence, defined as the need for most normal, non-hematopoietic cells to adhere firmly to a surface in order to survive [8, 43, 44]. Loss of anchorage dependence may allow cancer cells to survive following the penetration through the vasculature, and to float through the bloodstream before reaching distant colonization sites.

Equally important may be the increase in stiffness in many tumors relative to their normal counterpart or the surrounding tissues [45–47]. Conversely, it was found that increased ECM stiffness acted to promote tumorigenesis in an integrin and cytoskeletal contractility dependent manner [48, 49]. The pathology may involve two potential aspects. First is the possible stimulation of extracellular matrix production/assembly by the surrounding fibroblasts, as evidenced by the involvement of "carcinoma-associated fibroblasts" in cancer progression [50]. Second is the stimulated durotaxis as a result of increased stiffness, which may cause tumor cells to migrate away from the home tissue, and blood vessels to grow into the tumor to provide nutrients. Clearly mechanotransduction pathways offer exciting new targets for cancer therapy.

Insights from basic mechanobiology research will also facilitate engineering control of man-made tissues in regenerative medicine, a field with seemingly unlimited potential but challenged by limited success thus far. To guide stem cells toward desirable differentiation pathways and target sites, tissue engineers have begun to realize the critical need to control not only the chemical environment but also parameters such as stiffness and topography of the surrounding adhesive materials. For example, consistent with the tendency of adherent cells to disperse on stiff substrates and to form tissue-like aggregates on soft matrices [51], stem cells form organoids only under minimal mechanical input such as in hanging drops, and grow as spread monolayer on conventional tissue culture dishes [52]. Thus not only is it important to select scaffold materials of proper mechanical characteristics, but to develop new "smart" materials that allow the modulation of these parameters spatially and temporally. Complex tissues may be successfully engineered only through the regulation of mechanical and topographic environment at a matching complexity.

In summary, knowledge in basic cellular mechanotransduction is finding rapid translation into medical applications. Conversely, lessons learned from clinical outcomes of cellular mechanical manipulations may complement basic research

in understanding both normal mechanisms and pathological defects. Such interplay between basic "translatable" research and clinical research is likely to lead to significant breakthroughs and make the coming decade an exciting time for fruitful manipulations of cellular adhesive interactions.

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