7.10 Cell-Extracellular Matrix Mechanobiology in Cancer

S Kumar, University of California, Berkeley, CA, USA

Abbreviations

ACS	American Cancer Society
ADAM	a disintegrin and metalloprotease
AFM	atomic force microscopy
ATP/ADP	adenosine triphosphate/diphosphate
CAM	cell adhesion molecule
CNF-1	cytotoxic necrotizing factor-1
CRM	confocal reflectance microscopy
DIC	differential interference contrast
ECM	extracellular matrix
EMT	epithelial-mesenchymal transition
ERK	extracellular signal-related kinase
FAK	focal adhesion kinase
FC	focal complex
FDA	Food and Drug Administration (USA)
GAP	GTPase activating factor
GBM	glioblastoma multiforme
GEF	guanine nucleotide exchange factor
GTP/GDP	guanosine triphosphate/diphosphate
hMSC	human mesenchymal stem cell
IgSF	immunoglobulin superfamily
LOX	lysyl oxidase
MAT	mesenchymal amoeboid transition
mESC	mouse embryonic stem cell
MLC	myosin light chain
MLCK	myosin light chain kinase
MMP	matrix metalloprotease
NMMII	nonmuscle myosin II
ROCK	Rho-associated kinase
SEM	scanning electron microscopy
SHG	second harmonic generation
VEGF	vascular-endothelial growth factor

Glossary

Angiogenesis The formation of new blood vessels from preexisting vessels.

Cytoskeleton The three-dimensional, intracellular biopolymeric structural network that contributes to cellular morphology, mechanics, motility, and directional

 doi:10.1016/B978-0-12-374920-8.00712-8

intracellular transport. Key components of the cytoskeleton include actin filaments (F-actin), microtubules, and intermediate filaments.

Epithelial-mesenchymal transition A biological process through which epithelial structures adopt specific characteristics of mesenchymal tissues, including loss of cell-cell adhesions and increased motility. It is both critical to normal developmental processes and a common feature of the early progression of epithelial tumors.

Extracellular matrix The biopolymeric material scaffold to which cells adhere in tissues.

Focal contact A multimolecular, nano/microscale plaque-like complex that anchors cells to the ECM. Focal contacts may exist in various forms, including focal complexes, fibrillar adhesions, and focal adhesions, as defined by morphology, molecular composition, and other factors. Note that the term 'focal adhesion' is widely used in the field to represent all cell-ECM adhesive complexes.

Glioblastoma multiforme A grade IV astrocytoma, the most common and aggressive primary brain tumor.

Integrins A type of transmembrane cell adhesion receptor that is an obligate heterodimer consisting of an α and a β subunit.

Mechanobiology A field that seeks to understand the role of mechanical forces in biological systems at length scales ranging from single molecules to whole organisms.

Mechanotransduction The process through which living cells and tissues sense, process, and respond to mechanical cues in their environment.

Metastasis The process by which tumor cells spread from a primary organ of presentation to a distal (nonadjacent) organ.

Ras homology (Rho) family GTPases A family of monomeric GTPases classically associated with regulation of the actin cytoskeleton to establish shape polarity, mechanical properties, and migration. Examples include Rho, Rac, and Cdc42.

7.10.1 Introduction

The American Cancer Society (ACS) defines cancer as 'a group of diseases characterized by uncontrolled growth and spread of abnormal cells.' For 2009, the ACS estimated that nearly 1.5 million new diagnoses of cancer would be made in the United States, and that various types of cancer would claim over 500 000 lives (25% of all deaths), second only to heart disease as a cause of death.[1] At a fundamental level, cancer is a disease in which cell and tissue homeostasis is converted from a normal phenotype, in which cell proliferation, death, and differentiation are tightly choreographed, to a tumor-like phenotype, in which these events become grossly unbalanced. Thus, two central challenges in the field of cancer biology have been to identify cues that trigger transformation into the tumor phenotype and to understand the mechanisms through which cells process these cues to give rise to behaviors that promote tumor growth and spread. Traditionally, the field has focused the bulk of its attention on the role of primary genetic lesions in this process[2–6] and, more recently, epigenetic modifications that might alter transcription of genes relevant to tumorigenesis.[7–10]

While there is no question that these cell-intrinsic factors play crucial roles in all aspects of tumor growth and spread, it has recently become clear that dysfunctional crosstalk between the cell and its external microenvironment also plays a significant role.[11–15] This microenvironment consists of a complex combination of soluble signals (e.g., growth factors), other cells, and the extracellular matrix (ECM), which is the solid-state biopolymeric scaffold that surrounds cells in living tissues. While all of these components are capable of regulating cell behavior through classical receptor-mediated signal transduction pathways, it is increasingly appreciated that a critical component of this microenvironmental influence is biophysical in nature, including mechanical forces exchanged between cells and their surroundings. In fact, one can construct a 'mechanical force journey' through which tumor cells progress during growth, invasion, and metastasis that involves characteristic changes in mechanical interactions between the cells and their microenvironment, and parallels the genetic and epigenetic transformations traditionally associated with cancer (**Figure 1**).[16] This discovery has come in the context of a recent and very exciting explosion of work that illustrates the power of mechanical signals to control fundamental behaviors of a wide variety of cell types, including stem cells, in the context of normal development, tissue homeostasis, and biotechnology. Together, these efforts have given rise to the new field of cellular mechanobiology, whose goal is to understand how cells sense, process, and respond to mechanical inputs, and the role of these inputs and signaling systems in biology and disease.[17,18]

Viewed in retrospect from a clinical perspective, a close relationship between mechanical force and tumorigenesis seems almost obvious. Patients and physicians often initially detect superficial tumors by manual palpation, with apparent stiffness frequently yielding powerful predictive insight into prognosis.[19,20] Ultrasound imaging, which derives its contrast from gradients in tissue stiffness and density, has proven surprisingly effective at delineating tumor tissue, in some studies outperforming even magnetic resonance imaging.[21–25] The stereotypical dissemination pattern of many tumors hints at the importance of mechanical and other biophysical cues in guiding their spread; for example, malignant brain tumors often infiltrate the brain along white matter tracts, vascular beds, and other sharply defined mechanical and topological interfaces.[26]

This chapter reviews the role of cellular mechanobiology in the development, growth, and spread of tumors. While many of the concepts are generally applicable to a wide variety of biophysical inputs, the focus here is on mechanical interactions between tumor cells and the ECM. First an overview of cell-ECM mechanobiology is provided, including the contributions of adhesion receptors, focal adhesion complexes, mechanotransductive signaling pathways, and the cellular cytoskeleton. The role of mechanobiological signaling in tumor initiation, angiognesis, tissue invasion, and metastasis

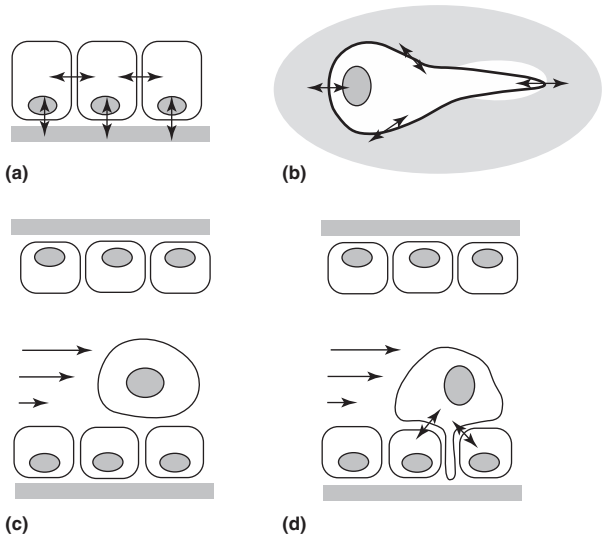


Figure 1 The force journey of a tumor cell. At all stages of tumor growth and spread, tumor cells and their progenitors are subject to mechanical forces in their environment. (a) Normal tissue homeostasis. Even in nominally normal static tissues, such as epithelia and endothelia, cells exert mechanical force on each other and the ECM that contribute to the mechanical stability of the tissue. This force balance is commonly disrupted in dysplastic tissues, even prior to the onset of frank tumor growth. (b) Invasion. As tumor cells invade the tissue parenchyma, they use mechanical force to rearrange ECM components and translocate in a directional fashion. (c) Circulation. If a tumor cell successfully escapes the primary tissue of presentation, it may enter the vasculature, where it is subject to shear and compressive forces. (d) Metastasis. In order for a tumor cell to leave the vasculature and establish itself in a distal site, it must undergo diapedesis through the endothelium, which requires expression of a new complement of adhesion receptors, alterations in endothelial cell-cell adhesion, and coordinated changes in the mechanics of both tumor and endothelial cells. Reproduced from Kumar, S.; Weaver, V. Mechanics, malignancy, and metastasis: The force journey of a tumor cell. *Cancer Metastasis Rev.* **2009**, *28*, 113–127, with kind permission of Springer Science and Business Media.

is then discussed. Next, a case study exemplifying these principles is considered: the mechanobiology of the malignant brain tumor glioblastoma multiforme (GBM). Finally, recent efforts to leverage cellular mechanobiological systems to develop novel anticancer strategies are reviewed, followed by a brief summary and discussion of future challenges in the field.

7.10.2 Cellular Mechanotransdution: Background and Overview

Before engaging in a detailed discussion of mechanobiological signaling in cancer specifically, a general overview of mechanotransductive signaling is given, with an emphasis on the molecular mechanisms through which cells sense and process mechanical signals encoded in the ECM. Although the bulk of the discussion will focus on cells that are either adhered to or surrounded by a static solid-state ECM, many of the molecular components also play central roles in mechanotransduction in cells subjected to dynamic force inputs or at solid-liquid interfaces, such as metastatic cells under shear.

7.10.2.1 Integrins: Structure, Function, and Regulation

Cells recognize specific molecules in the ECM through a variety of cell adhesion receptors, including selectins, immunoglobulin superfamily cell adhesion molecules (IgSF CAMs, e.g., NCAMs, VCAM-1, PECAM-1), and integrins.[27–31] Integrins are by far the most well studied members of this group, particularly with respect to their ability to convert ECM-imposed stresses and strains into activation of specific intracellular signaling pathways. Many lines of evidence support the concept that integrins provide a critical mechanical linkage between the ECM and the cellular cytoskeleton.[32–35] Integrins are heterodimeric transmembrane receptors composed of paired α and β subunits; the human genome encodes 18 α and eight β subunits, and the specific $\alpha\beta$ combination determines which ECM protein(s) the integrin will bind (e.g., fibronectin, collagen, laminin) (see also Chapter 7.2). Although combinatorial calculations might lead one to expect more than 100 possible $\alpha\beta$ dimers, only around 24 pairs are observed in nature. The biological activity of integrins depends on at least two molecular parameters, integrin conformation and integrin clustering, concepts that will be revisited in more detail below.[36]

The structural biology of integrins has been extensively studied and described in great detail elsewhere.[36–39] Briefly, both integrin subunits generally consist of very large extracellular domains ($\sim$800 amino acids), transmembrane domains ($\sim$20 amino acids), and short cytoplasmic domains (13–70 amino acids). Viewed at low resolution, the integrin heterodimer grossly resembles a large head (i.e., the extracellular-most portion of the heterodimer) poised atop two stalks or legs (the remainder of the two subunits). Many of the various domains of the α integrin subunits are named with direct reference to the leg analogy (e.g., the thigh domain, the genu (knee) domain, and two calf domains), and as the names would imply, the heterodimer is capable of bending about the genu between a 'bent' or 'closed' conformation and an 'extended' or 'open' conformation. Critically, these two conformational states bear very different biological activities, with the closed conformation representing an inactive, low-ECM affinity state and the open conformation representing an active, high-ECM affinity state. The specific submolecular mechanism through which heterodimer conformation controls ECM ligand binding affinity remains the subject of some controversy, and several competing models have been proposed to explain this relationship.[40–43]

Integrins may be activated by a variety of stimuli that include specific divalent cations and binding of both ECM proteins and intracellular ligands. Perhaps the most well understood intracellular activator of integrins is the focal adhesion protein talin. An NPxY motif within the head domain of talin directly binds to the β subunit of integrins in a highly specific manner, and overexpression of this domain is sufficient to activate $\alpha_{IIb}\beta_3$ integrin pairs.[44,45] It has also been determined that while the head domain of talin is sufficient to

bind and activate the β subunit, full-length talin, which contains additional β subunit binding sites, is needed to initiate formation of fully developed focal adhesion structures and provide full mechanical coupling to the cytoskeleton.[46] Recently, Ye and colleagues creatively combined high-resolution cryoelectron microscopy with lipid membrane reconstitution approaches to demonstrate that talin is sufficient to activate and extend $\alpha_{IIb}\beta_3$ integrins *in vitro*.[47] In addition to specific molecular binding events, mounting evidence suggests that mechanical inputs alone can trigger integrin activation in the absence of other external ligands; for example, shear has been demonstrated to activate integrins on the surface of endothelial cells,[48–51] and steered molecular dynamics simulations have suggested that application of tensile forces can accelerate integrin extension.[52] Based on these studies, integrin activation is often regarded as a positive-feedback system in which specific stimuli trigger integrin activation, which in turn renders these integrins more sensitive to further activation.

The ability of integrin engagement to transduce a downstream signal also depends strongly on lateral association of multiple integrin pairs into 'clusters', as evidenced by the findings that multivalent integrin antibodies can trigger adhesive signaling in the absence of ECM protein.[53] Indeed, simply enhancing heterodimer-heterodimer affinity by introducing asparagine residues into the transmembrane domain of β integrin subunits to create intermolecular hydrogen-bonded networks between the carboxamide side chains can significantly amplify integrin-dependent mechanotransductive signaling.[54,55] Conversely, integrin signaling can be interrupted when heterodimers are physically precluded from clustering. This principle was directly demonstrated by an elegant series of experiments by Spatz and co-workers, who used block-copolymer micelle-based lithography to pattern single-integrin binding sites (cyclic RGD peptides) on solid supports at interligand spacings ranging from 28 to 85 nm. At ligand spacings exceeding 73 nm, cells are no longer able to spread effectively or form well defined adhesive or cytoskeletal structures, because individual heterodimers cannot laterally associate or trigger cluster-dependent signaling when held apart at this distance.[56,57]

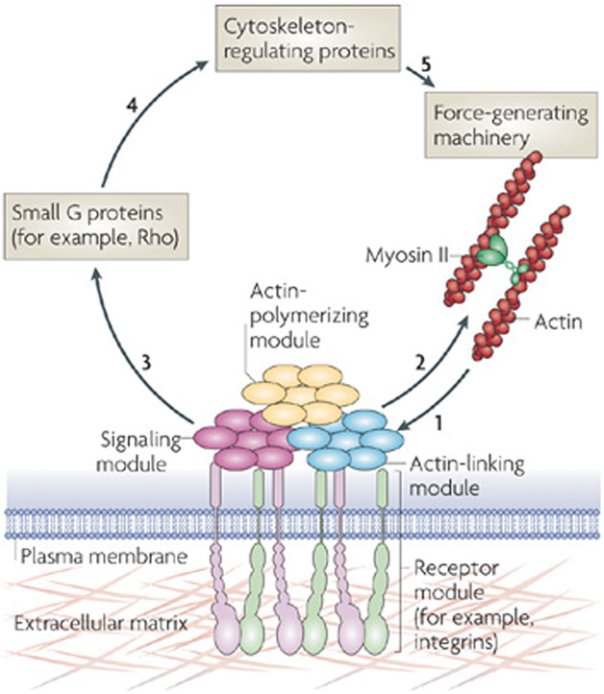


Figure 2 Mechanochemical feedback and cell-ECM adhesions. Cells engage ECM components through integrins and other cell adhesion receptors. Integrin clustering is accompanied by recruitment of a variety of intracellular proteins to the cell-ECM interface, some of which interact with and activate Rho family GTPases. Activation of these proteins facilitates assembly of actin cytoskeletal structure and force generation, which in turn provides a mechanism to exert tractional forces on the ECM through the adhesive plaque. Reproduced from Geiger, B.; Spatz, J. P.; Bershadsky, A. D. Environmental sensing through focal adhesions. *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 21–33, with permission. Copyright by Nature.

7.10.2.2 Cell-ECM Adhesions

Once integrins have formed a nascent cluster at the cell membrane, they begin to recruit a variety of factors to the intracellular face of the plasma membrane. Together, this accumulation of proteins creates discrete, micron-scale, plaque-like structures known as focal complexes (FCs), to which some >80 proteins have been identified to localize (**Figure 2**). The assembly and function of FCs have been the subject of several very comprehensive reviews,[58–61] but to summarize, FCs serve at least three key functions. First, they house proteins whose biochemical properties depend strongly on applied mechanical force, thus providing a set of 'mechanosensors' capable of communicating ECM-imposed forces and deformations into biochemical signals within the cell. Indeed, the development of FCs is strongly force dependent, with application of mechanical loads capable of converting FCs from an immature, punctate morphology ('focal contacts') to a larger and more elongated morphology as additional components are recruited ('focal adhesions'). It is worth noting that many in the field use the term 'focal adhesion' to describe all cell-matrix adhesion plaques, particularly where state of maturity is not critical to a given context. An incomplete list of proposed mechanisms for this mechanosensing includes force-dependent unfolding of specific molecules,[62–64] mechanical exposure of cryptic binding sites,[65–67] and force-dependent binding affinity.[68–72] Second, FCs physically couple integrins to the cellular cytoskeleton, as evidenced by the fact that many FC proteins can simultaneously bind both specific integrin subunits and cytoskeletal filaments. Consider, for example, the case of talin described earlier, which is one of the earliest molecules recruited to the FC and plays a central role in activating and clustering integrin heterodimers. Similarly, α-actinin, which is recruited to FCs relatively late in their development, binds both the intracellular domain of β integrin subunits and F-actin, thereby serving as a molecular 'glue' that couples the ECM to the cellular structural machinery.[73] Third, FCs serve as nodes that coordinate and locally concentrate molecular components of

signal transduction cascades, some of which may be directly relevant to growth and proliferation. For example, epidermal growth factor receptor (EGFR), a receptor tyrosine kinase that is widely mutated in certain tumors[74] and whose activation stimulates the rapid proliferation of many tumor cell types, strongly co-localizes to FCs, suggesting that these structures may serve to locally concentrate EGFR-mediated signaling. Similarly, focal adhesion kinase (FAK), extracellular signal-related kinase (ERK), and Src family kinases, all of which can powerfully stimulate proliferation, all co-localize to FCs.[75–81]

As described above, one of the most important roles played by FCs is to spatially concentrate the generation of mechanotransductive signals. The close relationship between force application, integrin engagement, and initiation of signal transduction was directly and elegantly demonstrated by Wang et al.,[82] who affixed RGD-coated colloidal beads to cells transfected with a FRET-based sensor of activation of the proto-oncoprotein Src. When they applied force to a bead using an optical tweezer, they observed 'waves' of Src activation propagating away from the bead in a temporally and spatially coordinated fashion. Remarkably, subsequent work showed that mechanical force is capable of activating Src more than two orders of magnitude more rapidly than treatment of the cell with growth factors.[83] Indeed, it appears that, under some circumstances, application of mechanical force can even 'bypass' Src and directly activate other signaling molecules typically regarded as downstream effectors of Src (e.g., Rac GTPase).[84] Together, these and other findings have raised the prospect that mechanical force and soluble growth factors may activate the same signaling networks through fundamentally distinct biochemical and biophysical mechanisms, although the details of these putative differences remain to be elucidated.

7.10.2.3 Rho Family GTPases

Of all the signal transduction pathways associated with FCs, perhaps the ones most closely associated with mecahanotransductive signaling and most intensely studied in that particular context involve the Rho (*R*as *ho*mology) family GTPases.[85–88] These small GTPases switch between an active, GTP-bound state and an inactive, GDP-bound state, with inactivation triggered by GTP hydrolysis and activation triggered by exchange of GDP for GTP. Both activation and inactivation are facilitated by the action of specific accessory proteins, including guanine nucleotide exchange factors (GEFs), which promote GDP-GTP exchange, and GTPase activating factors (GAPs), which promote GTP hydrolysis. There are three canonical GTPases, Rho, Rac, and Cdc42, all of which exist as multiple human isoforms and act through specific downstream effectors. While there is considerable crosstalk between the functions of these three molecules, each one is classically associated with regulation of a specific aspect of cytoskeletal assembly fundamental to dynamic regulation of cellular mechanics and motility. Specifically, Rho activation is associated with assembly of stress fibers and other contractile actin-based structures, Rac is associated with formation of lamellipodial protrusions, and Cdc42 is associated with formation of filopodial protrusions.[89] Importantly, the activation of these species is highly localized in time and space, as evidenced by the observation that when a specific GTPase is locally activated in a living cell, it induces formation of the associated structure specifically at the site of activation.[90,91] In addition, there are well described positive and negative cooperative relationships between these molecules; for example, Rho activation indirectly reduces Rac activation in many contexts, and vice versa.[90,92–94] Rho, Rac, and Cdc42 all localize to specific migratory and adhesive processes (e.g., FCs, lamellipodia), display strongly adhesion-dependent activation, and can contribute to mechanochemical feedback in complex ways.[95–97] For example, activation of Rho at FCs can in turn activate its downstream effectors mDia and Rho-associated kinase (ROCK), which promote actin polymerization and actomyosin contractility respectively.[98,99] The synergistic actions of these effectors enable the cell to nucleate focal adhesions from FCs, which cells then use to generate more contractile force on adhesions, which triggers greater integrin clustering, activation of FC-based mechanosensors, and adhesion maturation.[100] In addition, Rho GTPases and their accessory proteins are increasingly recognized to intersect with other signaling systems. For example, as will be discussed in greater detail later, p190RhoGAP physically interacts with the VEGF-dependent transcription factor GATA2 and impedes its entry into the nucleus, thereby providing a mechanistic explanation for the observation that angiogenesis depends strongly on the mechanical microenvironment.[101]

7.10.2.4 Nonmuscle Myosin II (NMMII)-Based Contractility

As described above, Rho, Rac, and Cdc42 all promote the assembly and function of specific subcellular structures that permit the cell to mechanically communicate with its environment. Although a detailed discussion of the mechanisms through which this occurs is overly complex to review here, an example of the activation by Rho of nonmuscle myosin II (NMMII), the primary actin-based motor in nonmuscle cells, is considered. As described earlier, Rho directly activates its downstream effector ROCK, which promotes contractility of stress fibers and other actomyosin bundles.[102] ROCK in turn both phosphorylates and inactivates myosin light chain (MLC) phosphatase[103,104] and directly phosphorylates MLC,[105] thereby both directly and indirectly promoting MLC phosphorylation. MLC phosphorylation, in turn, promotes association of NMMII filaments with actin filaments in actomyosin bundles and permits the former to slide against the latter, giving rise to bundle contraction. Several pharmacological tools have been developed or discovered to interrupt various components of this pathway, and consistent with the concept that adhesion growth can be reinforced by mechanical force, treatment of many cell types with these inhibitors disrupts stress fibers, focal adhesions, and mechanotransductive signaling. For example, C3 exoenzyme, Y27632, and blebbistatin inhibit Rho, ROCK, and NMMII respectively,[106–108] although the precise enzymological mechanisms through which this occurs remain the subject of active study.[109] NMMII may also be activated in a manner that is nominally Rho independent by MLC kinase (MLCK), which directly phosphorylates MLC via a rapid Ca^{2+}/calmodulin-dependent mechanism.[110,111] The relative roles and

importance of Rho/ROCK- and MLCK-based activation of NMMII within the cell remain unclear and the subject of considerable speculation, although one especially intriguing hypothesis proposes that Rho and ROCK primarily control stress fibers located in central regions of the cell, whereas MLCK primarily controls stress fibers located along the cell periphery.[104,112,113] It is physically intuitive that NMMII-based contractility regulates the ability of cells to exert forces against each other and the ECM in normal tissues, as well as the ability of tumor cells to change shape and squeeze through tissue microstructures during invasion and metastasis.[114] What perhaps may be more surprising, however, is that Rho/ROCK/NMMII-based signaling also appears to contribute to epigenetic regulation of gene expression. For example, inhibition of ROCK or MLCK in gastric carcinoma cells alters histone acetylation,[115] as does restriction of cell spreading in mammary epithelial cells.[116] The molecular mechanisms that define these connections and the extent to which they overlap with canonical epigenetic regulatory mechanisms remain important open questions.

7.10.3 Mechanobiology of Tumor Initiation

7.10.3.1 Force as a Regulator of Normal Development

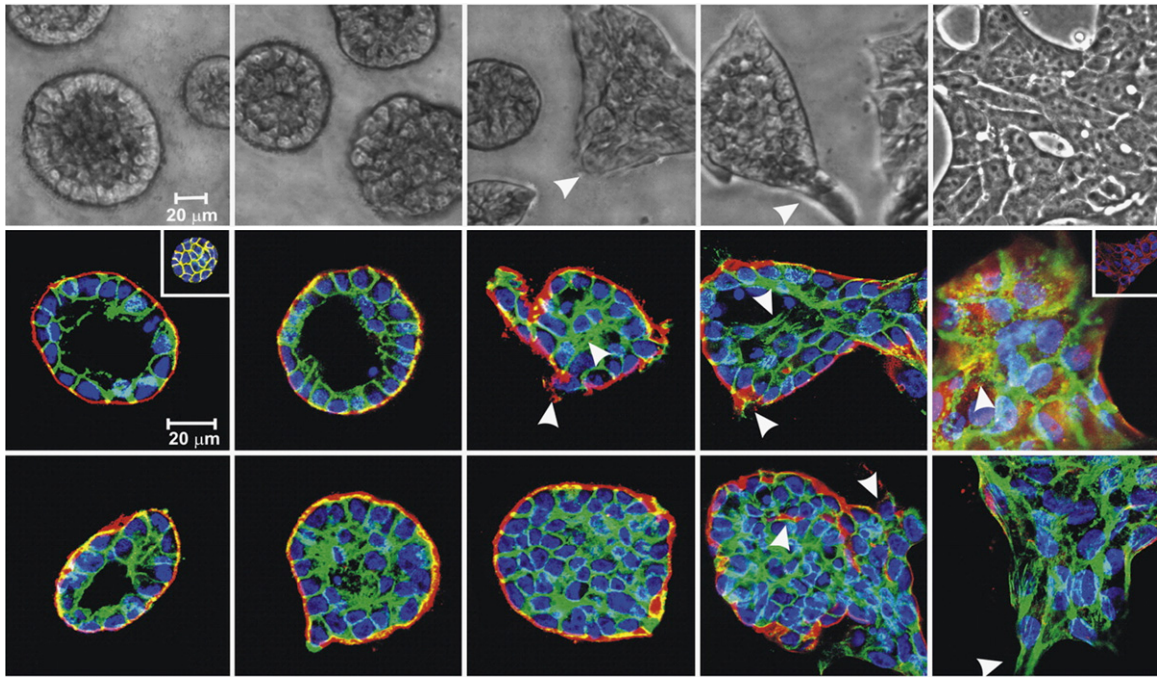
It is easy to understand the potential importance of mechanotransductive signaling to tissues that are classically regarded as load bearing or load generating, such as muscle, vasculature, and connective tissue. At first glance, this mode of signaling may seem considerably less relevant to tissues that are macroscopically static and not subjected to chronic external loads, such as epithelia. However, the reality is that cells in any multicellular tissue participate in a complex mechanical balance that involves the interchange of mechanical force against adjacent cells and the ECM. In many cases, these forces are channeled through integrin-dependent signaling systems described above;[117] in other cases, they may be channeled through analogous systems associated with cell-cell junctions.[118–120] *In vitro*, mechanical forces exchanged between cells in static two-dimensional culture have been demonstrated to strongly affect cell proliferation and may provide a mechanical basis for contact inhibition.[121] Superimposed on this cell-cell and cell-ECM force balance are mechanical loads nonspecifically applied to the entire tissue, including compressive, tensile, and shear loads.[18] In this sense, the field has come to conceptualize cells as participating in a 'dynamic mechanical reciprocity' with their surroundings, in which cells receive a variety of force-based signals from their mechanical microenvironment, integrate them by triggering mechanotransductive signaling pathways, and ultimately respond by exerting compensatory contractile forces against the environment to maintain mechanical equilibrium.[16] The notion that static tissues are 'prestressed' in this way has been extensively explored by Ingber and colleagues and lies at the heart of the tensegrity model of cell and tissue mechanics.[122] In recent years, there has been increasing appreciation for the importance of these cell-derived contractile forces for controlling organismal embryogenesis and organ development. For example, Farge, Beaurepaire and colleagues showed that applying force to the developing *Drosophila* embryo induces expression of the mechanosensitive gene *Twist* and subsequent ventralization and that developmental deficits in mutants with abnormal *Twist* expression can be rescued by application of compressive forces.[123] Subsequent work has validated the use of femtosecond laser ablation to quantify and directly manipulate cell-generated forces in the *Drosophila* embryo.[124,125] More recently, a high-resolution magnetic tweezer system was used to show that localizing the site of force application can provide an additional level of control.[126] These force interactions are also directly relevant to vertebrate embryogenesis and development;[127] for example, disruption of cellular contractility can interfere with branching morphogenesis in lung[128] and breast.[128]

7.10.3.2 Mechanoregulation of Stem Cells

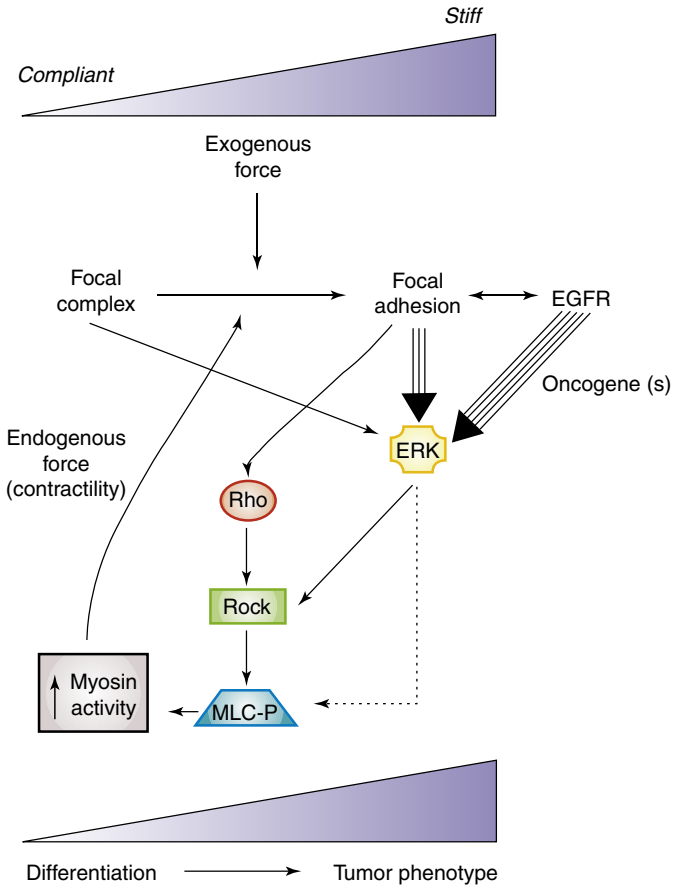
Both direct application of mechanical force and manipulation of the mechanical properties of culture scaffolds have been explored as a design tool for steering stem cell differentiation *in vitro*.[129] While the goal of these studies has primarily been to direct stem cell differentiation for tissue engineering and regenerative medicine applications, they are worth discussing here in light of the increasingly appreciated idea that some tumor cells may bear stem-like properties, which might imply that stimuli useful for the control of stem cell behavior may also prove useful for the control of tumor cells.[130–134] Human mesenchymal stem cells (hMSCs) cultured on mechanically compliant ECMs preferentially differentiate into neurons or adipocytes, whereas hMSCs cultured on rigid ECMs preferentially differentiate into osteocytes. The underlying model is that signals encoded in the mechanical rigidity of the ECM direct hMSCs to differentiate towards a tissue type whose stiffness matches that rigidity.[135,136] Analogous results have been observed when hMSCs are either allowed to spread fully and generate high contractile forces or confined to small adhesive areas,[137] an idea that has been extended to engineer stem cell differentiation in multicellular patterned tissues.[138] Such control mechanisms have even been observed in stem cell populations not traditionally regarded as 'load bearing'; for example, adult neural stem cells cultured on rigid ECMs and in the presence of mixed differentiation cues tend to yield astrocytic cultures, whereas those cultured on compliant ECMs under equivalent media conditions yield predominantly neuronal cultures.[139,140] Recently, Wang and colleagues extended these concepts to mouse embryonic stem cells (mESCs) by showing that sustained local application of mechanical force to mESCs via magnetic twisting cytometry could induce MLC activation, spreading, and – remarkably – loss of pluripotency markers.[141] This raises the exciting prospect that local mechanical manipulation of stem cells may be used to control self-renewal and differentiation as an alternative or complementary strategy to wholesale materials engineering of the mechanical microenvironment.

7.10.3.3 Mechanobiological Systems and Malignant Transformation

There is much evidence to suggest that altered mechanical interactions between cells and their environment can



(a)



(b)

contribute to the tissue dysplasia typically associated with the first pathologically evident stages of tumor development. Altered integrin subtype expression is an early molecular signature of many tumor types and may play a role in initial detachment from the ECM. For example, a meta-analysis of integrin expression for seven different tumor types revealed that elevated expression of $\alpha_5\beta_1$ heterodimer correlates with low levels of malignant transformation, whereas the opposite appears to be true of $\alpha_v\beta_3$ heterodimer.[142] In addition, overexpression of specific integrin subtypes in culture can reverse the malignant phenotype in cancer cells[143] and, as will be discussed later, integrins have emerged as valuable exploratory therapeutic targets in cancer. Finally, the seminal and highly influential work of Bissell and colleagues has clearly demonstrated that the malignant phenotype can be reversed through provision or interruption of specific ECM-based signals,[144–149] and more recent work has suggested that some of these signals may act through cellular mechanotransductive signaling systems.[116,150]

In addition to cell adhesion receptors, there is much evidence to support alterations in FC-based proteins in malignant transformation. Later, one example will be considered in detail in the context of malignant brain tumors: α-actinin. Another relevant and well characterized example is FAK, which is widely overexpressed and activated in tumor cells.[151–154] It has been hypothesized that the ability of many tumor cells to express FAK at high levels in the absence of well defined adhesion plaques underlies their ability to undergo anchorage-independent growth.[155] FAK also appears to play a central role in early ECM remodeling events, in part because suppression of FAK in ovarian cancer cells reduces expression of specific matrix metalloproteases.[156] For all of these reasons, FAK has emerged as an important therapeutic target in cancer. While it is clear that FAK figures centrally in all biomechanical steps in tumor transformation, detachment, invasion, and distal metastasis, understanding of the molecular mechanisms through which this occurs remains incomplete.

Gross alterations in both the composition and architecture of the cellular cytoskeleton are strongly associated with tumor transformation. In breast, both cellular morphology and intermediate filament expression are commonly used as measures of epithelial de-differentiation, with a central hallmark of the epithelial-to-mesenchymal transition (EMT) in mammary tumors being loss of a keratin-based cytoskeleton and gain of a vimentin-based cytoskeleton.[157–159] Moreover, Ras-transformed fibroblasts are capable of spreading, proliferating, and generating high tractional forces on compliant (soft) substrates that do not permit growth of normal cells,[160] a principle that underlies the predictive utility of the soft agar assay. Given the close connection between the cytoskeleton and tumor transformation, it is perhaps not surprising that the Rho family GTPases have also been implicated in early stages of tumor transformation. Inhibition of some Rho GTPases can prevent Ras-mediated transformation of fibroblasts, and constitutive activation of Rho GTPases can mimic certain features of oncogenic transformation.[161–164] The mechanisms through which aberrant Rho GTPase signaling might alter cell cycle progression have been well described and reviewed extensively elsewhere.[87] As an example, Rho-dependent ROCK activation can enhance expression of cyclin D1, which facilitates passage through the G1 phase of the cell cycle.[165] In addition, constitutive RhoA activation can overcome blocks on G1/S progression imposed by restricting cell spreading by stimulating expression of Skp2.[166]

Given the tight coupling between cytoskeletal assembly, mechanotransductive signaling, and cell cycle progression, one would expect that manipulation of the mechanical microenvironment might be capable of altering cell proliferation thorough activation of mechanobiological signaling events. These connections were recently and thoroughly investigated by Klein and colleagues, who cultured breast epithelial cells, osteoblasts, fibroblasts, and vascular smooth muscle cells on variable stiffness ECMs and asked how microenvironmental stiffness might regulate cell cycle progression. They found that use of ECMs whose elasticities resemble physiologic tissue inhibited cell cycle progression through a variety of mechanisms, including suppressing the FAK–Rac–cyclin D1 pathway.[167] A regulatory role of ECM stiffness in controlling tumor transformation has also been validated *in vivo*, including a landmark study in which Weaver and colleagues directly tested the hypothesis that mechanical signals from the ECM can independently facilitate mammary epithelial transformation (**Figure 3**). By culturing normal (nontumorigenic) mammary epithelial cells on ECM substrates spanning a range of stiffnesses, they showed that increasing ECM stiffness alters integrin subtype expression, enhances focal adhesion assembly, disrupts acinar architecture, and promotes invasion into three-dimensional (3-D) ECMs. The mechanistic origin of this effect is increased clustering of integrins, which in turn amplifies activation of Rho GTPase and growth factor-based signaling, as discussed earlier.[55,167] In a followup study, these investigators directly demonstrated the relevance of these principles to tumor formation and growth *in vivo* by showing that breast tumorigenesis is accompanied by significant increases in collagen crosslinking, tissue stiffness, and formation of focal adhesions,

Figure 3 ECM rigidity and the tumor phenotype. (a) Effect of ECM rigidity on mammary epithelial assembly. Phase contrast (top row) and immunofluoresence (middle and bottom rows) images of mammary epithelial cells (MECs) cultured on hydrogel ECMs with elasticities ranging from 150 to 1050 Pa, and on glass (>5000 Pa). Immunoflurescence images depict nuclear DNA (blue), β-catenin (green, middle row), β_4 integrin (red, middle row), F-actin (green, bottom row), and laminin basement membrane (red, bottom row). Cells cultured on ECMs with comparable elasticities to normal mammary tissue (~150 Pa) form patent acinar structures with well defined cell-cell junctions and cell-ECM adhesions. As ECM rigidity is increased, cell-cell junctions and integrin distributions change, lumina lose patency, and acinar architecture completely breaks down. (b) Model of relationship between mechanotransduction and growth control. ECM rigidity cues influence assembly of integrins and cell-ECM adhesions, which can in turn activate both myosin-dependent cell contractility and ERK-dependent growth programs. This represents one potential mechanism of crosstalk between mechanobiological signaling and growth factor-mediated signaling. Figures reproduced from Paszek, M. J.; Zahir, N.; Johnson, K. R.; Lakins, J. N.; Rozenberg, G. I.; Gefen, A.; Reinhart-King, C. A.; Margulies, S. S.; Dembo, M.; Boettiger, D.; Hammer, D. A.; Weaver, V. M. Tensional homeostasis and the malignant phenotype. *Cancer Cell* **2005**, *8*, 241–254.

and that these changes could be detected in the stroma of premalignant tissue, prior to the development of frank tumor invasion. Suppression of collagen crosslinking and stiffening through the inhibition of lysyl oxidase (LOX) was capable of reducing malignant transformation and tumor incidence in mice, raising the extremely exciting prospect that small-molecule LOX inhibitors may prove valuable in tumor therapy.[168]

Keely and co-workers have also shown that similar effects may be triggered by culturing transformed mammary epithelial cells on ECM gels affixed to a rigid substrate that are capable of sustaining large tractional forces (versus free-floating gels).[169] They later extended these studies to demonstrate that increasing ECM density *in vivo* can promote an invasive phenotype through the activation of a signaling network that includes FAK, ERK, and Rho.[170] In an independent but closely related effort, they used Cre/LoxP technology to ablate FAK expression in the mammary epithelium of mice and demonstrated that FAK is critical for tumor invasion.[171] Further collaborative efforts to gain insight into these findings from the Weaver, Keely, and Calderwood laboratories have led to the finding that the adhesion protein filamin A is needed for mammary epithelial cells to contract and remodel compliant collagen gels and that concentration of filamin A by reducing its degradation can induce remodeling of even highly dense collagen gels.[172] Together, these data paint an emerging picture in which ECM rigidity governs epithelial tissue assembly and transformation by mobilizing specific mechanosensors (e.g., filamin A) and activating specific mechanosensory signaling networks (e.g., RhoA) that may in turn trigger mitogenic signaling and lead to loss of tissue architecture and uncontrolled cell proliferation.

7.10.4 Mechanobiology of Angiogenesis

7.10.4.1 Overview of Angiogenesis

The viability of both normal and tumor cells depends on a constant supply of oxygen and soluble nutrients, as well as a mechanism to remove metabolic waste products. The simplest physical mechanism to accomplish both of these tasks is diffusion and, for this reason, mammalian cells are typically located within 100–200 μm of blood vessels, a distance within the approximate diffusion limit for oxygen in tissue.[173] As a solid tumor mass expands beyond a critical size limit of 1–2 mm,[174] it begins to outstrip the capacity of diffusion to deliver nutrients throughout the tumor and stops growing absent new perfusion mechanisms. Some four decades ago, Judah Folkman famously proposed that tumors circumvent this limitation through the process of tumor angiogenesis, in which the tumor coaxes the host vasculature to extend new capillary networks to supply the growing tumor.[175] Two key implications of this landmark proposition are that tumor growth and metastasis should be strongly angiogenesis dependent, and that inhibition of the angiogenic process may offer a therapeutic handle with which to treat solid tumors. Although highly controversial when first proposed, Folkman's hypothesis is now widely accepted, and both of these implications have been demonstrated in many experimental and clinical settings.[176–181] Indeed, the field has come to describe tumor cells as activating an 'angiogenic switch' when this process is needed to serve the metabolic needs of the tumor. The underlying assumption is that both tumor and host cells are capable of secreting soluble factors that both inhibit and stimulate angiogenesis; when the switch turns on, the proangiogenic cues overwhelm the antiangiogenic cues, and tumor cells compel endogenous capillary networks to direct microcirculation towards the tumor.

Thus, over the past four decades, a central challenge in this field has been to identify both conditions that turn the angiogenic switch on and off, and secreted factors that promote angiogenesis. In pursuit of the latter question, these efforts have produced an extensive list of soluble growth factors that act to promote angiogenesis, the most prominent and widely studied of which are members of the vascular-endothelial growth factor (VEGF) and angiopoietin families.[182,183] These findings have served as the basis for novel antiangiogenic pharmacotherapies now in clinical use; for example, antibodies against VEGF (e.g., bevacizumab) are FDA approved as a component of combination therapy in colorectal cancer and are being actively explored in a wide variety of other cancers.[181,184] While it is clear that soluble pro- and antiangiogenic signals play key roles in regulating capillary sprouting, some aspects of *in vivo* angiogenesis have been difficult to reconcile with a model based solely on the actions of these biochemical cues. In particular, angiogenic events are frequently highly localized in space; in fact, it was observed over 70 years ago – long before the importance of capillary angiogenesis to tumor growth was widely appreciated or understood – that nearby capillaries in the same tissue can simultaneously undergo proliferation, death, and differentiation.[185] This, in turn, fostered the hypothesis that microscale, solid-state elements in the tumor microenvironment might regulate angiogenesis independently or in concert with soluble signals.

7.10.4.2 Mechanobiological Regulation of Angiogenesis by the ECM

The influential work of Ingber and colleagues has demonstrated that mechanical signals from the ECM likely represent one important such cue.[121,186–190] Building on previous histological observations that angiogenic capillary sprouting is often accompanied by local thinning of the adjacent basement membrane,[191] Ingber hypothesized that basement membranes become more compliant as they thin, and that capillary endothelial cells sense this change as an alteration in the force balance between the cell and ECM. Evidence that alterations in ECM density are sufficient to trigger such wholesale phenotypic shifts comes from observations that when capillary endothelial cells are cultured on surfaces coated with low, medium, and high densities of fibronectin (but under identical media conditions), they spread to increasingly large projected areas and preferentially undergo apoptosis, differentiation, and proliferation, respectively.[192] However, this result leaves unclear whether the differences in cell behavior are due to alterations in cell shape or altered degrees of integrin occupancy. To distinguish between these possibilities and isolate cell shape as an 'independent variable', Ingber, Whitesides, and colleagues used

microcontact printing to culture single endothelial cells on small, medium, or large fibronectin-coated islands; in these studies, cells cultured on 50-μm-diameter islands and allowed to spread preferentially underwent proliferation, whereas cells restricted to 20-μm-diameter islands (and thus prevented from spreading) preferentially underwent apoptosis despite saturating concentrations of growth factor. Cells could even be induced to proliferate when presented with 5-μm-diameter fibronectin-coated islands, as long as these very small islands were spaced closely enough to one another to enable the cell to bridge across many islands. Thus, cell shape rather than total integrin-ECM occupancy appears to be the key governing parameter in this system.[193] Later studies revealed that cellular prestress and traction force generation correlated closely with projected cell area,[194] further strengthening potential connections between cell shape, cell-ECM mechanics, and the angiogenic switch.

These ideas have now been validated in 3-D ECMs by several investigators. For example, Gooch and colleagues cultured a series of human endothelial cell culture lines in either free-floating or surface-attached collagen I gels of various densities and found that endothelial cells in floating gels formed multicellular capillary structures with small lumina, whereas capillaries formed by cells in attached gels were thinner, had larger lumina, and developed prominent stress fibers.[195] Kuzuya and colleagues demonstrated that differences in capillary formation could be induced by enzymatically crosslinking collagen gels of fixed density in a manner that is largely independent of soluble angiogenic cues.[196] In a recent and very revealing set of studies, Putnam and colleagues have also explored connections between ECM stiffness in 3-D and capillary angiogenesis by culturing endothelial cells on microcarrier beads embedded in fibrin matrices of various densities and in the presence of fibroblasts secreting proangiogenic factors.[197,198] These studies revealed that increasing fibrin density, which concomitantly stiffens the matrix and reduces diffusivity, significantly retards the growth of capillary networks. Moreover, this effect relies upon the ability of the endothelial cells to generate traction forces, as inhibition of elements of the NMMII signaling pathway alters both gel contraction and capillary network formation. Similar effects may also be observed *in vivo*; treatment of lung bud explants in culture with ROCK inhibitors markedly disrupts capillary network formation, whereas pharmacological activation of Rho with cytotoxic necrotizing factor-1 (CNF-1) increases capillary elongation and extension.[199]

In addition to promoting angiogenic growth through local stimulation of capillary branching, mechanical signals from the ECM can also directly drive higher-order neovascularization during inflammation and tumorigenesis. In a chick chorioallantoic membrane model of wound healing, fibrin/collagen-based granulation tissue is tightly remodeled and contracted by infiltrating fibrobasts, which then leads to translocation of nearby vessels and their preexisting capillary networks into the tissue. The degree and dynamics of these processes closely follow gel contraction, suggesting that the fibroblasts may be creating mechanical gradients within the granulation tissue to direct vessel migration. Critically, however, neovascularization is abrogated in this model when the implanted collagen gel is rendered contraction resistant through incorporation of inert glass fibers that interfere with collagen fiber bundling, or by chemically crosslinking the fibers themselves. These results suggest that spatially and temporally directed mechanical force might be used to target and accelerate vascularization during wound healing, either by applying external hardware (analogous to vacuum-assisted wound closure devices) or by pharmacologically targeting mechanobiological signaling pathways in fibroblasts critical to tension generation.[200]

7.10.4.3 Crosstalk Between VEGF Signaling and Mechanotransductive Signaling

Together, these and many other experimental observations paint a convincing picture that capillary endothelial cells sense local variations in ECM density as mechanical signals, and that these signals are processed through mechanobiolgical signaling networks to ultimately yield cell behaviors that drive angiogenesis. An open question in this field is whether and to what extent these proangiogenic mechanobiological signaling events communicate with classically described proangiogenic pathways stimulated by VEGF, angiopoietins, and other soluble signals. Mammoto and colleagues recently made major strides towards addressing this question by elucidating specific interactions between adhesion-dependent signaling pathways and activation of transcription factors traditionally associated with VEGF-mediated signaling (**Figure 4**).[100] Using culture models, they showed that p190RhoGAP, an inhibitor of Rho GTPase activity, binds to and cytoplasmically sequesters GATA2, a transcription factor that strongly promotes expression of the *VEGFR2* gene, which encodes VEGFR2 and sensitizes endothelial cells to VEGF-based angiogenesis. GATA2 competes for the *VEGF2* promoter with a second transcription factor, TFII-I, which is normally resident at saturating concentrations in the nucleus, and which strongly suppresses expression of VEGFR2. In other words, p190RhoGAP 'tips the balance' between the antagonistic effects of TFII-I and GATA2, favoring angiogenesis when the latter is dominant. To test the notion that this balance might be affected by ECM-based mechanical cues, these authors cultured endothelial cells on ECMs of varying mechanical compliance and found that increasing ECM stiffness strongly stimulated nuclear translocation of GATA2 and VEGFR2 expression. Under these conditions, Rho activation and p190RhoGAP activity would be expected to be high and low respectively, consistent with the notion that biomechanical activation of Rho acts through GATA2 to promote VEGF-based angiogenesis. In addition, the authors creatively tested this hypothesis *in vivo* using a retinal angiogenesis assay and a Matrigel implant assay, in which they controlled implant stiffness by crosslinking the Matrigel with glutaraldehyde. Together, these results support a model in which increases in matrix stiffness increase Rho activation and reduced p190RhoGAP activity, which in turn frees GATA2 to stimulate VEGFR2 expression. This study represents perhaps the clearest demonstration to date that ECM-derived mechanical cues and soluble angiogenic factors access common pathways to trigger angiogenesis, and that these principles hold *in vivo* as well as in culture.

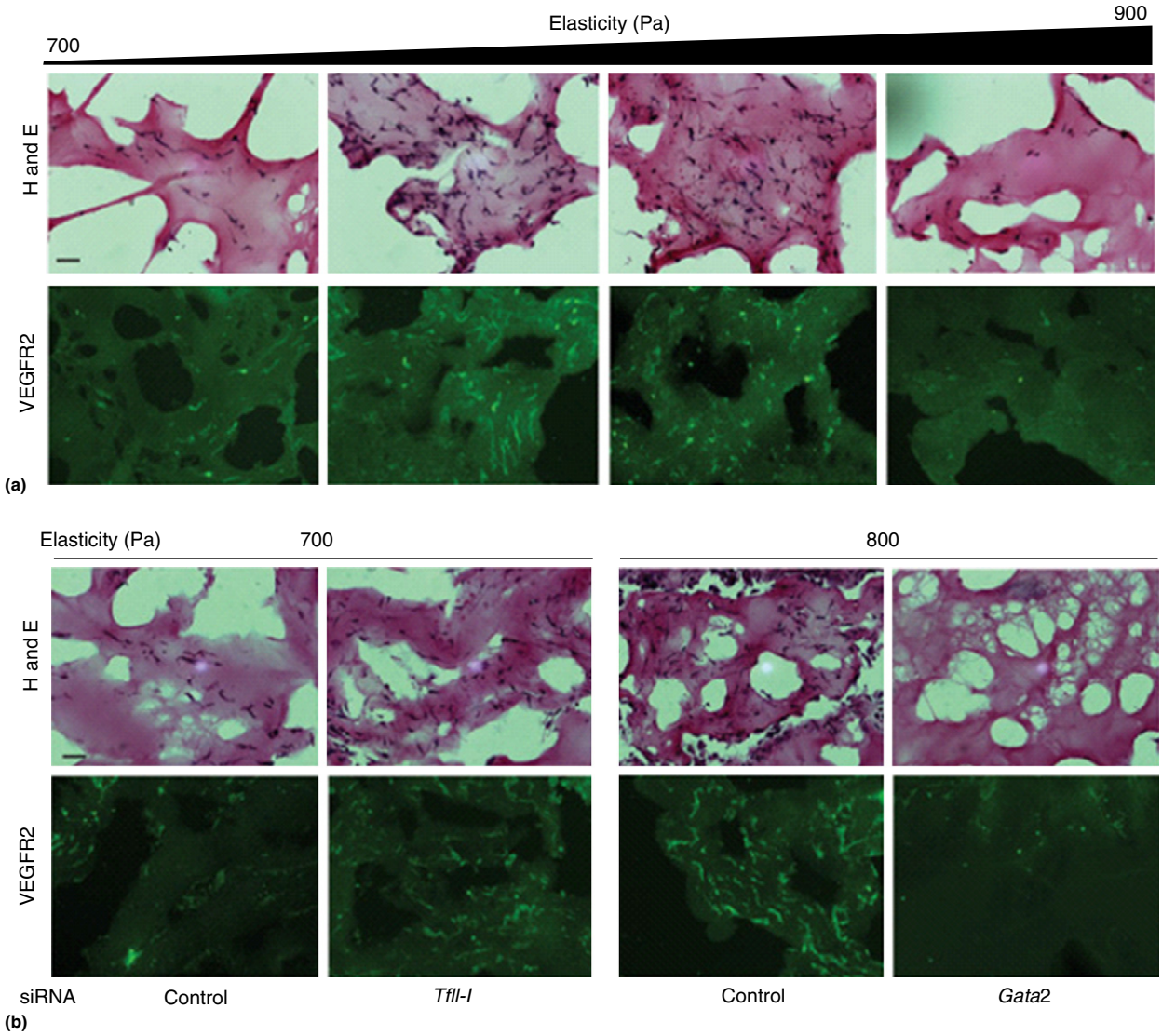


Figure 4 Mechanobiological regulation of angiogenesis. Subcutaneous implantation of Matrigel plugs into mice triggers capillary infiltration and capillary formation, which enables one to probe the relationship between plug material properties and angiogenesis. (a) Effect of ECM rigidity. As the rigidity of the Matrigel implant is increased through introduction of transglutaminase-mediated crosslinks, one observes increases in endothelial cell infiltration and vessel formation (hematoxylin and eosin stains, top row) and VEGFR2 expression (green immunofluorescence, bottom row). (b) Antagonistic regulation of VEGFR2 by TFII-I and GATA2. At tissue-like ECM rigidities, siRNA-mediated suppression of TFII-I enhances cell infiltration, capillary formation, and VEGFR2 expression relative to control (left two columns), whereas suppression of GATA2 reduces all of these values (right two columns). Stains are as in (a), and scale bars are 25 μm. Figure reproduced from Mammoto, A.; Connor, K. M.; Mammoto, T.; Yung, C. W.; Huh, D.; Aderman, C. M.; Mostoslavsky, G.; Smith, L. E. H.; Ingber, D. E. A mechanosensitive transcriptional mechanism that controls angiogenesis. *Nature* **2009**, *457*, 1103–1108 with permission. Copyright by Nature.

7.10.5 Mechanobiology of Tumor Invasion and Metastasis

Following their emergence at a primary site of presentation, many tumors begin to invade the surrounding parenchyma en route to infiltrating the primary tissue and potentially metastasizing to distal tissues. In order for this to occur, tumor cells must detach from neighboring cells and the ECM in the primary tumor and translocate into normal tissue. As described earlier, tumor transformation is accompanied by greatly altered expression of cell-cell and cell-ECM adhesion proteins, which likely contribute to this process. Moreover, the invasive behavior of specific tumor cells *in vivo* correlates strongly with expression of ECM proteins, actin binding proteins, intermediate filaments, and other components of the mechanobiological machinery.[201] In parallel, many tumor cells both proteolytically degrade existing ECM and synthesize new ECM proteins that may favor proliferation and/or migration. Thus, invasion and metastasis reflect the reciprocal effects of cell-mediated remodeling of ECM and dynamic control of cell morphology and mechanics to traverse the ECM.

7.10.5.1 Matrix Metalloproteases

Matrix metalloproteases (MMPs) represent perhaps the best characterized class of secreted proteolytic enzymes.[202] MMPs are classified by domain structure into eight categories, five of which are secreted and three of which are membrane associated (the membrane type or MT-MMPs). Collectively, these endopeptidases are capable of proteolyzing virtually any ECM protein, which serves at least two purposes. First, MMP-mediated digestion of ECM proteins permits clearance of pericellular material, thereby reducing steric barriers to motility. Second, ECM protein cleavage can yield proteolytic fragments with tumor-promoting bioactivity[203] or liberate matrix-bound growth factors.[204] The latter is also particularly true of the closely related ADAM (*a d*isintegrin *a*nd *m*etalloprotease) family of transmembrane proteases.[205] MMP expression and activity have long been known to be strongly influenced by applied mechanical force in both the musculoskeletal[206–208] and cardiovascular[209–211] systems, and it is becoming increasingly appreciated that force-dependent MMP expression may play a role in tumor growth and angiogenesis as well. Specific MMPs can induce EMT in breast epithelial cells through a Rac-based mechanism, and limiting cell spreading can block this effect, suggesting deep connections between cell shape, MMP activity, and tumor transformation.[212,213]

7.10.5.2 ECM Proteolysis and Tumor Cell Migration

Much exciting work over the past decade has examined the relationship between ECM proteolysis, actomyosin contractility, and cell migration, which have served to further strengthen connections between MMP function and mechanobiology.[214–220] As tumor cells proteolytically degrade the matrix, they are often observed to exhibit a mesenchymal mode of motility in which secreted MMPs clear ECM material at the leading edge of the cell and cells form adhesions along their length with ECM fibers and pull themselves forward in a persistent, directional fashion using contractile actomyosin bundles (**Figure 5(a)**). In some cases, proteolysis may also serve to selectively cleave fibers that present direct steric barriers (e.g., those oriented perpendicularly to the direction of motility), which the cell then bundles along with other ECM fibers to create contact guidance tracks.[220] In the absence of proteolytic capabilities (or when proteolysis is pharmacologically inhibited), cells transition into an amoeboid mode of motility in which they use ROCK-based contractility to extrude themselves through preexisting ECM pores. Integrin distributions are diffusely distributed throughout the cell, and polarity is typically significantly reduced (**Figure 5(b)**).[218] Subsequent studies from the Weiss[216] and Wirtz[221] laboratories have suggested that the propensity of cells to undergo amoeboid motility depends on the degree of crosslinking and effective pore size in the collagen matrix, which is in turn a strong function of how the collagen is prepared. For example, in acid-extracted collagen I, which retains extensive interfiber crosslinks, inhibition or depletion of MT1-MMP does not trigger amoeboid motility and dramatically reduces cell motility. Cells need not detach from one another to invade; in a third mode of motility known as collective motility, cells invade as multicellular sheets, with intact cell-cell adhesions and polarized proteolysis at the invasive front driven by chemotactic cues (**Figure 5(c)**). Recently, Friedl and Wolf captured the cooperative roles of force generation and proteolysis in mesenchymal motility into a five-step model consisting of: (1) pseudopodial elongation through matrix pores; (2) matrix adhesion and force generation; (3) focal proteolysis at the leading edge and center of the cell; (4) actomyosin contraction to pull the cell forward, in the process remodeling proteolyzed matrix fibers to be parallel to the direction of motility; and (5) retraction of the rear and translocation. If the ability to progress through this cycle is lost, cells may undergo MAT and convert to proteolysis-independent amoeboid motility.[215] It is important to note that details of models such as these are under active debate within the field, as exemplified by the discussion of collagen preparation and crosslinking above and the recent observation that pseudopodial protrusions may track fibers rather than the spaces between fibers.[222]

7.10.5.3 Invadopodia

The structures that cells use to spatially direct proteolysis, known as invadopodia (called podosomes in nontransformed cells), are themselves microscale mechanosensors. These structures are filled with parallel arrays of F-actin along with a variety of actin binding proteins that permit actin-based assembly and protrusion (e.g., Arp 2/3 and N-WASP).[223,224] They also include cortical rings of NMMII, which enable the podosome to generate tractional forces.[224] Moreover, increasing ECM stiffness, which concomitantly increases traction force, also increases the number and activity of invadopodia. Similar effects may be achieved by overexpression of molecular mechanosensors typically associated with adhesion plaques, including FAK and Cas.[226] While the analogy to FCs and other adhesive structures seems clear, the two structures bear important differences; for example, the assembly of FCs typically begins with integrin engagement whereas the assembly of podosomes and invadopodia does not, and the mechanical maturation of FCs appears to require greater forces applied over longer times than typically seen in podosomes and invadopodia.[227]

7.10.5.4 Traversing Endothelial Barriers During Metastasis

If a tumor cell can successfully invade through its primary tissue and enter the vasculature, it may metastasize to distal tissues by crossing the endothelial barrier in those tissues. Diapedesis, the process through which cells cross endothelial cell-cell junctions, requires profound local and dynamic changes in the mechanics of both tumor and endothelial cells driven by cytoskeletal remodeling.[228,229] In parallel with these mechanical changes, tumor cells undergoing diapedesis also express an altered complement of adhesion molecules, which facilitates a return to cell-ECM adhesion as the cell enters the parenchyma of the new tissue.[230,231] Two recent studies have illustrated the potential importance of mechanobiological signaling in metastasis. First, Mierke et al. recently screened 51 tumor lines for their ability to transmigrate endothelial

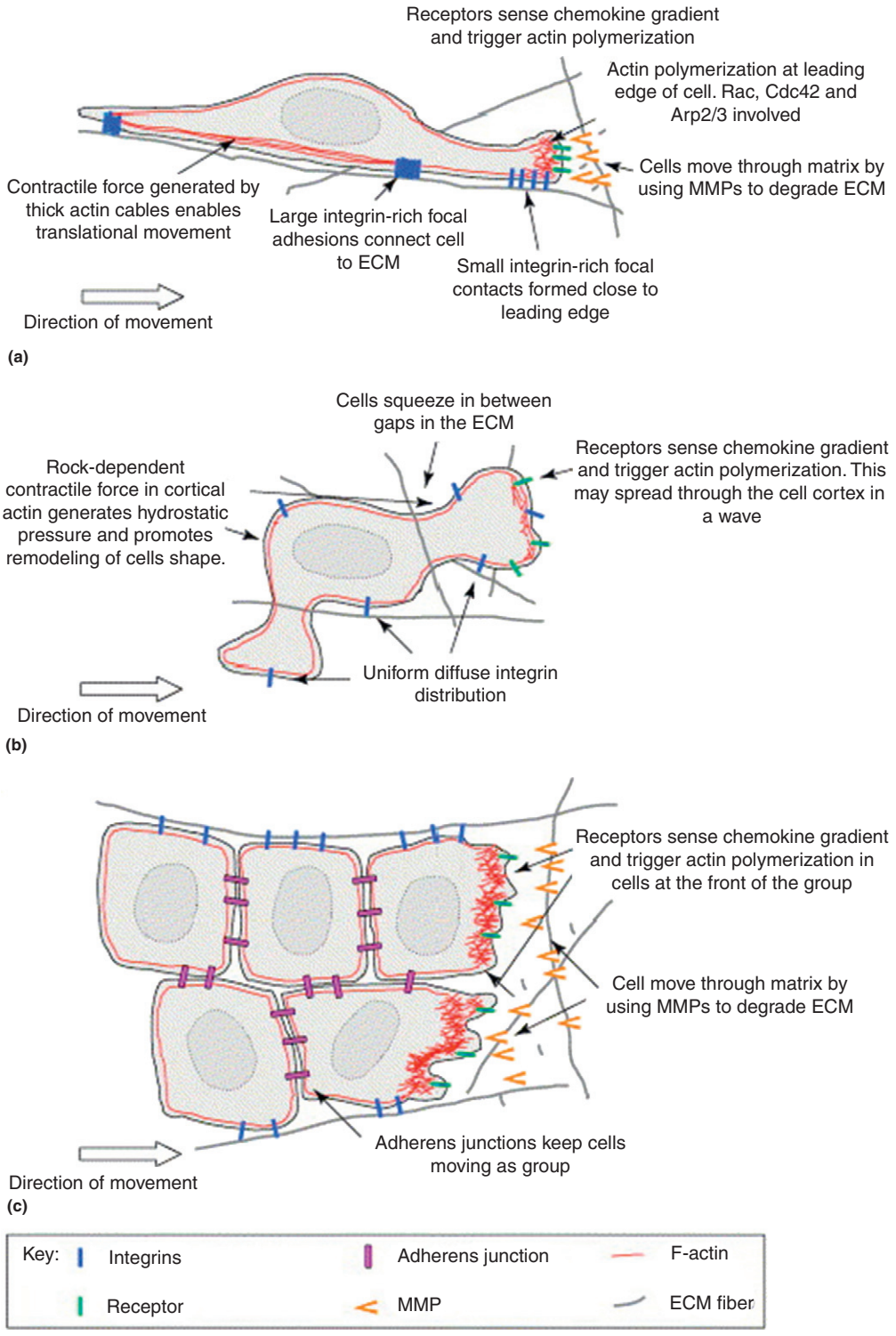


Figure 5 Modes of motility during tumor invasion. (a) Mesenchymal motility. Mesenchymal motility is characterized by well defined cell polarization, coordinated protelysis and protrusion at the leading edge of the cell, and contraction driven by thick actomyosin cables. (b) Amoeboid motility. In amoeboid motility, cells squeeze through preexisting pores in the ECM using cortical actin contractility to drive shape changes. Cell polarization is relatively poorly defined, and integrins are diffusely distributed rather than organized into adhesion plaques. (c) Collective motility. In collective motility, cells migrate as multicellular structures with intact cell-cell adhesions. Proteolysis is focused at the leading edge of the frontmost group of cells, often in response to a chemotactic gradient. Figure components reproduced from Sahai, E. Mechanisms of cancer cell invasion. *Curr. Opin. Genet. Dev.* **2005**, *15*, 87–96.

barriers in culture and showed that the propensity of a cell line to invade correlated strongly with expression of the chemokine receptor CXCR2. Magnetic tweezer measurements of cellular mechanics revealed that CXCR2 expression increases cytoskeletal remodeling dynamics and contractility, implying that CXCR2-mediated signaling promotes tumor cell transmigration by increasing shape plasticity and enhancing force generation.[232] Second, Kostic et al. recently explored the possibility that ECM stiffness might play a role in governing the target tissue to which breast tumors preferentially metastasize (i.e., tissue tropism).[233] After culturing previously established single-cell populations (SCPs)[234,235] derived from MDA-MB-231 breast cancer cells with known tissue tropism in collagen-based ECMs of varying stiffness, they found that the stiffness of optimum proliferation and migration correlated strongly with the stiffness of the preferred target tissue. For example, SCPs tropic towards bone grew optimally on highly rigid ECMs, an effect that could be blunted by siRNA suppression of the adhesive mechanosensor Fyn kinase. Thus, mechanobiological signaling may play a significant role both in initial access to metastatic tissue and in successful establishment of a distal focus of tumor.

7.10.6 Case Study: Mechanobiology of Glioblastoma Multiforme

7.10.6.1 Glioblastoma Multiforme: Background and Overview

Having discussed some of the general principles through which mechanical inputs can regulate various stages of tumor transformation, growth, invasion, and metastasis, a specific case study will now be considered in detail: the growth and invasion of malignant gliomas in the brain. Gliomas are tumors that arise from glial cells or their progenitors, with increasing evidence suggesting that the cells of origin may be a population of 'brain tumor stem cells', which are capable of self-renewing or differentiating into mature neural lineages and could theoretically arise either from resident neural stem cells or from de-differentiated neurons or astrocytes.[129–133] As with all solid tumors, gliomas are assigned a 'grade' of I–IV based on their histopathological properties (e.g., nuclear size and morphology, frequency of mitotic figures, endothelial cell proliferation, and necrosis), with higher grades correlating with greater malignant potential. Grade IV gliomas are collectively referred to as glioblastoma multiforme (GBM) and are characterized by extensive molecular and cellular heterogeneity within and across tumors.[74] GBM is the most common primary intracranial tumor, with an incidence of nearly 13 000 new cases annually in the United States, exceeding all other glioma subtypes combined. GBM follows an extremely aggressive and dismal clinical course, with median survival times of 12–15 months, even with multiple surgical resections, medical therapy, and radiation therapy.[236–240]

The extreme refractoriness of GBM to therapy has been attributed to a large number of causative factors, one of which is the ability of individual tumor cells to invade the parenchyma of the normal brain, thereby rendering complete surgical resection extremely unlikely, if not impossible.[241] As described earlier, invasion is a multistep process that includes detachment from the primary tumor site, adhesion to the endogenous ECM, ECM degradation and remodeling, and motility. For this reason, considerable attention has been paid to understanding how GBM tumor cells interact with the ECM of normal brain and then to remodel it to create a tumor-like ECM microenvironment, and these efforts have produced a large catalog of ECM components and adhesion receptors that are present in one context and absent in another.[242] In some cases, these efforts have led to the identification of novel therapeutic targets; for example, tenascin-C is an ECM glycoprotein that is significantly upregulated in GBM, and the radioiodinated antitenascin antibody 81C6 (Neuradiab) has been evaluated in clinical trials as a means of specifically delivering lethal radiation to tumor cells and is currently undergoing Phase III clinical trials.[239,243–246]

7.10.6.2 Potential Roles of Mechanobiological Signaling in GBM

While the bulk of effort in this area has been focused on identifying molecules enriched in the GBM tumor microenvironment with the goal of targeting them therapeutically, there has been comparatively little exploration of mechanical interactions between tumor cells and the ECM. All of the steps critical to GBM tumor cell invasion described above would be expected to depend strongly on cellular mechanical properties, exchange of mechanical forces between the cell and ECM, mobilization of mechanotransductive signaling pathways, and other cellular components expected to figure centrally in cellular mechanobiology. In addition, several GBM-specific lines of evidence suggest that tumor invasion involves alterations in the mechanical microenvironment of the brain. For example, ultrasound elastography, which derives its contrast from variations in tissue density and heterogeneity, outperforms magnetic resonance imaging in elucidating GBM tumor margins (albeit at lower spatial resolution) and is widely employed as an intraoperative imaging modality during tumor resection.[23,25,26,247] Moreover, invasive GBM tumors express aberrant subtype distributions of integrins and increased levels of focal adhesion proteins (e.g., FAK)[248] and motor proteins (e.g., NMMII).[114] Manipulation of these molecular systems can alter sensitivity of GBM tumor cells to chemotherapeutic agents and ionizing radiation. For example, expression of integrins $\alpha2$, $\alpha3$, $\alpha5$, and $\beta1$ in drug-resistant glioma cells correlates with enhanced adhesion to specific ECM proteins known to preferentially localize to tumor tissue and basement membrane.[249] Finally, pharmacologic inhibition of extracellular fibronectin assembly can enhance sensitivity of GBM cells to nitrosourea chemotherapy *in vitro* and *in vivo*,[250] raising the intriguing possibility that pharmacological agents that do not require cellular uptake may form the basis of effective strategies in GBM.

A number of indirect lines of evidence have supported the notion that glioma cells and their related cell populations are sensitive to mechanical signals from the ECM. For example, Thomas and DiMilla cultured human SNB-19 glioma cells on silicone rubber sheets whose compliance could be modulated by heating and used this system to show that increasing

compliance reduced both cell spreading area and random motility speed.[251] Georges and co-workers incubated mixed cortical cultures on ECM substrates of varying compliance and demonstrated that softer ECMs select for neuron-rich cultures whereas more rigid ECMs select for astrocyte-rich cultures.[252] Using a similar materials system, Saha and colleagues cultured adult neural stem cells on defined-rigidity ECMs and showed that highly compliant ECMs tend to promote neural differentiation, whereas highly rigid ECMs promote astrocytic differentiation.[253] More recently, Shi and colleagues have shown that neuronal differentiation of adult neural stem cells is accompanied by changes in mechanical force generation, further hinting at a connection between ECM mechanical properties, cellular force generation, and propensity to proliferate and differentiate.[254]

7.10.6.3 Regulation of GBM Tumor Cell Behavior by the Mechanical Microenviornment

To more directly explore these relationships *in vitro*, we recently tested the hypothesis that ECM rigidity can control key behaviors of cultured glioma cells that underlie tumor growth and invasion (**Figure 6**).[255] We cultured a series of glioma cells on fibronectin-conjugated polyacrylamide substrates whose stiffnesses ranged from <100 Pa to >100 kPa and probed the effect of ECM stiffness on cell adhesion, cytoarchitecture, random motility, and proliferation. We found that on highly compliant ECMs, cells fail to spread effectively and form only punctate, immature focal contacts with poorly-developed actin cytoskeletons. On stiffer ECMs, cell spreading increases dramatically and is accompanied by

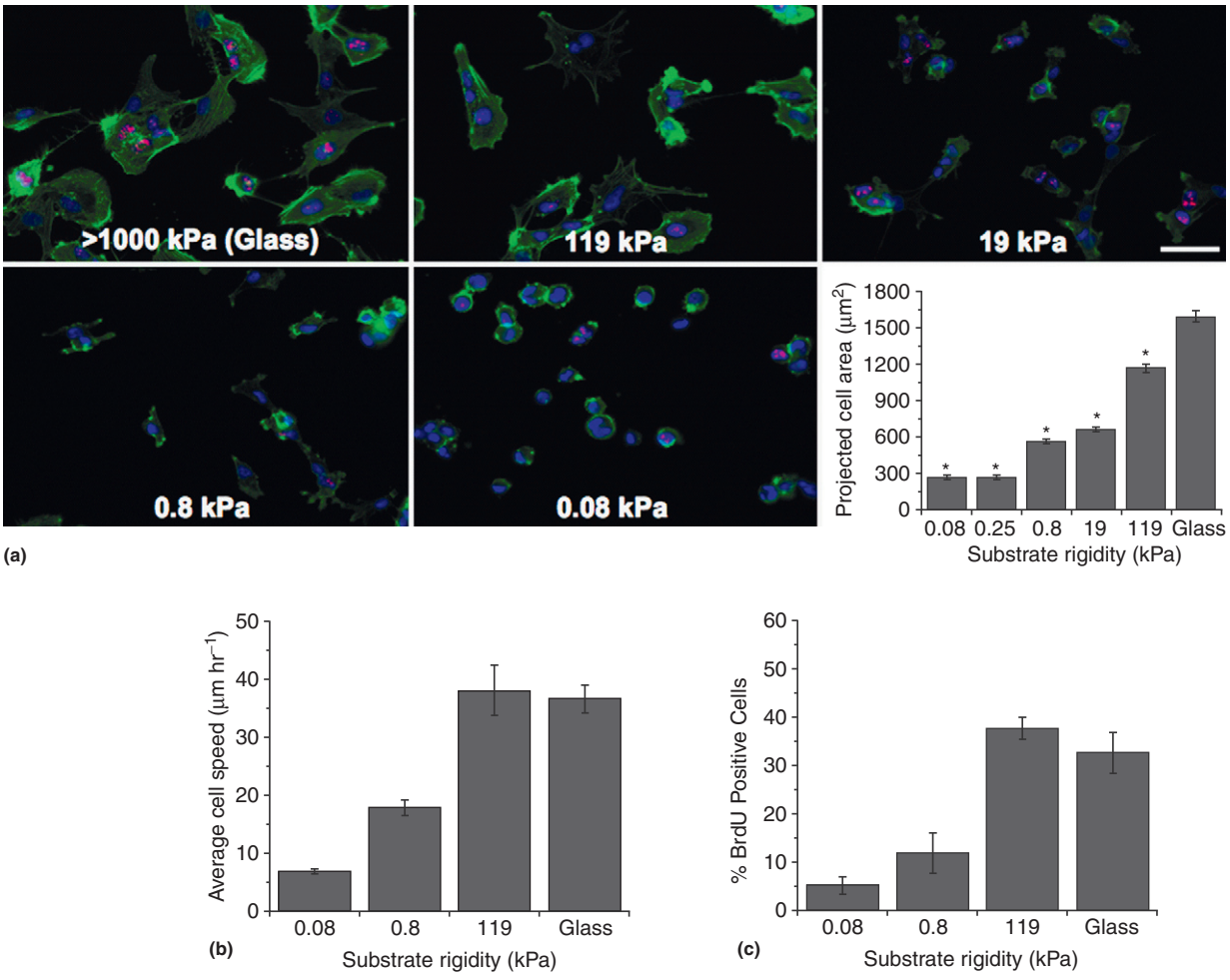


Figure 6 Mechanoregulation of GBM tumor cells. (a) Effect of ECM rigidity on cell spreading. On glass or on very stiff ECMs (119 kPa), U373 MG glioma cells spread extensively, with well defined stress fibers and focal adhesions. As ECM stiffness is reduced, cells progressively lose their ability to spread and to form mature stress fibers and focal adhesions. The plot illustrates the steep dependence of projected cell area on ECM stiffness. Immunofluorescence images depict nuclear DNA (blue), F-actin (green), and the proliferation marker Ki67 (red). The scale bar is 50 μm. (b) Effect of ECM rigidity on cell motility. Increasing ECM rigidity also increases the speed of random cell migration, which is accompanied by a transition from a filipodial to stick-slip to smooth and gliding mode of motility (not shown). (c) Effect of ECM rigidity on cell proliferation. Increasing ECM rigidity increases the percentage of dividing cells, as evidenced by bromodeoxyuridine (BrdU) incorporation. Figure components reproduced from Ulrich, T. A.; Pardo, E. M. D.; Kumar, S. The mechanical rigidity of the extracellular matrix regulates the structure, motility, and proliferation of glioma cells. *Cancer Res.* **2009**, *69*, 4167–4174, with permission. Copyright by American Association for Cancer Research.

formation of mature, elongated focal adhesions and stress fibers. Notably, adhesions and cytoskeletal structures observed on the stiffest ECMs are largely indistinguishable from those observed on fibronectin- or collagen-coated glass, suggesting that ECM stiffness governs assembly of these structures more strongly than the method of ligand presentation. When we performed time-lapse phase contrast imaging, we found that increasing ECM stiffness increases the speed of random migration, with migration speeds on ~100 kPa ECMs similar to speeds observed on glass. Intriguingly, these changes in migration speed are also accompanied by changes in the mode of motility, with highly compliant ECMs supporting only filopodial extension without appreciable locomotion, intermediate-stiffness ECMs supporting 'stick-slip' motility with advance of the leading edge largely uncoordinated with detachment of the trailing edge, and stiff ECMs supporting a smooth, gliding, form of motility similar to that observed on glass. Remarkably, proliferation is also strongly stiffness dependent, with the percentage of diving cells (as measured by uptake of the labeled nucleotide bromodeoxyuridine) increasing with increasing ECM stiffness. Importantly, NMMII-based contractility is critical for many of these stiffness-sensing behaviors, as treatment of cells with the NMMII inhibitor blebbistatin leads to similar morphologies across ECMs stiffnesses, and treatment with the ROCK inhibitor Y-27632 dramatically rescues motility on highly compliant ECMs. These results raise the exciting possibility that glioma cells may remodel and stiffen endogenous ECM as they invade the parenchyma of the brain, and that this stiffening may contribute to the growth and spread of the tumor by harnessing mechanotransductive signaling pathways to enhance motility and proliferation.

7.10.6.4 Contributions of α-Actinin to Glioma Cell Mechanobiology

In an effort to gain additional molecular mechanistic insight into this phenomenon, a later study focused on the focal adhesion protein α-actinin (**Figure 7**).[256] This focal adhesion protein is of significant interest in human glioma, in part because expression of this molecule has been previously correlated with glioma cell invasiveness *in vivo*.[257] Moreover, α-actinin plays a particularly important structural role in focal adhesions, as it is capable of both ligating β integrin subunits

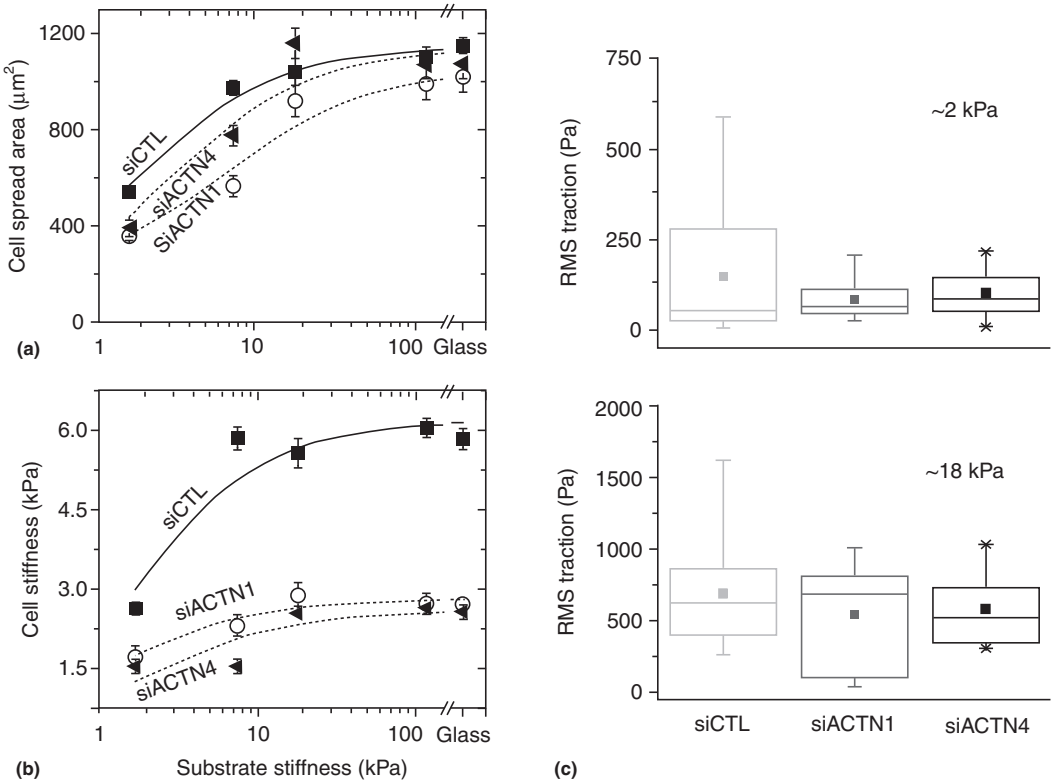


Figure 7 Role of α-actinin in glioma cell mechanobiology. (a) Effect on adaptation of cell spreading to ECM rigidity. When ECM rigidity is increased, glioma cells transfected with control siRNAs (siCTL) increase their spread areas. This adaptation is modestly compromised at low ECM stiffnesses by siRNA-mediated suppression of either α-actinin-1 (siACTN1) or α-actinin-4 (siACTN4). (b) Effect on adaptation of cell mechanics to ECM rigidity. As ECM rigidity is increased, control cells adapt their own stiffness in response, as measured by AFM. This stiffness adaptation response is strongly impaired by suppression of either α-actinin-1 or α-actinin-4. (c) Effect on generation of traction forces. Suppression of either α-actinin-1 or α-actinin-4 also impairs the ability of glioma cells to generate large tractional forces relative to control cells, as measured by traction force microscopy. This is true whether cells are cultured on relatively compliant (top) or stiff (bottom) ECMs, with larger overall traction forces being generated on stiffer substrates, as expected. Reproduced from Sen, S.; Dong, M.; Kumar, S. Isoform-specific contributions of alpha-actinin to glioma cell mechanobiology. *PLoS ONE* **2009**, *4*, e8427, under Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.5/). Copyright by PLoS ONE.

to F-actin and mechanically reinforcing F-actin into cross-linked networks, and is therefore regarded as a key player in mechanochemical feedback between the contractile and adhesive machineries in the cell.[73,258–260] First, we showed that the two nonmuscle isoforms of α-actinin, α-actinin-1 and α-actinin-4, localize to membrane ruffles, focal adhesions, and stress fibers in cultured glioma cells. Next, we examined isoform-specific contributions of α-actinin to glioma cell mechanobiology by specifically suppressing expression of each α-actinin-1 and α-actinin-4 using RNA interference. After optimizing this method to achieve 50–55% expression levels in U 373 MG human glioma cells relative to control cells transfected with nonspecific siRNAs and confirming the efficacy of knockdown by Western blot and immunofluorescence, we examined the contributions of each isoform to selected cellular mechanobiological properties. Suppression of either isoform led to a ~35% reduction in random motility speed, suggesting a role for both isoforms in modulating cell-ECM interactions. To probe this potential connection more deeply, we synthesized defined-stiffness ECMs coated with collagen I, glioma cells were cultured on these substrates, and projected cell area and cortical stiffness measured by AFM. As expected, control cells increased both projected cell area and cell stiffness as ECM stiffness was increased, consistent with previous reports.[100,261] When expression of either α-actinin isoform was suppressed, however, both stiffness responses were blunted. Specifically, α-actinin-depleted cells did not adapt their cortical stiffness as closely as control cells to increases in ECM stiffness, with α-actinin-depleted cells achieving plateau stiffnesses of ~2 kPa on glass substrates compared to ~6 kPa for control cells. Suppression of α-actinin also altered the relationship between projected cell area and ECM stiffness, although in more complex ways. On glass and highly rigid substrates, projected cell areas of α-actinin-depleted cells were comparable to control cells; however, on compliant ECMs, projected cell areas for α-actinin-depleted cells were significantly smaller than control cells. In other words, suppression of α-actinin on rigid substrates reduces cortical stiffness without compromising the ability of the cell to spread.

We reasoned that α-actinin-depleted cells might be able to preserve their ability to spread on rigid substrates through compensatory increases in expression and/or activity of other focal adhesion proteins. To test this possibility, we immunostained for the focal adhesion marker vinculin and found that suppression of either isoform indeed increases the mean area and aspect ratio of vinculin-positive focal adhesions, supporting the notion that vinculin might functionally substitute for α-actinin to preserve adhesion. Somewhat surprisingly, suppression of either α-actinin isoform also led to increased net expression of vinculin, implying that depletion of α-actinin from focal adhesions sufficiently alters cell-ECM mechanochemical feedback to mobilize mechanotransductive gene programs and/or alter protein stability. Together, these data suggest a complex functional interaction between α-actinin, expression and recruitment of vinculin to focal adhesions, and cell-ECM mechanotransduction. To confirm these connections, we treated U-373 MG cells treated with inhibitors of various elements of the NMMII contractility pathway, including ROCK, MLCK, and NMMII itself. Inhibition of each of these components substantially altered both localization of α-actinin to focal adhesions and the effect of α-actinin suppression on migration velocity. We also used traction force microscopy, in which cells are cultured on PA ECMs studded with fluorescent microparticles that report the stress and strain distribution within the ECM, to measure contributions of α-actinin to cellular contractility. Consistent with other studies in the author's lab, suppression of either isoform significantly reduces mean cellular tractional forces by ~40% relative to controls, closely mirroring the effects on cortical stiffness. Analogous to the vinculin result, these phenomena could be traced to expression level effects; suppression of α-actinin also reduces expression of NMMII and phosphorylation of MLC.

7.10.6.5 Probing GBM Tumor Cell Mechanobiology in Three-Dimensional ECMs

While these results provide valuable insight into the mechanosensitivity of gliomas, they were obtained using idealized two-dimensional substrates that fail to capture the complexity of the 3-D ECM environment found in the brain. As described earlier in the context of breast epithelial tumors, this difference in dimensionality may fundamentally alter the resulting biology and limit the ability to translate findings from *in vitro* systems to the *in vivo* disease. This has led to a tremendous effort to develop 3-D ECM systems appropriate for *in vitro* studies. However, the development of such matrices has proven challenging for a variety of reasons. One common synthetic strategy is based on inclusion of cell adhesion peptides (e.g., RGD) into synthetic polymer networks.[262–264] While this clearly enables independent control of ECM ligand density and elasticity, it does so by sacrificing the rich biochemical and topological information encoded in networks of full-length matrix proteins; for example, RGD peptides capture the cell-adhesive moiety of fibronectin but lose the critical functional contributions of the fibronectin synergy sequence, which strongly influences integrin-fibronectin binding affinity.[265] Another common strategy is based on manipulation of 3-D matrix properties by varying the concentration of a native ECM protein or other formulation, such as collagen I or Matrigel. However, these materials are intrinsically quite compliant and offer a relatively narrow dynamic range of elasticities; changing ECM protein concentration concurrently changes ECM mechanics, ligand density, microstructure, and other potentially biophysical parameters that may preclude clear interpretation of *in vitro* experiments.[266,267]

We recently sought to develop a new materials strategy that combines strengths of both approaches while also allowing us to investigate glioma mechanobiology in 3-D ECMs (**Figure 8**).[268] The approach was based on modulation of the biophysical properties of collagen I by adding agarose, a biologically inert polysaccharide sometimes used in tissue engineering applications. We showed that incorporation of agarose into relatively dilute (0.5 mg ml^{-1}) collagen I gels can increase the storage modulus of these gels from <10 Pa in the absence of agarose to nearly 1 kPa at an agarose concentration of 1% w/v, which is more than an order of magnitude stiffer than could be achieved by stiffening pure collagen gels by increasing the collagen concentration to 1.5 mg ml^{-1}. We then used a

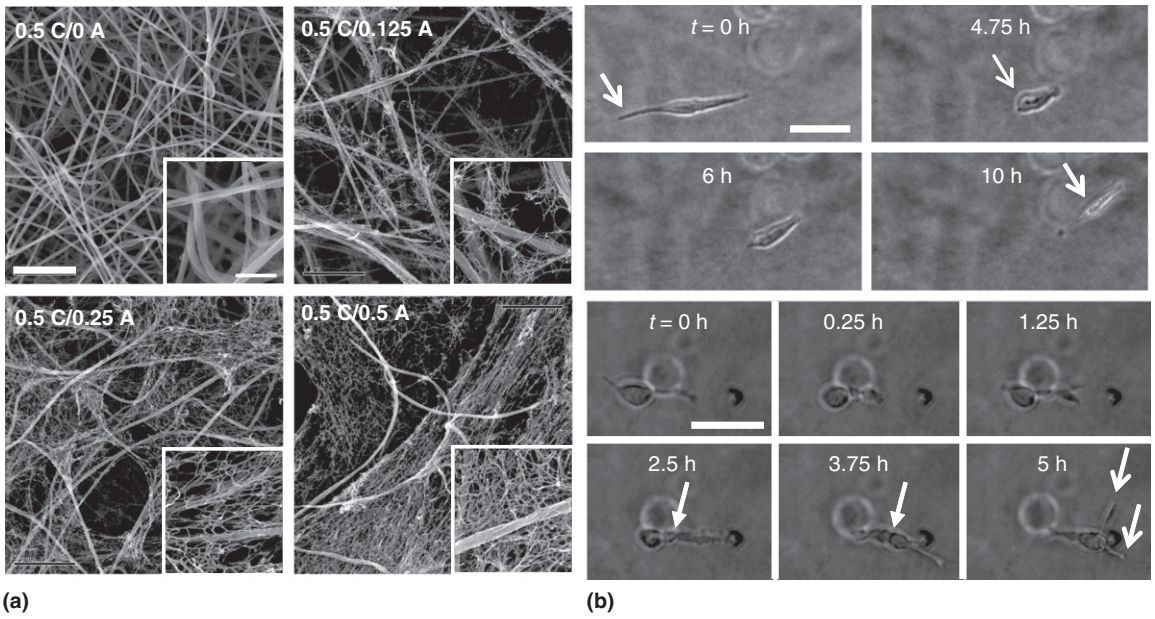


Figure 8 Use of collagen-agarose ECMs to probe tumor cell mechanobiology. (a) Effect of agarose on collagen microstructure. Pure Collagen I ECMs (upper left panel, 0.5 mg ml^{-1} collagen + 0% w/v agarose) consist of a fibrous network with large pore sizes as visualized by scanning electron microscopy (SEM). As agarose is incorporated into these ECMs, the agarose forms a fine, meshlike network between the collagen fibers that reduces the pore size of the matrix. Scale bars are 2 μm in main panels and 500 nm in high-magnification insets. (b) Effect of agarose on tumor cell motility. One consequence of the effect of agarose on mesh size is that glioma tumor cells migrate in a mesenchymal fashion in agarose-poor ECMs (top panels) and then resort to amoeboid motility in agarose-rich matrices (bottom). The open arrows denote processes formed at the leading edge of the cell, and the closed arrows denote constriction rings. Scale bars are 50 μm. Figure components reproduced from Ulrich, T. A.; Jain, A.; Tanner, K.; Mackay, J. L.; Kumar, S. Probing cellular mechanobiology in three-dimensional culture with collagen-agarose matrices. *Biomaterials* **2010**, *31*, 1875–1884.

combination of Nomarski differential interference contrast imaging (DIC), second harmonic generation (SHG) imaging, and confocal reflectance microscopy (CRM) imaging to demonstrate that the inclusion of agarose does not grossly interfere with the ability of collagen to form an entangled fiber network below 0.5% w/v agarose, and does so only modestly for agarose concentrations up to 1.0% w/v. In other words, addition of agarose is capable of modulating the elastic properties of collagen I over approximately three orders of magnitude with minimal alteration to collagen fiber architecture.

Thus, we next proceeded to use this materials platform to examine the relationship between tumor cell invasive behavior and ECM elasticity in 3-D. Because the motility of single cells is technically challenging to track and quantify in 3-D without the use of vital dyes and confocal microscopy, we instead chose to use a spheroid paradigm, where we grew 3-D, multicellular spheroids using hanging-drop culture and physically implanted these spheroids in the collagen/agarose gel prior to gelation. Approximately 6–12 hours postimplantation, cells detached from the spheroid and began to invade the surrounding gel in all directions; the radius of the invasive front, which can be readily obtained from low-magnification phase contrast imaging, provides a measure of the speed of motility. This approach has been extensively used to probe the invasive behavior of a wide variety of tumor cells, including glioma cells,[269–274] and thereby provided an opportunity to compare these results to those in the literature.

Based on the 2-D results described earlier, we expected that increasing agarose concentration, which increases gel elasticity, would increase the rate of motility. However, when we performed spheroid invasion assays, we found that increasing agarose concentration actually dramatically reduces spheroid invasion speed, with complete abrogation of motility at agarose concentrations in excess of 1% w/v. Importantly, this behavior stands in strong contrast to motility of cells through pure collagen I-based gels of increasing density, where there is no clear correlation between collagen concentration and invasiveness, as others had observed in the past.[272] In other words, enrichment of agarose inhibited motility in a manner largely independent of collagen ligand density or fiber architecture. To gain additional insight into the orgin of this behavior, we decided to obtain higher-resolution ultrastructural data for the agarose, which is inaccessible to the methods we used previously to image the collagen fibers (DIC, SHG, CRM). To accomplish this, the collagen/agarose formulations were subjected to critical point drying and scanning electron microscopy (SEM). These studies revealed that the agarose forms a filamentous, mesh-like network that intercalated between the collagen fibers. At relatively low agarose concentrations the agarose filaments adhere closely to the fibers, leaving large interstices, whereas at high concentrations the filaments occupied effectively all of the space between the collagen fibers and left very small mesh sizes. In other words, the mesh sizes of pure collagen or agarose-poor matrices are largely defined by the large spaces between the collagen fibers,

whereas the mesh sizes of agarose-rich matrices were defined by the small spaces between the agarose filaments.

Based on these results, it was hypothesized that the agarose might be presenting steric barriers to motility, such that invading glioma cells, which are incapable of degrading agarose-based filaments, could only migrate by 'squeezing past' them. This process would become increasingly difficult at high agarose concentrations and manifest itself in terms of lower rates of invasion. If this was the case, then one might expect motility to transition from a mesenchymal-like phenotype, characteristic of migration through large pore size, degradable matrices, to an amoeboid-like phenotype, characteristic of migration through small pore size, nondegradable matrices (i.e., MAT). To determine if this might be the case, we used phase-contrast imaging to track single glioma cells migrating through the various collagen/agarose formulations. In agarose-poor formulations, cells established clearly polarized morphologies and moved in a directionally persistent fashion, consistent with mesenchymal motility. As the agarose concentration was raised, cells adopted a rounded morphology and advanced by extending thin projections in multiple directions and then squeezing forward with a segmented morphology, with bleb-like portions of the cell separated by constriction rings. In addition to this MAT, the agarose fundamentally alters how the collagen fibers dissipate cell-induced stresses during migration, as visualized by DIC imaging. In pure collagen or agarose-poor matrices, cells primarily deformed the ECM via the bending and buckling of individual collagen fibers, whereas cells in agarose-rich matrices deformed the surrounding matrix in a more continuum-like fashion, with stresses and strains distributed more isotropically along all surrounding fibers. Importantly, the ability of collagen I and other fibrous biopolymers to dissipate stresses in this nonaffine fashion underlies these materials' highly nonlinear elastic properties (e.g., strain stiffening) and has been shown to facilitate mechanical communication between cells in 3-D matrices.[275] Indeed, in experiments at the author's lab, spheroids cultured millimeters away from one another in agarose-poor matrices remodeled the material between them into fibers, which leads to migration of cells from one spheroid to another. By contrast, spheroids placed tens of microns apart in agarose-rich matrices do not exhibit this topological communication and fail to preferentially remodel the interstitial material. Thus, although agarose is indeed capable of stiffening collagen matrices, its primary influences on cell behavior derive from its effects on collagen microstructure and nonlinear elastic properties.

7.10.7 Mechanobiological Signaling Pathways as Therapeutic Targets in Cancer

The finding that many key steps in tumor transformation, growth, angiogenesis, invasion, and metastasis depend critically on the function of mechanobiological signaling systems has raised tremendous interest in the prospect of leveraging these systems as therapeutic targets. Such inhibitor strategies have been devised against many elements of the mechanotransductive signaling machinery, including adhesion receptors, FC proteins, and activators of NMMII-based contractility. Clearly, host vs. tumor selectivity remains a critical ongoing challenge in this area, because mechanobiological signaling systems play key roles in the physiology of normal cells, even those that are terminally differentiated. Nonetheless, there is much evidence to support the validity of the concept of attacking tumors through cellular mechanobiological signaling, and some of these agents are now approved for clinical use, as discussed below.

Therapeutic antibodies against integrins have been extensively explored as a means of disrupting cell-ECM adhesion in angiogenesis and tumor progression, the best known example of which may be LM609, a monocloncal mouse IgG directed against $\alpha_v\beta_3$ integrins.[276–279] Local or systemic administration of LM609 has been shown to reduce angiogenesis and tumor growth in animal models of many tumor types, including breast cancer,[276] Kaposi sarcoma,[280] and melanoma.[281] Key mediators of integrin-dependent signaling have also been widely examined as anticancer therapies, including drugs directed against FAK and Src.[282] Dasatinib is a small-molecule Src inhibitor that acts by impeding ATP binding site and is currently in use as a second-line therapy in chronic myelogenous leukemia.[283] Similarly, the FAK inhibitors TAE226 and PF-562271 have shown strong antitumor activity in culture and in mouse models, and are currently in clinical trials.[284,285]

Finally, several studies have explored the therapeutic potential of antagonists of elements of the Rho GTPase-based contractility pathway. While efforts to develop direct inhibitors of Rho GTPase activity have met with mixed results,[286] it appears that HMG CoA reductase inhibitors (the 'statins'), which are commonly used to treat hypercholesterolemia, surprisingly can dampen Rho signaling by impairing Rho prenylation, which is needed for optimum activity. For example, atorvastatin (Lipitor) reduces the invasive properties of melanoma cells *in vitro* and their metastatic properties in mice.[287] Efforts to develop and apply direct inhibition strategies against Rho effectors has met with considerably greater success. The ROCK inhibitor Y-27632, which is widely used in cell culture experiments to relax cell contractility, reduces extrahepatic metastases in a mouse model of hepatocellular carcinoma[288] and reduces metastasis of breast tumors to bone.[289] Fasudil, another ROCK inhibitor, strongly reduces breast tumor progression in a number of animal models and is already in clinical use in Japan for cerebral vasospasm, raising its promise as a clinically tolerable agent.[290]

7.10.8 Conclusions and Future Directions

The field of cancer biology has increasingly begun to appreciate the role of mechanical force and other biophysical inputs in the development and dissemination of tumor cells. In this chapter, fundamental aspects of cellular mechanobiology have been reviewed, as well as the direct relevance of this field to tumor transformation and growth, angiogenesis, tissue invasion, and metastasis. The chapter has also explored how these concepts are exemplified in the growth and spread of the malignant brain tumor GBM and identified ongoing efforts to target various components of the cell mechanobiological signaling machinery in novel anticancer therapies.

While the field's understanding of the importance of mechanobiology in cancer has grown dramatically over the past two decades, many open questions remain with respect to underlying mechanisms and therapeutic potential. For example, when a tumor cell is placed in an ECM environment of defined stiffness, topology, or geometry, what matrix property is it ultimately sensing and how? And do multiple different microenvironmental inputs converge on common pathways? Moreover, what are the critical points of intersection between mechanobiological signaling systems and signaling systems traditionally associated with cell cycle control, proliferation, differentiation, and apoptosis? Finally, if alterations in the mechanical microenvironment can critically regulate tumor growth, how might one modulate this environment *in vivo* to prevent or arrest tumor growth? While it is difficult to envision directing ECM assembly or mechanics in the same way one might deliver a small-molecule drug to inhibit an enzyme or block a binding site, it is important to remember that ECM stiffening *in vivo* often requires the action of accessory proteins and enzymes, all of which may represent potential drug targets. Recent success with LOX inhibitors in mice to disrupt collagen crosslinking in breast tumors offers some optimism for this approach.[168] Whether all of these questions can be convincingly addressed and how much more time and energy will be required to do so remains to be seen. But as the examples discussed in this chapter demonstrate, little question remains that cancer is, at least in part, a disease of aberrant cellular mechanobiology. Thus, while the questions are large and the challenges grand, history suggests that the investment will be a fruitful one.

Acknowledgments

Work in the author's laboratory is supported in part by the Arnold and Mabel Beckman Young Investigator Award, the NSF (CMMI 0727420; CMMI 1055965), a Presidential Early Career Award for Scientists and Engineers from the ARO (PECASE W911NF-09–1-0507), and the NIH Director's New Innovator Award (1DP2OD004213), a part of the NIH Roadmap for Medical Research. The author extends his sincere regrets to the many investigators whose excellent contributions to this field were inadvertently overlooked or could not be discussed because of space limitations.

References

[1] American Cancer Society. *Cancer Facts and Figures*; ACS: Oklahoma City, 2009.
[2] Hartwell, L. H.; Kastan, M. B. Cell-cycle control and cancer. *Science* **1994**, *266*, 1821–1828.
[3] Hoeijmakers, J. H. J. Genome maintenance mechanisms for preventing cancer. *Nature* **2001**, *411*, 366–374.
[4] Lengauer, C.; Kinzler, K. W.; Vogelstein, B. Genetic instabilities in human cancers. *Nature* **1998**, *396*, 643–649.
[5] Levine, A. J.; Momand, J.; Finlay, C. A. The p53 tumor suppressor gene. *Nature* **1991**, *351*, 453–456.
[6] Sherr, C. J. Cancer cell cycles. *Science* **1996**, *274*, 1672–1677.
[7] Baylin, S. B.; Herman, J. G. DNA hypermethylation in tumorigenesis – epigenetics joins genetics. *Trends Genet.* **2000**, *16*, 168–174.
[8] Jaenisch, R.; Bird, A. Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. *Nat. Genet.* **2003**, *33*, 245–254.
[9] Jones, P. A.; Baylin, S. B. The fundamental role of epigenetic events in cancer. *Nat. Rev. Genet.* **2002**, *3*, 415–428.
[10] Minucci, S.; Pelicci, P. G. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nat. Rev. Cancer* **2006**, *6*, 38–51.
[11] Coussens, L. M.; Werb, Z. Inflammation and cancer. *Nature* **2002**, *420*, 860–867.
[12] Gupta, G. P.; Massague, J. Cancer metastasis: Building a framework. *Cell* **2006**, *127*, 679–695.
[13] Kalluri, R. Basement membranes: Structure, assembly and role in tumour angiogenesis. *Nat. Rev. Cancer* **2003**, *3*, 422–433.
[14] Mueller, M. M.; Fusenig, N. E. Friends or foes – Bipolar effects of the tumour stroma in cancer. *Nat. Rev. Cancer* **2004**, *4*, 839–849.
[15] Nelson, C. M.; Bissell, M. J. Of extracellular matrix, scaffolds, and signaling: Tissue architecture regulates development, homeostasis, and cancer. *Annu. Rev. Cell Dev. Biol.* **2006**, *22*, 287–309.
[16] Kumar, S.; Weaver, V. Mechanics, malignancy, and metastasis: The force journey of a tumor cell. *Cancer Metastasis Rev.* **2009**, *28*, 113–127.
[17] Discher, D. E.; Janmey, P.; Wang, Y. L. Tissue cells feel and respond to the stiffness of their substrate. *Science* **2005**, *310*, 1139–1143.
[18] Ingber, D. E. Mechanobiology and diseases of mechanotransduction. *Ann. Med.* **2003**, *35*, 564–577.
[19] Fattovich, G.; Giustina, G.; Schalm, S. W.; Hadziyannis, S.; Sancheztapias, J.; Almasio, P.; Christensen, E.; Krogsgaard, K.; Degos, F.; Demoura, M. C.; Solinas, A.; Noventa, F.; Realdi, G.; Alberti, A.; Quero, C.; Savvas, S.; Mas, A.; Craxi, A.; Olsen, J. F.; Delarocque, E.; Rocha, P.; Tocco, A.; Cossu, P. A. Occurrence of hepatocellular-carcinoma and decompensation in western-European patients with cirrhosis type-b. *Hepatology* **1995**, *21*, 77–82.
[20] Colpaert, C.; Vermeulen, P.; Van Marck, E.; Dirix, L. The presence of a fibrotic focus is an independent predictor of early metastasis in lymph node-negative breast cancer patients. *Am. J. Surg. Pathol* **2001**, *25*, 1557–1558.
[21] Selbekk, T.; Bang, J.; Unsgaard, G. Strain processing of intraoperative ultrasound images of brain tumours: Initial results. *Ultrasound Med. Biol.* **2005**, *31*, 45–51.
[22] Unsgaard, G.; Selbekk, T.; Muller, T. B.; Ommedal, S.; Torp, S. H.; Myhr, G.; Bang, J.; Hernes, T. A. N. Ability of navigated 3D ultrasound to delineate gliomas and metastases – comparison of image interpretations with histopathology. *Acta Neurochir.* **2005**, *147*, 1259–1269.
[23] Unsgaard, G.; Rygh, O. M.; Selbekk, T.; Muller, T. B.; Kolstad, F.; Lindseth, F.; Hernes, T. A. Intra-operative 3D ultrasound in neurosurgery. *Acta Neurochir.* **2006**, *148*, 235–253, discussion 253.
[24] Rygh, O. M.; Selbekk, T.; Torp, S. H.; Lydersen, S.; Hernes, T. A. N.; Unsgaard, G. Comparison of navigated 3D ultrasound findings with histopathology in subsequent phases of glioblastoma resection. *Acta Neurochir.* **2008**, *150*, 1033–1042.
[25] Gulati, S.; Berntsen, E. M.; Solheim, O.; Kvistad, K. A.; Haberg, A.; Selbekk, T.; Torp, S. H.; Unsgaard, G. Surgical resection of high-grade gliomas in eloquent regions guided by blood oxygenation level dependent functional magnetic resonance imaging, diffusion tensor tractography, and intraoperative navigated 3D ultrasound. *Min. Invas. Neurosurg* **2009**, *52*, 17–24.
[26] Scherer, H. J. The forms of growth in gliomas and their practical significance. *Brain* **1940**, *63*, 1–35.
[27] Ashkenas, J.; Muschler, J.; Bissell, M. J. The extracellular matrix in epithelial biology: Shared molecules and common themes in distant phyla. *Dev. Biol.* **1996**, *180*, 433–444.
[28] Blankenberg, S.; Barbaux, S.; Tiret, L. Adhesion molecules and atherosclerosis. *Atherosclerosis* **2003**, *170*, 191–203.
[29] Howlett, A. R.; Bissell, M. J. The influence of tissue microenvironment (stroma and extracellular-matrix) on the development and function of mammary epithelium. *Epithel. Cell Biol.* **1993**, *2*, 79–89.
[30] Ley, K. Molecular mechanisms of leukocyte recruitment in the inflammatory process. *Cardiovasc. Res.* **1996**, *32*, 733–742.
[31] Ruoslahti, E. RGD and other recognition sequences for integrins. *Annu. Rev. Cell Dev. Biol.* **1996**, *12*, 697–715.
[32] Ridley, A. J.; Schwartz, M. A.; Burridge, K.; Firtel, R. A.; Ginsberg, M. H.; Borisy, G.; Parsons, J. T.; Horwitz, A. R. Cell migration: Integrating signals from front to back. *Science* **2003**, *302*, 1704–1709.
[33] Roca-Cusachs, P.; Gauthier, N. C.; del Rio, A.; Sheetz, M. P. Clustering of alpha(5)beta(1) integrins determines adhesion strength whereas alpha(v)beta(3) and talin enable mechanotransduction. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 16245–16250.
[34] Vogel, V.; Sheetz, M. P. Cell fate regulation by coupling mechanical cycles to biochemical signaling pathways. *Curr. Opin. Cell Biol.* **2009**, *21*, 38–46.

[35] Wang, N.; Butler, J. P.; Ingber, D. E. Mechanotransduction across the cell-surface and through the cytoskeleton. *Science* **1993**, *260*, 1124–1127.

[36] Moser, M.; Legate, K. R.; Zent, R.; Fassler, R. The tail of integrins, talin, and kindlins. *Science* **2009**, *324*, 895–899.

[37] Baker, E. L.; Zaman, M. H. The biomechanical integrin. *J. Biomech.* **2010**, *43*, 38–44.

[38] Hynes, R. O. Integrins: Bidirectional, allosteric signaling machines. *Cell* **2002**, *110*, 673–687.

[39] Luo, B. H.; Carman, C. V.; Springer, T. A. Structural basis of integrin regulation and signaling. *Annu. Rev. Immunol.* **2007**, *25*, 619–647.

[40] Carman, C. V.; Springer, T. A. Integrin avidity regulation: Are changes in affinity and conformation underemphasized? *Curr. Opin. Cell Biol.* **2003**, *15*, 547–556.

[41] Kinashi, T. Intracellular signalling controlling integrin activation in lymphocytes. *Nat. Rev. Immunol.* **2005**, *5*, 546–559.

[42] Shimaoka, M.; Takagi, J.; Springer, T. A. Conformational regulation of integrin structure and function. *Annu. Rev. Biophys. Biomol. Struct.* **2002**, *31*, 485–516.

[43] Xiong, J. P.; Stehle, T.; Goodman, S. L.; Arnaout, M. A. New insights into the structural basis of integrin activation. *Blood* **2003**, *102*, 1155–1159.

[44] Calderwood, D. A.; Tai, V.; Di Paolo, G.; De Camilli, P.; Ginsberg, M. H. Competition for talin results in trans-dominant inhibition of integrin activation. *J. Biol. Chem.* **2004**, *279*, 28889–28895.

[45] Calderwood, D. A.; Yan, B. X.; de Pereda, J. M.; Alvarez, B. G.; Fujioka, Y.; Liddington, R. C.; Ginsberg, M. H. The phosphotyrosine binding-like domain of talin activates integrins. *J. Biol. Chem.* **2002**, *277*, 21749–21758.

[46] Zhang, X.; Jiang, G.; Cai, Y.; Monkley, S. J.; Critchley, D. R.; Sheetz, M. P. Talin depletion reveals independence of initial cell spreading from integrin activation and traction. *Nat. Cell Biol.* **2008**, *10*, 1062–1068.

[47] Ye, F.; Hu, G.; Taylor, D.; Ratnikov, B.; Bobkov, A. A.; McLean, M. A.; Sligar, S. G.; Taylor, K. A.; Ginsberg, M. H. Recreation of the terminal events in physiological integrin activation. *J. Cell Biol.* **2010**, *188*(1), 157–173.

[48] Chen, K. D.; Li, Y. S.; Kim, M.; Li, S.; Yuan, S.; Chien, S.; Shyy, J. Y. J. Mechanotransduction in response to shear stress – Roles of receptor tyrosine kinases, integrins, and Shc. *J. Biol. Chem.* **1999**, *274*, 18393–18400.

[49] Jalali, S.; del Pozo, M. A.; Chen, K. D.; Miao, H.; Li, Y. S.; Schwartz, M. A.; Shyy, J. Y. J.; Chien, S. Integrin-mediated mechanotransduction requires its dynamic interaction with specific extracellular matrix (ECM) ligands. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 1042–1046.

[50] Shyy, J. Y.; Chien, S. Role of integrins in endothelial mechanosensing of shear stress. *Circ. Res.* **2002**, *91*, 769–775.

[51] Wang, Y. X.; Miao, H.; Li, S.; Chen, K. D.; Li, Y. S.; Yuan, S. L.; Shyy, J. Y. J.; Chien, S. Interplay between integrins and FLK-1 in shear stress-induced signaling. *Am. J. Physiol. – Cell Physiol.* **2002**, *283*, C1540–C1547.

[52] Puklin-Faucher, E.; Gao, M.; Schulten, K.; Vogel, V. How the headpiece hinge angle is opened: New insights into the dynamics of integrin activation. *J. Cell Biol.* **2006**, *175*, 349–360.

[53] Miyamoto, S.; Akiyama, S. K.; Yamada, K. M. Synergistic roles for receptor occupancy and aggregation in integrin transmembrane function. *Science* **1995**, *267*, 883–885.

[54] Li, R. H.; Mitra, N.; Gratkowski, H.; Vilaire, G.; Litvinov, R.; Nagasami, C.; Weisel, J. W.; Lear, J. D.; DeGrado, W. F.; Bennett, J. S. Activation of integrin alpha IIb beta 3 by modulation of transmembrane helix associations. *Science* **2003**, *300*, 795–798.

[55] Paszek, M. J.; Zahir, N.; Johnson, K. R.; Lakins, J. N.; Rozenberg, G. I.; Gefen, A.; Reinhart-King, C. A.; Margulies, S. S.; Dembo, M.; Boettiger, D.; Hammer, D. A.; Weaver, V. M. Tensional homeostasis and the malignant phenotype. *Cancer Cell* **2005**, *8*, 241–254.

[56] Arnold, M.; Cavalcanti-Adam, E. A.; Glass, R.; Blummel, J.; Eck, W.; Kantlehner, M.; Kessler, H.; Spatz, J. P. Activation of integrin function by nanopatterned adhesive interfaces. *Chemphyschem* **2004**, *5*, 383–388.

[57] Cavalcanti-Adam, E. A.; Micoulet, A.; Blummel, J.; Auernheimer, J.; Kessler, H.; Spatz, J. P. Lateral spacing of integrin ligands influences cell spreading and focal adhesion assembly. *Eur. J. Cell Biol.* **2006**, *85*, 219–224.

[58] Burridge, K.; Chrzanowska Wodnicka, M. Focal adhesions, contractility, and signaling. *Annu. Rev. Cell Dev. Biol.* **1996**, *12*, 463–518.

[59] Geiger, B.; Spatz, J. P.; Bershadsky, A. D. Environmental sensing through focal adhesions. *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 21–33.

[60] Lauffenburger, D. A.; Horwitz, A. F. Cell migration: A physically integrated molecular process. *Cell* **1996**, *84*, 359–369.

[61] Wolfenson, H.; Henis, Y. I.; Geiger, B.; Bershadsky, A. D. The heel and toe of the cell's foot: A multifaceted approach for understanding the structure and dynamics of focal adhesions. *Cell Motil. Cytoskel* **2009**, *66*, 1017–1029.

[62] Brown, A. E. X.; Discher, D. E. Conformational changes and signaling in cell and matrix physics. *Curr. Biol.* **2009**, *19*, R781–R789.

[63] Carl, P.; Kwok, C. H.; Manderson, G.; Speicher, D. W.; Discher, D. E. Forced unfolding modulated by disulfide bonds in the Ig domains of a cell adhesion molecule. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 1565–1570.

[64] Smith, M. L.; Gourdon, D.; Little, W. C.; Kubow, K. E.; Eguiluz, R. A.; Luna-Morris, S.; Vogel, V. Force-induced unfolding of fibronectin in the extracellular matrix of living cells. *PLoS Biol.* **2007**, *5*, 2243–2254.

[65] Hytonen, V. P.; Vogel, V. How force might activate talin's vinculin binding sites: SMD reveals a structural mechanism. *PLoS Comput. Biol.* **2008**, *4*, e24.

[66] Lee, S. E.; Chunsrivirot, S.; Kamm, R. D.; Mofrad, M. R. K. Molecular dynamics study of talin-vinculin binding. *Biophys. J.* **2008**, *95*, 2027–2036.

[67] Lee, S. E.; Kamm, R. D.; Mofrad, M. R. K. Force-induced activation of Talin and its possible role in focal adhesion mechanotransduction. *J. Biomech.* **2007**, *40*, 2096–2106.

[68] Lele, T. P.; Pendse, J.; Kumar, S.; Salanga, M.; Karavitis, J.; Ingber, D. E. Mechanical forces alter zyxin unbinding kinetics within focal adhesions of living cells. *J. Cell. Physiol.* **2006**, *207*, 187–194.

[69] Lele, T. P.; Thodeti, C. K.; Ingber, D. E. Force meets chemistry: Analysis of mechanochemical conversion in focal adhesions using fluorescence recovery after photobleaching. *J. Cell. Biochem.* **2006**, *97*, 1175–1183.

[70] Lele, T. P.; Thodeti, C. K.; Pendse, J.; Ingber, D. E. Investigating complexity of protein-protein interactions in focal adhesions. *Biochem. Biophys. Res. Commun.* **2008**, *369*, 929–934.

[71] Mohl, C. C.; Kirchgessner, N.; Schafer, C.; Kupper, K.; Born, S.; Diez, G.; Goldmann, W. H.; Merkel, R.; Hoffmann, B. Becoming stable and strong: The interplay between vinculin exchange dynamics and adhesion strength during adhesion site maturation. *Cell Motil. Cytoskel* **2009**, *66*, 350–364.

[72] von Wichert, G.; Haimovich, B.; Feng, G. S.; Sheetz, M. P. Force-dependent integrin-cytoskeleton linkage formation requires downregulation of focal complex dynamics by Shp2. *EMBO J.* **2003**, *22*, 5023–5035.

[73] Otey, C. A.; Carpen, O. Alpha-actinin revisited: A fresh look at an old player. *Cell Motil. Cytoskel* **2004**, *58*, 104–111.

[74] Chin, L.; Meyerson, M.; Aldape, K.; Bigner, D.; Mikkelsen, T.; VandenBerg, S.; Kahn, A.; Penny, R.; Ferguson, M. L.; Gerhard, D. S.; Getz, G.; Brennan, C.; Taylor, B. S.; Winckler, W.; Park, P.; Ladanyi, M.; Hoadley, K. A.; Verhaak, R. G. W.; Hayes, D. N.; Spellman, P. T.; Absher, D.; Weir, B. A.; Ding, L.; Wheeler, D.; Lawrence, M. S.; Cibulskis, K.; Mardis, E.; Zhang, J. H.; Wilson, R. K.; Donehower, L.; Wheeler, D. A.; Purdom, E.; Wallis, J.; Laird, P. W.; Herman, J. G.; Schuebel, K. E.; Weisenberger, D. J.; Baylin, S. B.; Schultz, N.; Yao, J.; Wiedemeyer, R.; Weinstein, J.; Sander, C.; Gibbs, R. A.; Gray, J.; Kucherlapati, R.; Lander, E. S.; Myers, R. M.; Perou, C. M.; McLendon, R.; Friedman, A.; Van Meir, E. G.; Brat, D. J.; Mastrogianakis, G. M.; Olson, J. J.; Lehman, N.; Yung, W. K. A.; Bogler, O.; Berger, M.; Prados, M.; Muzny, D.; Morgan, M.; Scherer, S.; Sabo, A.; Nazareth, L.; Lewis, L.; Hall, O.; Zhu, Y. M.; Ren, Y. R.; Alvi, O.; Yao, J. Q.; Hawes, A.; Jhangiani, S.; Fowler, G.; San Lucas, A.; Kovar, C.; Cree, A.; Dinh, H.; Santibanez, J.; Joshi, V.; Gonzalez-Garay, M. L.; Miller, C. A.; Milosavljevic, A.; Sougnez, C.; Fennell, T.; Mahan, S.; Wilkinson, J.; Ziaugra, L.; Onofrio, R.; Bloom, T.; Nicol, R.; Ardlie, K.; Baldwin, J.; Gabriel, S.; Fulton, R. S.; McLellan, M. D.; Larson, D. E.; Shi, X. Q.; Abbott, R.; Fulton, L.; Chen, K.; Koboldt, D. C.; Wendl, M. C.; Meyer, R.; Tang, Y. Z.; Lin, L.; Osborne, J. R.; Dunford-Shore, B. H.; Miner, T. L.; Delehaunty, K.; Markovic, C.; Swift, G.; Courtney, W.; Pohl, C.; Abbott, S.; Hawkins, A.; Leong, S.; Haipek, C.; Schmidt, H.; Wiechert, M.; Vickery, T.; Scott, S.; Dooling, D. J.; Chinwalla, A.; Weinstock, G. M.; O'Kelly, M.; Robinson, J.; Alexe, G.; Beroukhim, R.; Carter, S.; Chiang, D.; Gould, J.; Gupta, S.; Korn, J.; Mermel, C.; Mesirov, J.; Monti, S.; Nguyen, H.; Parkin, M.; Reich, M.; Stransky, N.; Garraway, L.; Golub, T.; Protopopov, A.; Perna, I.; Aronson, S.; Sathiamoorthy, N.; Ren, G.; Kim, H.; Kong, S. K.; Xiao, Y. H.; Kohane, I. S.; Seidman, J.; Cope, L.; Pan, F.; Van Den Berg, D.; Van Neste, L.; Yi, J. M.; Li, J. Z.; Southwick, A.; Brady, S.; Aggarwal, A.; Chung, T.; Sherlock, G.; Brooks, J. D.; Jakkula, L. R.; Lapuk, A. V.; Marr, H.; Dorton, S.; Choi, Y. G.; Han, J.; Ray, A.; Wang, V.; Durinck, S.; Robinson, M.; Wang, N. J.; Vranizan, K.; Peng, V.; Van Name, E.; Fontenay, G. V.; Ngai, J.; Conboy, J. G.; Parvin, B.; Feiler, H. S.; Speed, T. P.; Socci, N. D.; Olshen, A.; Lash, A.; Reva, B.; Antipin, Y.; Stukalov, A.; Gross, B.; Cerami, E.; Wang, W. Q.; Qin, L. X.; Seshan, V. E.; Villafania, L.; Cavatore, M.; Borsu, L.; Viale, A.; Gerald, W.; Topal, M. D.; Qi, Y.; Balu, S.; Shi, Y.; Wu, G.; Bittner, M.; Shelton, T.; Lenkiewicz, E.; Morris, S.; Beasley, D.; Sanders, S.; Sfeir, R.; Chen, J.; Nassau, D.; Feng, L.; Hickey, E.; Schaefer, C.; Madhavan, S.;

Buetow, F.; Barker, A.; Vockley, J.; Compton, C.; Vaught, J.; Fielding, P.; Collins, F.; Good, P.; Guyer, M.; Ozenberger, B.; Peterson, J.; Thomson, E. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* **2008**, *455*, 1061–1068.

[75] Besson, A.; Davy, A.; Robbins, S. M.; Yong, V. W. Differential activation of ERKs to focal adhesions by PKC epsilon is required for PMA-induced adhesion and migration of human glioma cells. *Oncogene* **2001**, *20*, 7398–7407.

[76] Mainiero, F.; Pepe, A.; Yeon, M.; Ren, Y. L.; Giancotti, F. G. The intracellular functions of alpha(6)beta(4) integrin are regulated by EGF. *J. Cell Biol.* **1996**, *134*, 241–253.

[77] Munger, J. S.; Huang, X. Z.; Kawakatsu, H.; Griffiths, M. J. D.; Dalton, S. L.; Wu, J. F.; Pittet, J. F.; Kaminski, N.; Garat, C.; Matthay, M. A.; Rifkin, D. B.; Sheppard, D. The integrin alpha v beta 6 binds and activates latent TGF beta 1: A mechanism for regulating pulmonary inflammation and fibrosis. *Cell* **1999**, *96*, 319–328.

[78] Schlaepfer, D. D.; Hauck, C. R.; Sieg, D. J. Signaling through focal adhesion kinase. *Prog. Biophys. Mol. Biol.* **1999**, *71*, 435–478.

[79] Sieg, D. J.; Hauck, C. R.; Ilic, D.; Klingbeil, C. K.; Schaefer, E.; Damsky, C. H.; Schlaepfer, D. D. FAK integrates growth-factor and integrin signals to promote cell migration. *Nat. Cell Biol.* **2000**, *2*, 249–256.

[80] Thomas, S. M.; Brugge, J. S. Cellular functions regulated by Src family kinases. *Annu. Rev. Cell Dev. Biol.* **1997**, *13*, 513–609.

[81] Wang, F.; Weaver, V. M.; Petersen, O. W.; Larabell, C. A.; Dedhar, S.; Briand, P.; Lupu, R.; Bissell, M. J. Reciprocal interactions between beta 1-integrin and epidermal growth factor receptor in three-dimensional basement membrane breast cultures: A different perspective in epithelial biology. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 14821–14826.

[82] Wang, Y. X.; Botvinick, E. L.; Zhao, Y. H.; Berns, M. W.; Usami, S.; Tsien, R. Y.; Chien, S. Visualizing the mechanical activation of Src. *Nature* **2005**, *434*, 1040–1045.

[83] Na, S.; Collin, O.; Chowdhury, F.; Tay, B.; Ouyang, M. X.; Wang, Y. X.; Wang, N. Rapid signal transduction in living cells is a unique feature of mechanotransduction. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 6626–6631.

[84] Poh, Y. C.; Na, S.; Chowdhury, F.; Ouyang, M.; Wang, Y.; Wang, N. Rapid activation of Rac GTPase in living cells by force is independent of Src. *PLoS ONE* **2009**, *4*, e7886.

[85] Berdeaux, R. L.; Diaz, B.; Kim, L.; Martin, G. S. Active Rho is localized to podosomes induced by oncogenic Src and is required for their assembly and function. *J. Cell Biol.* **2004**, *166*, 317–323.

[86] Burridge, K.; Wennerberg, K. Rho and Rac take center stage. *Cell* **2004**, *116*, 167–179.

[87] Hall, A. The cytoskeleton and cancer. *Cancer Metastasis Rev.* **2009**, *28*, 5–14.

[88] Karlsson, R.; Pedersen, E. D.; Wang, Z.; Brakebusch, C. Rho GTPase function in tumorigenesis. *Biochim. Biophys. Acta – Rev. Cancer* **2009**, *1796*, 91–98.

[89] Nobes, C. D.; Hall, A. Rho, Rac, and CDC42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell* **1995**, *81*, 53–62.

[90] Machacek, M.; Hodgson, L.; Welch, C.; Elliott, H.; Pertz, O.; Nalbant, P.; Abell, A.; Johnson, G. L.; Hahn, K. M.; Danuser, G. Coordination of Rho GTPase activities during cell protrusion. *Nature* **2009**, *461*, 99–103.

[91] Wu, Y. I.; Frey, D.; Lungu, O. I.; Jaehrig, A.; Schlichting, I.; Kuhlman, B.; Hahn, K. M. A genetically encoded photoactivatable Rac controls the motility of living cells. *Nature* **2009**, *461*, 104–111.

[92] Katsumi, A.; Milanini, J.; Kiosses, W. B.; del Pozo, M. A.; Kaunas, R.; Chien, S.; Hahn, K. M.; Schwartz, M. A. Effects of cell tension on the small GTPase Rac. *J. Cell Biol.* **2002**, *158*, 153–164.

[93] Rottner, K.; Hall, A.; Small, J. V. Interplay between Rac and Rho in the control of substrate contact dynamics. *Curr. Biol.* **1999**, *9*, 640–648.

[94] Sander, E. E.; ten Klooster, J. P.; van Delft, S.; van der Kammen, R. A.; Collard, J. G. Rac downregulates Rho activity: Reciprocal balance between both GTPases determines cellular morphology and migratory behavior. *J. Cell Biol.* **1999**, *147*, 1009–1021.

[95] Amano, M.; Chihara, K.; Kimura, K.; Fukata, Y.; Nakamura, N.; Matsuura, Y.; Kaibuchi, K. Formation of actin stress fibers and focal adhesions enhanced by Rho-kinase. *Science* **1997**, *275*, 1308–1311.

[96] Clark, E. A.; King, W. G.; Brugge, J. S.; Symons, M.; Hynes, R. O. Integrin-mediated signals regulated by members of the Rho family of GTPases. *J. Cell Biol.* **1998**, *142*, 573–586.

[97] Price, L. S.; Leng, J.; Schwartz, M. A.; Bokoch, G. M. Activation of Rac and Cdc42 by integrins mediates cell spreading. *Mol. Biol. Cell* **1998**, *9*, 1863–1871.

[98] Narumiya, S.; Tanji, M.; Ishizaki, T. Rho signaling, ROCK and mDia1, in transformation, metastasis and invasion. *Cancer Metastasis Rev.* **2009**, *28*, 65–76.

[99] Watanabe, N.; Kato, T.; Fujita, A.; Ishizaki, T.; Narumiya, S. Cooperation between mDia1 and ROCK in Rho-induced actin reorganization. *Nat. Cell Biol.* **1999**, *1*, 136–143.

[100] Peyton, S. R.; Putnam, A. J. Extracellular matrix rigidity governs smooth muscle cell motility in a biphasic fashion. *J. Cell. Physiol.* **2005**, *204*, 198–209.

[101] Mammoto, A.; Connor, K. M.; Mammoto, T.; Yung, C. W.; Huh, D.; Aderman, C. M.; Mostoslavsky, G.; Smith, L. E. H.; Ingber, D. E. A mechanosensitive transcriptional mechanism that controls angiogenesis. *Nature* **2009**, *457*, 1103–1108.

[102] Matsui, T.; Amano, M.; Yamamoto, T.; Chihara, K.; Nakafuku, M.; Ito, M.; Nakano, T.; Okawa, K.; Iwamatsu, A.; Kaibuchi, K. Rho-associated kinase, a novel serine threonine kinase, as a putative target for the small GTP binding protein Rho. *EMBO J.* **1996**, *15*, 2208–2216.

[103] Kimura, K.; Ito, M.; Amano, M.; Chihara, K.; Fukata, Y.; Nakafuku, M.; Yamamori, B.; Feng, J. H.; Nakano, T.; Okawa, K.; Iwamatsu, A.; Kaibuchi, K. Regulation of myosin phosphatase by Rho and Rho-Associated kinase (Rho-kinase). *Science* **1996**, *273*, 245–248.

[104] Totsukawa, G.; Yamakita, Y.; Yamashiro, S.; Hartshorne, D. J.; Sasaki, Y.; Matsumura, F. Distinct roles of ROCK (Rho-kinase) and MLCK in spatial regulation of MLC phosphorylation for assembly of stress fibers and focal adhesions in 3T3 fibroblasts. *J. Cell Biol.* **2000**, *150*, 797–806.

[105] Amano, M.; Ito, M.; Kimura, K.; Fukata, Y.; Chihara, K.; Nakano, T.; Matsuura, Y.; Kaibuchi, K. Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). *J. Biol. Chem.* **1996**, *271*, 20246–20249.

[106] Aktories, K.; Braun, U.; Rosener, S.; Just, I.; Hall, A. The Rho gene-product expressed in Escherichia-coli is a substrate of botulinum ADP-ribosyltransferase-C3. *Biochem. Biophys. Res. Commun.* **1989**, *158*, 209–213.

[107] Cheung, A.; Westwood, N. J.; Chen, I.; Mitchison, T. J.; Straight, A. F. Blebbistatin: A cell permeable inhibitor of non-muscle myosin II. *Mol. Biol. Cell* **2001**, *12*, 1480.

[108] Ishizaki, T.; Uehata, M.; Tamechika, I.; Keel, J.; Nonomura, K.; Maekawa, M.; Narumiya, S. Pharmacological properties of Y-27632, a specific inhibitor of Rho-associated kinases. *Mol. Pharmacol.* **2000**, *57*, 976–983.

[109] Kovacs, M.; Toth, J.; Hetenyi, C.; Malnasi-Csizmadia, A.; Sellers, J. R. Mechanism of blebbistatin inhibition of myosin II. *J. Biol. Chem.* **2004**, *279*, 35557–35563.

[110] Saitoh, M.; Ishikawa, T.; Matsushima, S.; Naka, M.; Hidaka, H. Selective-inhibition of catalytic activity of smooth-muscle myosin light chain kinase. *J. Biol. Chem.* **1987**, *262*, 7796–7801.

[111] Pfitzer, G. Signal transduction in smooth muscle – Invited review: Regulation of myosin phosphorylation in smooth muscle. *J. Appl. Physiol.* **2001**, *91*, 497–503.

[112] Katoh, K.; Kano, Y.; Amano, M.; Kaibuchi, K.; Fujiwara, K. Stress fiber organization regulated by MLCK and Rho-kinase in cultured human fibroblasts. *Am. J. Physiol. – Cell Physiol.* **2001**, *280*, C1669–C1679.

[113] Katoh, K.; Kano, Y.; Ookawara, S. Rho-kinase dependent organization of stress fibers and focal adhesions in cultured fibroblasts. *Genes to Cells* **2007**, *12*, 623–638.

[114] Beadle, C.; Assanah, M. C.; Monzo, P.; Vallee, R.; Rosenfeld, S. S.; Canoll, P. The role of myosin II in glioma invasion of the brain. *Mol. Biol. Cell* **2008**, *19*, 3357–3368.

[115] Kim, Y. B.; Yu, J. Y.; Lee, S. Y.; Lee, M. S.; Ko, S. G.; Ye, S. K.; Jong, H. S.; Kim, T. Y.; Bang, Y. J.; Lee, J. W. Cell adhesion status-dependent histone acetylation is regulated through intracellular contractility-related signaling activities. *J. Biol. Chem.* **2005**, *280*, 28357–28364.

[116] Le Beyec, J.; Xu, R.; Lee, S. Y.; Nelson, C. M.; Rizki, A.; Alcaraz, J.; Bissell, M. J. Cell shape regulates global histone acetylation in human mammary epithelial cells. *Exp. Cell Res.* **2007**, *313*, 3066–3075.

[117] Wang, N.; Butler, J. P.; Ingber, D. E. Mechanotransduction across the cell surface and through the cytoskeleton. *Science* **1993**, *260*, 1124–1127.

[118] Halbleib, J. M.; Nelson, W. J. Cadherins in development: Cell adhesion, sorting, and tissue morphogenesis. *Genes Dev.* **2006**, *20*, 3199–3214.

[119] Bajpai, S.; Correia, J.; Feng, Y. F.; Figueiredo, J.; Sun, S. X.; Longmore, G. D.; Suriano, G.; Wirtz, D. alpha-Catenin mediates initial E-cadherin-dependent cell-cell recognition and subsequent bond strengthening. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 18331–18336.

[120] Bajpai, S.; Feng, Y. F.; Krishnamurthy, R.; Longmore, G. D.; Wirtz, D. Loss of alpha-catenin decreases the strength of single E-cadherin bonds between human cancer cells. *J. Biol. Chem.* **2009**, *284*, 18252–18259.

[121] Nelson, C. M.; Jean, R. P.; Tan, J. L.; Liu, W. F.; Sniadecki, N. J.; Spector, A. A.; Chen, C. S. Emergent patterns of growth controlled by multicellular form and mechanics. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 11594–11599.

[122] Ingber, D. E.; Tensegrity., I. Cell structure and hierarchical systems biology. *J. Cell Sci.* **2003**, *116*, 1157–1173.

[123] Farge, E. Mechanical induction of Twist in the Drosophila foregut/stomodeal primordium. *Curr. Biol* **2003**, *13*, 1365–1377.

[124] Supatto, W.; Debarre, D.; Moulia, B.; Brouzes, E.; Martin, J. L.; Farge, E.; Beaurepaire, E. In vivo modulation of morphogenetic movements in Drosophila embryos with femtosecond laser pulses. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 1047–1052.

[125] Hutson, M. S.; Veldhuis, J.; Ma, X. Y.; Lynch, H. E.; Cranston, P. G.; Brodland, G. W. Combining laser microsurgery and finite element modeling to assess cell-level epithelial mechanics. *Biophys. J.* **2009**, *97*, 3075–3085.

[126] Desprat, N.; Supatto, W.; Pouille, P. A.; Beaurepaire, E.; Farge, E. Tissue deformation modulates twist expression to determine anterior midgut differentiation in Drosophila embryos. *Dev. Cell* **2008**, *15*, 470–477.

[127] Wei, L.; Roberts, W.; Wang, L.; Yamada, M.; Zhang, S.; Zhao, Z.; Rivkees, S. A.; Schwartz, R. J.; Imanaka-Yoshida, K. Rho kinases play an obligatory role in vertebrate embryonic organogenesis. *Development* **2001**, *128*, 2953–2962.

[128] Ewald, A. J.; Brenot, A.; Duong, M.; Chan, B. S.; Werb, Z. Collective epithelial migration and cell rearrangements drive mammary branching morphogenesis. *Dev. Cell* **2008**, *14*, 570–581.

[129] Guilak, F.; Cohen, D. M.; Estes, B. T.; Gimble, J. M.; Liedtke, W.; Chen, C. S. Control of stem cell fate by physical interactions with the extracellular matrix. *Cell Stem Cell* **2009**, *5*, 17–26.

[130] Fine, H. A. Glioma stem cells: Not all created equal. *Cancer Cell* **2009**, *15*, 247–249.

[131] Lee, J.; Kotliarova, S.; Kotliarov, Y.; Li, A. G.; Su, Q.; Donin, N. M.; Pastorino, S.; Purow, B. W.; Christopher, N.; Zhang, W.; Park, J. K.; Fine, H. A. Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. *Cancer Cell* **2006**, *9*, 391–403.

[132] Pollard, S. M.; Yoshikawa, K.; Clarke, I. D.; Danovi, D.; Stricker, S.; Russell, R.; Bayani, J.; Head, R.; Lee, M.; Bernstein, M.; Squire, J. A.; Smith, A.; Dirks, P. Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens. *Cell Stem Cell* **2009**, *4*, 568–580.

[133] Singh, S. K.; Clarke, I. D.; Hide, T.; Dirks, P. B. Cancer stem cells in nervous system tumors. *Oncogene* **2004**, *23*, 7267–7273.

[134] Vescovi, A. L.; Galli, R.; Reynolds, B. A. Brain tumour stem cells. *Nat. Rev. Cancer* **2006**, *6*, 425–436.

[135] Engler, A. J.; Sen, S.; Sweeney, H. L.; Discher, D. E. Matrix elasticity directs stem cell lineage specification. *Cell* **2006**, *126*, 677–689.

[136] Winer, J. P.; Janmey, P. A.; McCormick, M. E.; Funaki, M. Bone marrow-derived human mesenchymal stem cells become quiescent on soft substrates but remain responsive to chemical or mechanical stimuli. *Tissue Eng. Part A* **2008**, *15*, 147–154.

[137] McBeath, R.; Pirone, D. M.; Nelson, C. M.; Bhadriraju, K.; Chen, C. S. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* **2004**, *6*, 483–495.

[138] Ruiz, S. A.; Chen, C. S. Emergence of patterned stem cell differentiation within multicellular structures. *Stem Cells* **2008**, *26*, 2921–2927.

[139] Saha, K.; Keung, A.; Irwin, E.; Li, Y.; Little, L.; Schaffer, D.; Healy, K. E. Substrate modulus directs neural stem cell behavior. *Biophys. J.* **2008**, *95*, 4426–4438.

[140] Keung, A. J.; de Juan-Pardo, E. M.; Schaffer, D. V.; Kumar, S. Rho GTPases mediate the mechanosensitive lineage commitment of neural stem cells. *Stem Cells* **2011**, in press. doi: 10.1002/stem.746.

[141] Chowdhury, F.; Na, S.; Li, D.; Poh, Y. C.; Tanaka, T. S.; Wang, F.; Wang, N. Material properties of the cell dictate stress-induced spreading and differentiation in embryonic stem cells. *Nat. Mater.* **2010**, *9*, 82–88.

[142] Mizejewski, G. J. Role of integrins in cancer: Survey of expression patterns. *Proc. Soc. Exp. Biol. Med.* **1999**, *222*, 124–138.

[143] Chen, F. A.; Repasky, E. A.; Bankert, R. B. Human lung-tumor associated antigen identified as an extracellular-matrix adhesion molecule. *J. Exp. Med.* **1991**, *173*, 1111–1119.

[144] Bissell, M. J.; LaBarge, M. A. Context, tissue plasticity, and cancer: Are tumor stem cells also regulated by the microenvironment? *Cancer Cell* **2005**, *7*, 17–23.

[145] Bissell, M. J.; Radisky, D. Putting tumours in context. *Nat. Rev. Cancer* **2001**, *1*, 46–54.

[146] Boudreau, N.; Sympson, C. J.; Werb, Z.; Bissell, M. J. Suppression of ice and apoptosis in mammary epithelial-cells by extracellular-matrix. *Science* **1995**, *267*, 891–893.

[147] Muthuswamy, S. K.; Li, D. M.; Lelievre, S.; Bissell, M. J.; Brugge, J. S. ErbB2, but not ErbB1, reinitiates proliferation and induces luminal repopulation in epithelial acini. *Nat. Cell Biol.* **2001**, *3*, 785–792.

[148] Weaver, V. M.; Petersen, O. W.; Wang, F.; Larabell, C. A.; Briand, P.; Damsky, C.; Bissell, M. J. Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. *J. Cell Biol.* **1997**, *137*, 231–245.

[149] Roskelley, C. D.; Srebrow, A.; Bissell, M. J. A hierarchy of ECM-mediated signaling regulates tissue-specific gene-expression. *Curr. Opin. Cell Biol.* **1995**, *7*, 736–747.

[150] Alcaraz, J.; Xu, R.; Mori, H.; Nelson, C. M.; Mroue, R.; Spencer, V. A.; Brownfield, D.; Radisky, D. C.; Bustamante, C.; Bissell, M. J. Laminin and biomimetic extracellular elasticity enhance functional differentiation in mammary epithelia. *EMBO J.* **2008**, *27*, 2829–2838.

[151] Gabarra-Niecko, V.; Schaller, M. D.; Dunty, J. M. FAK regulates biological processes important for the pathogenesis of cancer. *Cancer Metastasis Rev.* **2003**, *22*, 359–374.

[152] Mitra, S. K.; Hanson, D. A.; Schlaepfer, D. D. Focal adhesion kinase: In command and control of cell motility. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 56–68.

[153] Parsons, J. T. Focal adhesion kinase: The first ten years. *J. Cell Sci.* **2003**, *116*, 1409–1416.

[154] Schlaepfer, D. D.; Mitra, S. K.; Ilic, D. Control of motile and invasive cell phenotypes by focal adhesion kinase. *Biochim. Biophys. Acta – Mol. Cell Res* **2004**, *1692*, 77–102.

[155] Zhao, J.; Guan, J. L. Signal transduction by focal adhesion kinase in cancer. *Cancer Metastasis Rev.* **2009**, *28*, 35–49.

[156] Shibata, K.; Kikkawa, F.; Nawa, A.; Thant, A. A.; Naruse, K.; Mizutani, S.; Hamaguchi, M. Both focal adhesion kinase and c-ras are required for the enhanced matrix metalloproteinase 9 secretion by fibronectin in ovarian cancer cells. *Cancer Res.* **1998**, *58*, 900–903.

[157] Kokkinos, M. I.; Wafai, R.; Wong, M. K.; Newgreen, D. F.; Thompson, E. W.; Waltham, M. Vimentin and epithelial-mesenchymal transition in human breast cancer – Observations in vitro and in vivo. *Cells Tissues Organs* **2007**, *185*, 191–203.

[158] Pagan, R.; Martin, I.; Alonso, A.; Llobera, M.; Vilaro, S. Vimentin filaments follow the preexisting cytokeratin network during epithelial-mesenchymal transition of cultured neonatal rat hepatocytes. *Exp. Cell Res.* **1996**, *222*, 333–344.

[159] Willipinski-Stapelfeldt, B.; Riethdorf, S.; Assmann, V.; Woelfle, U.; Rau, T.; Sauter, G.; Heukeshoven, J.; Pantel, K. Changes in cytoskeletal protein composition indicative of an epithelial-mesenchymal transition in human micrometastatic and primary breast carcinoma cells. *Clin. Cancer Res.* **2005**, *11*, 8006–8014.

[160] Wang, H. B.; Dembo, M.; Wang, Y. L. Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. *Am. J. Physiol. – Cell Physiol.* **2000**, *279*, C1345–C1350.

[161] Khosravi-Far, R.; Solski, P. A.; Clark, G. J.; Kinch, M. S.; Der, C. J. Activation of Rac1, RhoA, and mitogen-activated protein-kinases is required for Ras transformation. *Mol. Cell. Biol.* **1995**, *15*, 6443–6453.

[162] Lin, R.; Bagrodia, S.; Cerione, R.; Manor, D. Novel Cdc42Hs mutant induces cellular transformation. *Curr. Biol.* **1997**, *7*, 794–797.

[163] Qiu, R. G.; Chen, J.; McCormick, F.; Symons, M. A role for Rho in Ras transformation. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 11781–11785.

[164] Sahai, E.; Marshall, C. J. RHO-GTPases and cancer. *Nat. Rev. Cancer* **2002**, *2*, 133.

[165] Welsh, C. F.; Roovers, K.; Villanueva, J.; Liu, Y. Q.; Schwartz, M. A.; Assoian, R. K. Timing of cyclin D1 expression within G1 phase is controlled by Rho. *Nat. Cell Biol.* **2001**, *3*, 950–957.

[166] Mammoto, A.; Huang, S.; Moore, K.; Oh, P.; Ingber, D. E. Role of RhoA, mDia, and ROCK in cell shape-dependent control of the Skp2-p27(kip1) pathway and the G(1)/S transition. *J. Biol. Chem.* **2004**, *279*, 26323–26330.

[167] Klein, E. A.; Yin, L. Q.; Kothapalli, D.; Castagnino, P.; Byfield, F. J.; Xu, T. N.; Levental, I.; Hawthorne, E.; Janmey, P. A.; Assoian, R. K. Cell-cycle

control by physiological matrix elasticity and in vivo tissue stiffening. *Curr. Biol.* **2009**, *19*, 1511–1518.
[168] Levental, K. R.; Yu, H. M.; Kass, L.; Lakins, J. N.; Egeblad, M.; Erler, J. T.; Fong, S. F. T.; Csiszar, K.; Giaccia, A.; Weninger, W.; Yamauchi, M.; Gasser, D. L.; Weaver, V. M. Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* **2009**, *139*, 891–906.
[169] Wozniak, M. A.; Desai, R.; Solski, P. A.; Der, C. J.; Keely, P. J. ROCK-generated contractility regulates breast epithelial cell differentiation in response to the physical properties of a three-dimensional collagen matrix. *J. Cell Biol.* **2003**, *163*, 583–595.
[170] Provenzano, P. P.; Inman, D. R.; Eliceiri, K. W.; Keely, P. J. Matrix density-induced mechanoregulation of breast cell phenotype, signaling and gene expression through a FAK-ERK linkage. *Oncogene* **2009**, *28*, 4326–4343.
[171] Provenzano, P. P.; Inman, D. R.; Eliceiri, K. W.; Beggs, H. E.; Keely, P. J. Mammary epithelial-specific disruption of focal adhesion kinase retards tumor formation and metastasis in a transgenic mouse model of human breast cancer. *Am. J. Pathol.* **2008**, *173*, 1551–1565.
[172] Gehler, S.; Baldassarre, M.; Lad, Y.; Leight, J. L.; Wozniak, M. A.; Riching, K. M.; Eliceiri, K. W.; Weaver, V. M.; Calderwood, D. A.; Keely, P. J. Filamin A-beta 1 integrin complex tunes epithelial cell response to matrix tension. *Mol. Biol. Cell* **2009**, *20*, 3224–3238.
[173] Carmeliet, P.; Jain, R. K. Angiogenesis in cancer and other diseases. *Nature* **2000**, *407*, 249–257.
[174] Naumov, G. N.; Akslen, L. A.; Folkman, J. Role of angiogenesis in human tumor dormancy – Animal models of the angiogenic switch. *Cell Cycle* **2006**, *5*, 1779–1787.
[175] Folkman, J.; Bach, M.; Rowe, J. W.; Davidoff, F.; Lambert, P.; Hirsch, C.; Goldberg, A.; Hiatt, H. H.; Glass, J.; Henshaw, E. Tumor angiogenesis – Therapeutic implications. *New Engl. J. Med* **1971**, *285*, 1182.
[176] Carmeliet, P. Mechanisms of angiogenesis and arteriogenesis. *Nat. Med* **2000**, *6*, 389–395.
[177] Carmeliet, P. Angiogenesis in health and disease. *Nat. Med* **2003**, *9*, 653–660.
[178] Ferrara, N.; Gerber, H. P.; LeCouter, J. The biology of VEGF and its receptors. *Nat. Med* **2003**, *9*, 669–676.
[179] Folkman, J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat. Med.* **1995**, *1*, 27–31.
[180] Hanahan, D.; Folkman, J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* **1996**, *86*, 353–364.
[181] Jain, R. K. Normalization of tumor vasculature: An emerging concept in antiangiogenic therapy. *Science* **2005**, *307*, 58–62.
[182] Davis, S.; Yancopoulos, G. D. The angiopoietins: Yin and yang in angiogenesis. *Curr. Top. Microbiol. Immunol.* **1999**, *237*, 173–185.
[183] Hackett, S. F.; Ozaki, H.; Strauss, R. W.; Wahlin, K.; Suri, C.; Maisonpierre, P.; Yancopoulos, G.; Campochiaro, P. A. Angiopoietin 2 expression in the retina: Upregulation during physiologic and pathologic neovascularization. *J. Cell. Physiol.* **2000**, *184*, 275–284.
[184] Boere, I. A.; Hamberg, P.; Sleijfer, S. It takes two to tango: Combinations of conventional cytotoxics with compounds targeting the vascular endothelial growth factor-vascular endothelial growth factor receptor pathway in patients with solid malignancies. *Cancer Sci.* **2010**, *101*, 7–15.
[185] Clark, E. R.; Clark, E. L. Microscopic observations on the growth of blood capillaries in the living mammal. *Am. J. Anat.* **1939**, *64*, 251–301.
[186] Huang, S.; Ingber, D. E. The structural and mechanical complexity of cell-growth control. *Nat. Cell Biol.* **1999**, *1*, E131–E138.
[187] Ingber, D. E. Tensegrity: The architectural basis of cellular mechanotransduction. *Annu. Rev. Physiol.* **1997**, *59*, 575–599.
[188] Ingber, D. E. Mechanical signalling and the cellular response to extracellular matrix in angiogenesis and cardiovascular physiology. *Circ. Res* **2002**, *91*, 877–887.
[189] Ingber, D. E. Cellular mechanotransduction: Putting all the pieces together again. *FASEB J.* **2006**, *20*, 811–827.
[190] Ingber, D. E.; Folkman, J. How does extracellular-matrix control capillary morphogenesis. *Cell* **1989**, *58*, 803–805.
[191] Ausprunk, D. H.; Folkman, J. Migration and proliferation of endothelial cells in preformed and newly formed blood-vessels during tumor angiogenesis. *Microvasc. Res* **1977**, *14*, 53–65.
[192] Ingber, D. E. Fibronectin controls capillary endothelial-cell growth by modulating cell-shape. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 3579–3583.
[193] Chen, C. S.; Mrksich, M.; Huang, S.; Whitesides, G. M.; Ingber, D. E. Geometric control of cell life and death. *Science* **1997**, *276*, 1425–1428.
[194] Wang, N.; Ostuni, E.; Whitesides, G. M.; Ingber, D. E. Micropatterning tractional forces in living cells. *Cell Motil. Cytoskel* **2002**, *52*, 97–106.
[195] Sieminski, A. L.; Hebbel, R. P.; Gooch, K. J. The relative magnitudes of endothelial force generation and matrix stiffness modulate capillary morphogenesis in vitro. *Exp. Cell Res.* **2004**, *297*, 574–584.
[196] Kuzuya, M.; Asai, T.; Kanda, S.; Maeda, K.; Cheng, X. W.; Iguchi, A. Glycation cross-links inhibit matrix metalloproteinase-2 activation in vascular smooth muscle cells cultured on collagen lattice. *Diabetologia* **2001**, *44*, 433–436.
[197] Ghajar, C. M.; Chen, X.; Harris, J. W.; Suresh, V.; Hughes, C. C. W.; Jeon, N. L.; Putnam, A. J.; George, S. C. The effect of matrix density on the regulation of 3-D capillary morphogenesis. *Biophys. J.* **2008**, *94*, 1930–1941.
[198] Kniazeva, E.; Putnam, A. J. Endothelial cell traction and ECM density influence both capillary morphogenesis and maintenance in 3-D. *Am. J. Physiol. – Cell Physiol.* **2009**, *297*, C179–C187.
[199] Moore, K. A.; Polte, T.; Huang, S.; Shi, B.; Alsberg, E.; Sunday, M. E.; Ingber, D. E. Control of basement membrane remodeling and epithelial branching morphogenesis in embryonic lung by Rho and cytoskeletal tension. *Dev. Dyn.* **2005**, *232*, 268–281.
[200] Kilarski, W. W.; Samolov, B.; Petersson, L.; Kvanta, A.; Gerwins, P. Biomechanical regulation of blood vessel growth during tissue vascularization. *Nat. Med* **2009**, *15*, 657–664.
[201] Wang, W. G.; Wyckoff, J. B.; Frohlich, V. C.; Oleynikov, Y.; Huttelmaier, S.; Zavadil, J.; Cermak, L.; Bottinger, E. P.; Singer, R. H.; White, J. G.; Segall, J. E.; Condeelis, J. S. Single cell behavior in metastatic primary mammary tumors correlated with gene expression patterns revealed by molecular profiling. *Cancer Res.* **2002**, *62*, 6278–6288.
[202] Egeblad, M.; Werb, Z. New functions for the matrix metalloproteinases in cancer progression. *Nat. Rev. Cancer* **2002**, *2*, 161–174.
[203] Giannelli, G.; FalkMarzillier, J.; Schiraldi, O.; Stetler Stevenson, W. G.; Quaranta, V. Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5. *Science* **1997**, *277*, 225–228.
[204] Peschon, J. J.; Slack, J. L.; Reddy, P.; Stocking, K. L.; Sunnarborg, S. W.; Lee, D. C.; Russell, W. E.; Castner, B. J.; Johnson, R. S.; Fitzner, J. N.; Boyce, R. W.; Nelson, N.; Kozlosky, C. J.; Wolfson, M. F.; Rauch, C. T.; Cerretti, D. P.; Paxton, R. J.; March, C. J.; Black, R. A. An essential role for ectodomain shedding in mammalian development. *Science* **1998**, *282*, 1281–1284.
[205] Blobel, C. P. ADAMs: Key components in EGFR signalling and development. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 32–43.
[206] Rivilis, I.; Milkiewicz, M.; Boyd, P.; Goldstein, J.; Brown, M. D.; Egginton, S.; Hansen, F. M.; Hudlicka, O.; Haas, T. L. Differential involvement of MMP-2 and VEGF during muscle stretch-versus shear stress-induced angiogenesis. *Am. J. Physiol. – Heart Circ. Physiol.* **2002**, *283*, H1430–H1438.
[207] Blain, E. J.; Gilbert, S. J.; Wardale, R. J.; Capper, S. J.; Mason, D. J.; Duance, V. C. Up-regulation of matrix metalloproteinase expression and activation following cyclical compressive loading of articular cartilage in vitro. *Arch. Biochem. Biophys.* **2001**, *396*, 49–55.
[208] Honda, K.; Ohno, S.; Tanimoto, K.; Ijuin, C.; Tanaka, N.; Doi, T.; Kato, Y.; Tanne, K. The effects of high magnitude cyclic tensile load on cartilage matrix metabolism in cultured chondrocytes. *Eur. J. Cell Biol.* **2000**, *79*, 601–609.
[209] Haseneen, N. A.; Vaday, G. G.; Zucker, S.; Foda, H. D. Mechanical stretch induces MMP-2 release and activation in lung endothelium: Role of EMMPRIN. *Am. J. Physiol. – Lung Cell. Mol. Physiol.* **2003**, *284*, L541–L547.
[210] Milkiewicz, M.; Haas, T. L. Effect of mechanical stretch on HIF-1 alpha and MMP-2 expression in capillaries isolated from overloaded skeletal muscles: Laser capture microdissection study. *Am. J. Physiol. – Heart Circ. Physiol.* **2005**, *289*, H1315–H1320.
[211] Wang, B. W.; Chang, H.; Lin, S. K.; Kuan, P. L.; Shyu, K. G. Induction of matrix metalloproteinases-14 and -2 by cyclical mechanical stretch is mediated by tumor necrosis factor-alpha in cultured human umbilical vein endothelial cells. *Cardiovasc. Res* **2003**, *59*, 460–469.
[212] Nelson, C. M.; Khauv, D.; Bissell, M. J.; Radisky, D. C. Change in cell shape is required for matrix metalloproteinase-induced epithelial-mesenchymal transition of mammary epithelial cells. *J. Cell. Biochem.* **2008**, *105*, 25–33.
[213] Radisky, D. C.; Levy, D. D.; Littlepage, L. E.; Liu, H.; Nelson, C. M.; Fata, J. E.; Leake, D.; Godden, E. L.; Albertson, D. G.; Nieto, M. A.; Werb, Z.; Bissell, M. J. Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. *Nature* **2005**, *436*, 123–127.
[214] Friedl, P.; Wolf, K. Tumour-cell invasion and migration: Diversity and escape mechanisms. *Nat. Rev. Cancer* **2003**, *3*, 362–374.

[215] Friedl, P.; Wolf, K. Proteolytic interstitial cell migration: A five-step process. *Cancer Metastasis Rev.* **2009**, *28*, 129–135.

[216] Sabeh, F.; Shimizu-Hirota, R.; Weiss, S. J. Protease-dependent versus - independent cancer cell invasion programs: Three-dimensional amoeboid movement revisited. *J. Cell Biol.* **2009**, *185*, 11–19.

[217] Sahai, E. Mechanisms of cancer cell invasion. *Curr. Opin. Genet. Dev* **2005**, *15*, 87–96.

[218] Wolf, K.; Mazo, I.; Leung, H.; Engelke, K.; von Andrian, U. H.; Deryugina, E. I.; Strongin, A. Y.; Brocker, E. B.; Friedl, P. Compensation mechanism in tumor cell migration: Mesenchymal-amoeboid transition after blocking of pericellular proteolysis. *J. Cell Biol.* **2003**, *160*, 267–277.

[219] Wolf, K.; Wu, Y. I.; Liu, Y.; Geiger, J.; Tam, E.; Overall, C.; Stack, M. S.; Friedl, P. Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion. *Nat. Cell Biol.* **2007**, *9*, 893–904.

[220] Friedl, P.; Wolf, K. Plasticity of cell migration: A multiscale tuning model. *J. Cell Biol.* **2009**, *188*, 11–19.

[221] Bloom, R. J.; George, J. P.; Celedon, A.; Sun, S. X.; Wirtz, D. Mapping local matrix remodeling induced by a migrating tumor cell using three-dimensional multiple-particle tracking. *Biophys. J.* **2008**, *95*, 4077–4088.

[222] Fraley, S. I.; Feng, Y. F.; Krishnamurthy, R.; Kim, D. H.; Celedon, A.; Longmore, G. D.; Wirtz, D. A distinctive role for focal adhesion proteins in three-dimensional cell motility. *Nat. Cell Biol.* **2010**, *12*, 598–604.

[223] Yamaguchi, H.; Lorenz, M.; Kempiak, S.; Sarmiento, C.; Coniglio, S.; Symons, M.; Segall, J.; Eddy, R.; Miki, H.; Takenawa, T.; Condeelis, J. Molecular mechanisms of invadopodium formation: The role of the N-WASP-Arp2/3 complex pathway and cofilin. *J. Cell Biol.* **2005**, *168*, 441–452.

[224] Yamaguchi, H.; Wyckoff, J.; Condeelis, J. *Cell migration in tumors. Curr. Opin. Cell Biol.* **2005**, *17*, 559–564.

[225] Collin, O.; Na, S.; Chowdhury, F.; Hong, M.; Shin, M. E.; Wang, F.; Wang, N. Self-organized podosomes are dynamic mechanosensors. *Curr. Biol.* **2008**, *18*, 1288–1294.

[226] Alexander, N. R.; Branch, K. M.; Parekh, A.; Clark, E. S.; Lwueke, L. C.; Guelcher, S. A.; Weaver, A. M. Extracellular matrix rigidity promotes invadopodia activity. *Curr. Biol.* **2008**, *18*, 1295–1299.

[227] Albiges-Rizo, C.; Destaing, O.; Fourcade, B.; Planus, E.; Block, M. R. Actin machinery and mechanosensitivity in invadopodia, podosomes and focal adhesions. *J. Cell Sci.* **2009**, *122*, 3037–3049.

[228] Miles, F. L.; Pruitt, F. L.; van Golen, K. L.; Cooper, C. R. Stepping out of the flow: Capillary extravasation in cancer metastasis. *Clin. Exp. Metastasis* **2008**, *25*, 305–324.

[229] Worthylake, R. A.; Lemoine, S.; Watson, J. M.; Burridge, K. RhoA is required for monocyte tail retraction during transendothelial migration. *J. Cell Biol.* **2001**, *154*, 147–160.

[230] Stewart, D. A.; Cooper, C. R.; Sikes, R. A. Changes in extracellular matrix (ECM) and ECM-associated proteins in the metastatic progression of prostate cancer. *Reprod. Biol. Endocrinol.* **2004**, *2*, 2.

[231] Heino, J.; Massague, J. Transforming growth factor-beta switches the pattern of integrins expressed in MG-63 human osteosarcoma cells and causes a selective loss of cell adhesion to laminin. *J. Biol. Chem* **1989**, *264*, 21806–21811.

[232] Mierke, C. T.; Zitterbart, D. P.; Kollmannsberger, P.; Raupach, C.; Schlotzer-Schrehardt, U.; Goecke, T. W.; Behrens, J.; Fabry, B. Breakdown of the endothelial barrier function in tumor cell transmigration. *Biophys. J.* **2008**, *94*, 2832–2846.

[233] Kostic, A.; Lynch, C. D.; Sheetz, M. P. Differential matrix rigidity response in breast cancer cell lines correlates with the tissue tropism. *PLoS ONE* **2009**, *4*, e6361.

[234] Minn, A. J.; Gupta, G. P.; Padua, D.; Bos, P.; Nguyen, D. X.; Nuyten, D.; Kreike, B.; Zhang, Y.; Wang, Y. X.; Ishwaran, H.; Foekens, J. A.; van de Vijver, M.; Massague, J. Lung metastasis genes couple breast tumor size and metastatic spread. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 6740–6745.

[235] Minn, A. J.; Gupta, G. P.; Siegel, P. M.; Bos, P. D.; Shu, W. P.; Giri, D. D.; Viale, A.; Olshen, A. B.; Gerald, W. L.; Massague, J. Genes that mediate breast cancer metastasis to lung. *Nature* **2005**, *436*, 518–524.

[236] Friedman, H. S.; Kerby, T.; Calvert, H. Temozolomide and treatment of malignant glioma. *Clin. Cancer Res.* **2000**, *6*, 2585–2597.

[237] Giese, A.; Bjerkvig, R.; Berens, M. E.; Westphal, M. Cost of migration: Invasion of malignant gliomas and implications for treatment. *J. Clin. Oncol.* **2003**, *21*, 1624–1636.

[238] Kleihues, P.; Louis, D. N.; Scheithauer, B. W.; Rorke, L. B.; Reifenberger, G.; Burger, P. C.; Cavenee, W. K. The WHO classification of tumors of the nervous system. *J. Neuropathol. Exp. Neurol.* **2002**, *61*, 215–225.

[239] Reardon, D. A.; Rich, J. N.; Friedman, H. S.; Bigner, D. D. Recent advances in the treatment of malignant astrocytoma. *J. Clin. Oncol.* **2006**, *24*, 1253–1265.

[240] Schwartzbaum, J. A.; Fisher, J. L.; Aldape, K. D.; Wrensch, M. Epidemiology and molecular pathology of glioma. *Nat. Clin. Pract. Neurol.* **2006**, *2*, 494–503.

[241] Teodorczyk, M.; Martin-Villalba, A. Sensing invasion: Cell surface receptors driving spreading of glioblastoma. *J. Cell. Physiol.* **2009**, *222*, 1–10.

[242] Bellail, A. C.; Hunter, S. B.; Brat, D. J.; Tan, C.; Van Meir, E. G. Microregional extracellular matrix heterogeneity in brain modulates glioma cell invasion. *Int. J. Biochem. Cell Biol.* **2004**, *36*, 1046–1069.

[243] Blasberg, R. G.; Nakagawa, H. Bourdon, M. A.; Groothuis, D. R.; Patlak, C. S.; Bigner, D. D. Regional localization of a glioma-associated antigen defined by monoclonal-antibody 81C6 in vivo – Kinetics and implications for diagnosis and therapy. *Cancer Res.* **1987**, *47*, 4432–4443.

[244] Reardon, D. A.; Akabani, G.; Coleman, R. E.; Friedman, A. H.; Friedman, H. S.; Herndon, J. E. 2nd; McLendon, R. E.; Pegram, C. N.; Provenzale, J. M.; Quinn, J. A.; Rich, J. N.; Vredenburgh, J. J.; Desjardins, A.; Gururangan, S.; Badruddoja, M.; Dowell, J. M.; Wong, T. Z.; Zhao, X. G.; Zalutsky, M. R.; Bigner, D. D. Salvage radioimmunotherapy with murine iodine-131-labeled antitenascin monoclonal antibody 81C6 for patients with recurrent primary and metastatic malignant brain tumors: phase II study results. *J. Clin. Oncol* **2006**, *24*(1), 115–122.

[245] Reardon, D. A.; Akabani, G.; Coleman, R. E.; Friedman, A. H.; Friedman, H. S.; Herndon, J. E.; Cokgor, I.; McLendon, R. E.; Pegram, C. N.; Provenzale, J. M.; Quinn, J. A.; Rich, J. N.; Regalado, L. V.; Sampson, J. H.; Shafman, T. D.; Wikstrand, C. J.; Wong, T. Z.; Zhao, X. G.; Zalutsky, M. R.; Bigner, D. D. Phase II trial of murine I-131-labeled antitenascin monoclonal antibody 81C6 administered into surgically created resection cavities of patients with newly diagnosed malignant gliomas. *J. Clin. Oncol.* **2002**, *20*, 1389–1397.

[246] Reardon, D. A.; Desjardins, A.; Rich, J. N.; Vredenburgh, J. J. The emerging role of anti-angiogenic therapy for malignant glioma. *Curr. Treat. Options Oncol.* **2008**, *9*(1), 1–22.

[247] Unsgaard, G.; Rygh, O. M.; Selbekk, T.; Muller, T. B.; Kolstad, F.; Lindseth, F.; Hernes, T. A. N. Intra-operative 3D ultrasound in neurosurgery. *Acta Neurochir.* **2006**, *148*, 235–253.

[248] Demuth, T.; Berens, M. E. Molecular mechanisms of glioma cell migration and invasion. *J. Neuro-Oncol.* **2004**, *70*, 217–228.

[249] Hikawa, T.; Mori, T.; Abe, T.; Hori, S. The ability in adhesion and invasion of drug-resistant human glioma cells. *J. Exp. Clin. Cancer Res.* **2000**, *19*, 357–362.

[250] Yuan, L.; Siegel, M.; Choi, K.; Khosla, C.; Miller, C. R.; Jackson, E. N.; Piwnica-Worms, D.; Rich, K. M. Transglutaminase 2 inhibitor, KCC009, disrupts fibronectin assembly in the extracellular matrix and sensitizes orthotopic glioblastomas to chemotherapy. *Oncogene* **2007**, *26*, 2563–2573.

[251] Thomas, T. W.; DiMilla, P. A. Spreading and motility of human glioblastoma cells on sheets of silicone rubber depend on substratum compliance. *Med. Biol. Eng. Comput.* **2000**, *38*, 360–370.

[252] Georges, P. C.; Miller, W. J.; Meaney, D. F.; Sawyer, E. S.; Janmey, P. A. Matrices with compliance comparable to that of brain tissue select neuronal over glial growth in mixed cortical cultures. *Biophys. J.* **2006**, *90*, 3012–3018.

[253] Saha, K.; Keung, A. J.; Irwin, E. F.; Li, Y.; Little, L.; Schaffer, D. V.; Healy, K. E. Substrate modulus directs neural stem cell behavior. *Biophys. J.* **2008**, *95*, 4426–4438.

[254] Shi, P.; Shen, K.; Ghassemi, S.; Hone, J.; Kam, L. C. Dynamic force generation by neural stem cells. *Cell. Mol. Bioeng* **2009**, *2*, 464–474.

[255] Ulrich, T. A.; Pardo, E. M. D.; Kumar, S. The mechanical rigidity of the extracellular matrix regulates the structure, motility, and proliferation of glioma cells. *Cancer Res.* **2009**, *69*, 4167–4174.

[256] Sen, S.; Dong, M.; Kumar, S. Isoform-specific contributions of alpha-actinin to glioma cell mechanobiology. *PLoS ONE* **2009**, *4*, e8427.

[257] Belot, N.; Rorive, S.; Doyen, I.; Lefranc, F.; Bruyneel, E.; Dedecker, R.; Micik, S.; Brotchi, J.; Decaestecker, C.; Salmon, I.; Kiss, R.; Camby, I. Molecular characterization of cell substratum attachments in human glial tumors relates to prognostic features. *Glia* **2001**, *36*, 375–390.

[258] Kole, T. P.; Tseng, Y.; Jiang, I.; Katz, J. L.; Wirtz, D. Intracellular mechanics of migrating fibroblasts. *Mol. Biol. Cell* **2005**, *16*, 328–338.

[259] Tseng, Y.; Kole, T. P.; Wirtz, D. Micromechanical mapping of live cells by multiple-particle-tracking microrheology. *Biophys. J.* **2002**, *83*, 3162–3176.

[260] Xu, J. Y.; Wirtz, D.; Pollard, T. D. Dynamic cross-linking by alpha-actinin determines the mechanical properties of actin filament networks. *J. Biol. Chem.* **1998**, *273*, 9570–9576.

[261] Yeung, T.; Georges, P. C.; Flanagan, L. A.; Marg, B.; Ortiz, M.; Funaki, M.; Zahir, N.; Ming, W. Y.; Weaver, V.; Janmey, P. A. Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. *Cell Motil. Cytoskel* **2005**, *60*, 24–34.
[262] Kim, S.; Healy, K. E. Synthesis and characterization of injectable poly(N-isopropylacrylamide-co-acrylic acid) hydrogels with proteolytically degradable cross-links. *Biomacromolecules* **2003**, *4*, 1214–1223.
[263] Peyton, S. R.; Raub, C. B.; Keschrumrus, V. P.; Putnam, A. J. The use of poly(ethylene glycol) hydrogels to investigate the impact of ECM chemistry and mechanics on smooth muscle cells. *Biomaterials* **2006**, *27*, 4881–4893.
[264] Seliktar, D.; Zisch, A. H.; Lutolf, M. P.; Wrana, J. L.; Hubbell, J. A. MMP-2 sensitive, VEGF-bearing bioactive hydrogels for promotion of vascular healing. *J. Biomed. Mater. Res. A* **2004**, *68*, 704–716.
[265] Takagi, J. Structural basis for ligand recognition by RGD (Arg-Gly-Asp)-dependent integrins. *Biochem. Soc. Trans.* **2004**, *32*, 403–406.
[266] Willits, R. K.; Skornia, S. L. Effect of collagen gel stiffness on neurite extension. *J. Biomater. Sci. Polym. Ed.* **2004**, *15*, 1521–1531.
[267] Zaman, M. H.; Trapani, L. M.; Sieminski, A. L.; Mackellar, D.; Gong, H.; Kamm, R. D.; Wells, A.; Lauffenburger, D. A.; Matsudaira, P. Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 10889–10894.
[268] Ulrich, T. A.; Jain, A.; Tanner, K.; Mackay, J. L.; Kumar, S. Probing cellular mechanobiology in three-dimensional culture with collagen-agarose matrices. *Biomaterials* **2010**, *31*, 1875–1884.
[269] Deisboeck, T. S.; Berens, M. E.; Kansal, A. R.; Torquato, S.; Stemmer-Rachamimov, A. O.; Chiocca, E. A. Pattern of self-organization in tumour systems: Complex growth dynamics in a novel brain tumour spheroid model. *Cell Prolif.* **2001**, *34*, 115–134.
[270] Del Duca, D.; Werbowetski, T.; Del Maestro, R. F. Spheroid preparation from hanging drops: Characterization of a model of brain tumor invasion. *J. Neuro-Oncol.* **2004**, *67*, 295–303.
[271] Heese, O.; Disko, A.; Zirkel, D.; Westphal, M.; Lamszus, K. Neural stem cell migration toward gliomas in vitro. *Neuro-Oncology* **2005**, *7*, 476–484.
[272] Kaufman, L. J.; Brangwynne, C. P.; Kasza, K. E.; Filippidi, E.; Gordon, V. D.; Deisboeck, T. S.; Weitz, D. A. Glioma expansion in collagen I matrices: Analyzing collagen concentration-dependent growth and motility patterns. *Biophys. J.* **2005**, *89*, 635–650.
[273] MuellerKlieser, W. Three-dimensional cell cultures: From molecular mechanisms to clinical applications. *Am. J. Physiol. – Cell Physiol.* **1997**, *273*, C1109–C1123.
[274] Stein, A. M.; Demuth, T.; Mobley, D.; Berens, M.; Sander, L. M. A mathematical model of glioblastoma tumor spheroid invasion in a three-dimensional in vitro experiment. *Biophys. J.* **2007**, *92*, 356–365.
[275] Winer, J. P.; Oake, S.; Janmey, P. A. Non-linear elasticity of extracellular matrices enables contractile cells to communicate local position and orientation. *PLoS ONE* **2009**, *4*, e6382.
[276] Brooks, P. C.; Stromblad, S.; Klemke, R.; Visscher, D.; Sarkar, F. H.; Cheresh, D. A. Antiintegrin alpha-v-beta-3 blocks human breast-cancer growth and angiogenesis in human skin. *J. Clin. Invest.* **1995**, *96*, 1815–1822.
[277] Drake, C. J.; Cheresh, D. A.; Little, C. D. An antagonist of integrin alpha(v)beta(3) prevents maturation of blood-vessels during embryonic neovascularization. *J. Cell Sci.* **1995**, *108*, 2655–2661.
[278] Miyauchi, A.; Alvarez, J.; Greenfield, E. M.; Teti, A.; Grano, M.; Colucci, S.; Zamboninzallone, A.; Ross, F. P.; Teitelbaum, S. L.; Cheresh, D.; Hruska, K. A. Recognition of osteopontin and related peptides by an alpha-v-beta-3 integrin stimulates immediate cell signals in osteoclasts. *J. Biol. Chem.* **1991**, *266*, 20369–20374.
[279] Sipkins, D. A.; Cheresh, D. A.; Kazemi, M. R.; Nevin, L. M.; Bednarski, M. D.; Li, K. C. P. Detection of tumor angiogenesis in vivo by alpha(v)beta(3)-targeted magnetic resonance imaging. *Nat. Med* **1998**, *4*, 623–626.
[280] Rader, C.; Popkov, M.; Neves, J. A.; Barbas, C. F. Integrin alpha v beta 3-targeted therapy for Kaposi's sarcoma with an in vitro-evolved antibody. *FASEB J.* **2002**, *16*, 2000.
[281] Mitjans, F.; Sander, D.; Adan, J.; Sutter, A.; Martinez, J. M.; Jaggle, C. S.; Moyano, J. M.; Kreysch, H. G.; Piulats, J.; Goodman, S. L. An anti-alpha-v-integrin antibody that blocks integrin function inhibits the development of a human-melanoma in nude-mice. *J. Cell Sci.* **1995**, *108*, 2825–2838.
[282] Brunton, V. G.; Frame, M. C. Src and focal adhesion kinase as therapeutic targets in cancer. *Curr. Opin. Pharmacol.* **2008**, *8*, 427–432.
[283] Masiello, D.; Gorospe, G.; Yang, A. S. The occurrence and management of fluid retention associated with TKI therapy in CML, with a focus on dasatinib. *J. Hematol. Oncol.* **2009**, *2*, 46.
[284] Halder, J.; Lin, Y. G.; Merritt, W. M.; Spannuth, W. A.; Nick, A. M.; Honda, T.; Kamat, A. A.; Han, L. Y.; Kim, T. J.; Lu, C.; Tari, A. M.; Bornmann, W.; Fernandez, A.; Lopez-Berestein, G.; Sood, A. K. Therapeutic efficacy of a novel focal adhesion kinase inhibitor TAE226 in ovarian carcinoma. *Cancer Res.* **2007**, *67*, 10976–10983.
[285] Roberts, W. G.; Ung, E.; Whalen, P.; Cooper, B.; Hulford, C.; Autry, C.; Richter, D.; Emerson, E.; Lin, J.; Kath, J.; Coleman, K.; Yao, L.; Martinez-Alsina, L.; Lorenzen, M.; Berliner, M.; Luzzio, M.; Patel, N.; Schmitt, E.; LaGreca, S.; Jani, J.; Wessel, M.; Marr, E.; Griffor, M.; Vajdos, F. Antitumor activity and pharmacology of a selective focal adhesion kinase inhibitor, PF-562,271. *Cancer Res.* **2008**, *68*, 1935–1944.
[286] Lu, Q.; Longo, F. M.; Zhou, H. C.; Massa, S. M.; Chen, Y. H. Signaling through Rho GTPase pathway as viable drug target. *Curr. Med. Chem.* **2009**, *16*, 1355–1365.
[287] Collisson, E. A.; Kleer, C.; Wu, M.; De, A.; Gambhir, S. S.; Merajver, S. D.; Kolodney, M. S. Atorvastatin prevents RhoC isoprenylation, invasion, and metastasis in human melanoma cells. *Mol. Cancer Therapeut* **2003**, *2*, 941–948.
[288] Xue, F.; Zhang, J. J.; Qiu, F.; Zhang, M.; Chen, X. S.; Li, Q. G.; Han, L. Z.; Xi, Z. F.; Xia, Q. Rho signaling inhibitor, Y-27632, inhibits invasiveness of metastastic hepatocellular carcinoma in a mouse model. *Chinese Med. J* **2007**, *120*, 2304–2307.
[289] Liu, S. J.; Goldstein, R. H.; Scepansky, E. M.; Rosenblatt, M. Inhibition of Rho-associated kinase signaling prevents breast cancer metastasis to human bone. *Cancer Res.* **2009**, *69*, 8742–8751.
[290] Ying, H.; Biroc, S. L.; Li, W. W.; Alicke, B.; Xuan, J. A.; Pagila, R.; Ohashi, Y.; Okada, T.; Kamata, Y.; Dinter, H. The Rho kinase inhibitor fasudil inhibits tumor progression in human and rat tumor models. *Mol. Cancer Therapeut* **2006**, *5*, 2158–2164.