18 Mechanobiology in Health and Disease in the Central Nervous System

Theresa A. Ulrich and Sanjay Kumar

CONTENTS

18.1 INTRODUCTION

Over 2300 years have passed since Aristotle first identified "touch" as one of the five exteroceptive senses, marking one of the first discussions in the academic literature of mechanosensation, the ability to sense mechanical forces. In the intervening time, we have learned a great deal about how cells convert mechanical sensations into chemical signals, a process known as mechanotransduction. Importantly, we have learned that mechanotransduction serves not only as a sensory mechanism for cells and tissues, but as a regulatory mechanism as well. Numerous studies over the last several decades have revealed the robust sensitivity of mammalian cells to mechanical cues such as shear flow, cyclic strain, and microenvironmental stiffness, demonstrating that the mechanical microenvironment participates centrally in the homeostasis of cells and tissues, and that disruptions of these mechanical cues can contribute to the onset and progression of disease [1,2]. The field of mechanobiology, which examines how cells sense, process, and respond to mechanical stimuli, has emerged at the intersection of the physical and biological sciences to examine the often subtle yet complex relationship between mechanical force and cell and tissue behavior.

The central nervous system (CNS) has been the focus of extensive tissue biomechanics research for over three decades, spurred in part by widespread public interest in brain and spinal cord injury in automobile accidents, sports injuries, and other traumatic settings. Yet, despite this history of pioneering work in tissue-level biomechanics, investigation of cellular mechanobiology in the CNS has been comparatively limited. While the earliest work in this field stemmed directly from studies of neuronal trauma and regeneration (e.g., cell-level studies of axonal repair following injury), the last decade has seen rapid acceleration in the pace of research exploring how the properties of the normal (nontraumatized) mechanical microenvironment affect the health and disease of CNS cells and tissues.

Here, we provide an overview of the nascent field of cellular mechanobiology in the CNS, beginning with a description of the structure and mechanical microenvironment within the brain and spinal cord, continuing with an overview of the mechanobiological characteristics of cells in the CNS, and concluding with demonstrated and potential roles for how dynamic interactions between cells and their mechanical microenvironment can contribute to the onset or exacerbation of disease in the nervous system.

18.2 THE MECHANICAL MICROENVIRONMENT OF CNS TISSUES

No other organ system in the body is as carefully protected from external mechanical forces as the CNS by its encasement within the bony skull and vertebral column (and as a consequence, no other organ system is as vulnerable to the buildup of excess pressure during disease or injury, as we will discuss in the last section of this chapter). The soft tissues inside the CNS are further protected by three membranes known as meninges (the superficial dura mater, central arachnoid mater, and deep pia mater) and by the serum-like cerebrospinal fluid (CSF) that bathes both the brain and the spinal cord. A network of cavities deep within the brain, known as ventricles, is lined by a population of epithelial cells whose main function is to secrete CSF and to aid its circulation throughout the ventricles, the subarachnoid space (located between the arachnoid mater and pia mater), and the central canal of the spinal cord.

The brain parenchyma is organized into distinct anatomical structures (the cerebral hemispheres, diencephalon, brain stem, and cerebellum), which are crisscrossed by blood vessels and defined by a complex and highly organized neuroarchitecture of white matter (composed of neuronal axons) and gray matter (composed of nerve cell bodies). Far less is known about the structure and function of the extracellular matrix (ECM) in CNS tissues than in most other organ systems in the body. A well-defined ECM containing large amounts of collagen, fibronectin, and laminin exists in the basement membrane of the cerebral vasculature and in the meninges surrounding the brain cortex; however, these structures stand in stark contrast to the rest of the brain parenchyma, which consists of a relatively amorphous, anisotropic, and heterogeneous matrix containing mainly hyaluronic acid as well as various other glycosaminoglycans and proteoglycans [3,4].

The spinal cord is similarly heterogeneous, consisting of longitudinally oriented white matter tracts surrounding a central, butterfly shaped region of gray matter. Like the brain, the spinal cord is organized into distinct anatomical structures with specialized functions (e.g., the ascending and descending sensory and motor white matter tracts), and contains an anisotropic ECM that consists mostly of hyaluronic acid [5]. The structural inhomogeneities within both the brain and the spinal cord lead to extensive heterogeneity in the mechanical and biochemical microenvironment of resident cells, however, cyclic mechanical strain is generated throughout the CNS by pulsatile flow of CSF at a rate of approximately 1 Hz [6–8].

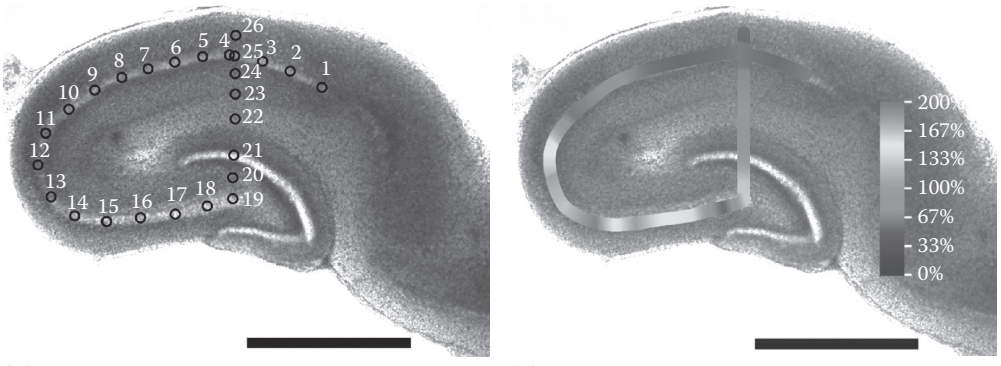
The mechanical stiffness of human tissues varies widely, ranging from very soft brain, fat, and mammary tissues with elastic moduli of less than 1 kPa to calcified bone tissues with elastic moduli near 10,000 kPa (10 MPa). Reported measurements of the mechanical properties of human and animal brain and spinal cord tissues have varied by more than an order of magnitude from several hundred Pa to several kPa, likely due to variations in species, age, sample size, and anatomic origin, sample preparation, testing conditions, and time postmortem [9–13].

The spectrum of available methods and published results for mechanical analysis of CNS tissues has recently been reviewed in considerable detail [14,15]. Here, we will focus our discussion on the two methods that are most commonly used today: rotational rheometry and magnetic resonance elastography (MRE). Rotational rheometry has been employed in materials characterization for decades and generally involves the small-amplitude oscillatory deformation of a sample of known geometry, measurement of the resulting stress within the sample, and calculation of material properties, such as the shear modulus and elastic modulus (see [16] for a straightforward and detailed explanation of rheometry theory and methods in the context of biological samples). MRE, on the other hand, is a noninvasive alternative that allows determination of tissue stiffness in living

subjects, and therefore has become increasingly popular for measuring the mechanical properties of brain tissue [17–20]. This technique involves application of acoustic shear waves to the tissue of interest; a phase-sensitive magnetic resonance imaging (MRI) sequence is then used to visualize and quantitatively measure propagating strain waves throughout the tissue, and an algorithm is subsequently used to generate a map of the elastic modulus of the imaged region [21].

A recent report by Vappou et al. demonstrated a surprisingly strong agreement between the linear viscoelastic behavior of brain tissues measured by rotational rheometry and MRE [22], which may facilitate longitudinal or comparative studies of brain tissue mechanics in healthy and diseased individuals in the future. However, an important caveat of both rotational rheometry and MRE is that both measurements require a macroscopic sample of tissue that may contain multiple cell types and ECM with microscale heterogeneities, thereby complicating inference of the local mechanical microenvironment of single cells. This limitation was illustrated by a recent atomic force microscopy (AFM) indentation study by Elkin et al., which measured the apparent elastic modulus of the cell-scale microenvironment in anatomical subregions of the rat hippocampus, revealing spatial heterogeneity in local tissue mechanics (Figure 18.1) [11].

The brain is generally considered to be a nonlinear viscoelastic material with mechanical properties that exhibit relatively low but measurable interspecies variability. These mechanical properties appear to be independent of perfusion pressure and cranial confinement as long as strains are modest. Importantly, the mechanics of brain tissue change rapidly and dramatically within hours after death [23], which may account for some of the variability in reported measurements of brain tissue elasticity. Rheological studies of immature and adult porcine and rat brain tissue have revealed that an additional source of variability may be a decrease in the mechanical stiffness of the brain parenchyma with age, an observation that has been attributed in part to the increasing lipid content associated with myelination of rapidly branching axonal and dendritic arbors during development as well as reduction in water content with age [24,25]. Dynamic MRI studies appear to support the idea of age-dependent alterations in the mechanical properties of mature CNS tissues [10]. While dynamic-phase contrast MRI is not traditionally thought of as a measurement of tissue mechanics, it can be used to measure the coupling of brain, spinal cord, and CSF pulsations to the driving vascular pulsations; because the biomechanical properties of CNS tissues exert strong influence over this coupling, observed differences between adult and



(A) (B)

FIGURE 18.1 (See color insert.) Biomechanical heterogeneity of the rat hippocampus. (A) Spatial distribution of indentation measurements. The elastic modulus of the rat hippocampus was measured via atomic force microscopy (AFM) indentation along a layer of pyramidal neuron cell bodies at the depicted locations. (B) Elasticity map of the hippocampus. Apparent elastic modulus normalized to the mean apparent elastic modulus of all indentations is depicted by the color bar. The material properties of the hippocampus were spatially heterogeneous. Scale bar is 1 mm. (Reprinted from Elkin, B.S. et al., *J. Neurotrauma*., 24, 812, 2007. With permission.)

elderly individuals provide indirect evidence that human CNS tissue mechanics progressively change with age.

In one of the earliest studies of brain tissue biomechanics, Metz et al. compared the viscoelastic properties of living tissue in anesthetized animals with postmortem and postfixation tissue samples; although the authors reported significant increases in the elastic modulus of the fixed tissue, they surprisingly observed very little change in the nonlinearity of the stress–strain relationship [26]. Numerous studies have since reported nonlinear stress–strain responses in mammalian brain tissue, similar to the characteristic strain-stiffening observed in collagenous soft tissues [27–32]; these nonlinearities feature prominently in models of traumatic injury, but their significance to cellular mechanobiology remains incompletely understood because the range of frequencies at which cells probe their environment is not yet well determined.

18.3 MECHANOBIOLOGY IN CNS DEVELOPMENT

The mechanical properties of the ECM can direct a wide range of cellular properties, including cell shape and cytoarchitecture [33–35], motility [36,37], matrix remodeling [38], differentiation [39–42], and the extension of functional cellular projections [43–46]. As the human brain develops, billions of cells are generated in the proliferative tissues lining the lateral ventricles of the brain. These cells migrate throughout the developing CNS, differentiate into neurons or glial cells, and establish a diverse array of organized structures with distinctive shapes and intricate internal architecture [47]. Neurogenesis continues, albeit in a much more limited way, into adulthood through the self-renewal and differentiation of adult neural stem cells (aNSCs) found within the hippocampus and subventricular zone [48]. The mechanosensitivity of aNSCs was recently explored by Saha et al., who demonstrated that the differentiation and self-renewal of aNSCs can be modulated by controlling the mechanical stiffness of the surrounding microenvironment [42]. In particular, culturing aNSCs on the surface of soft polymeric substrates with stiffnesses close to living brain tissue (~100–500 Pa) favored differentiation into neurons, whereas culturing aNSCs on polymeric substrates with identical surface chemistry but much greater mechanical rigidity (~1–10 kPa) favored differentiation into glial cell types. The latter finding raises the intriguing possibility that tissue stiffening may play an instructive role in glial scar formation rather than merely serving as a passive consequence of the process. This mechanosensitivity, coupled with the mechanical heterogeneity present throughout the normal and diseased brain, lends support to the hypothesis that mechanical cues may be dynamically involved in CNS development, in the maintenance of homeostasis, and in the development of disease.

The mechanisms governing morphogenesis (the development of shape, organization, and structure) in CNS tissues have long been the subject of vigorous investigation and debate, but one of the most elegant and robust hypotheses that has emerged is the tension-based theory of morphogenesis proposed by David Van Essen in 1997 [47]. This theory holds that mechanical tension along axons, neurites, and glial cell extensions is sufficient to generate many of the structural features of the mammalian CNS, including the folding patterns of the cortex and the observed compactness of the neural circuitry. This hypothesis is supported by the unique mechanical properties of neurons (described below), which would be expected to facilitate regulation of steady tension in neuronal processes during development through both passive and active mechanisms.

18.4 MECHANOBIOLOGY OF NEURONS IN THE CNS

The notion that mechanosensation is a central function of many neurons, such as the somatosensory neurons that transduce tactile and sound cues, was clear long before the recent surge of interest in cellular mechanotransduction mechanisms. It was less obvious whether differentiated neurons in the CNS, which are largely shielded from external mechanical loading by the presence of the cranium and vertebral column, should be expected to retain similar sensitivity to the

mechanical properties of their microenvironment. Yet, a growing body of work has indicated that the relationship between mature neurons and microenvironmental mechanics is as dynamic and delicate in the CNS as in tissues that routinely experience mechanical loading. For example, the growth and functionality of neurons appears to be tightly coupled to microenvironmental rigidity. Two recent studies by Janmey and coworkers revealed that substrate rigidity can modulate the outgrowth of cells from explants of the spinal cord [45] and cortical brain tissue [49] in a manner that mirrors the stiffness-dependence of aNSC differentiation discussed previously [42]. Specifically, explanting CNS tissues onto substrates that match the approximate stiffness of CNS tissues (several hundred Pa) was found to optimally support both neuronal and glial survival while suppressing overgrowth of the cultures by astrocytes, whereas explanting onto softer substrates selected for neuronal survival and growth and explanting onto stiffer substrates selected for glial survival and proliferation.

The extension and branching of neurites (thin projections from the cell body, including axons and dendrites) in culture can be similarly regulated by the rigidity of the cellular microenvironment (Figure 18.2) [43–46]; substrates that are either much softer or much stiffer than the normal brain

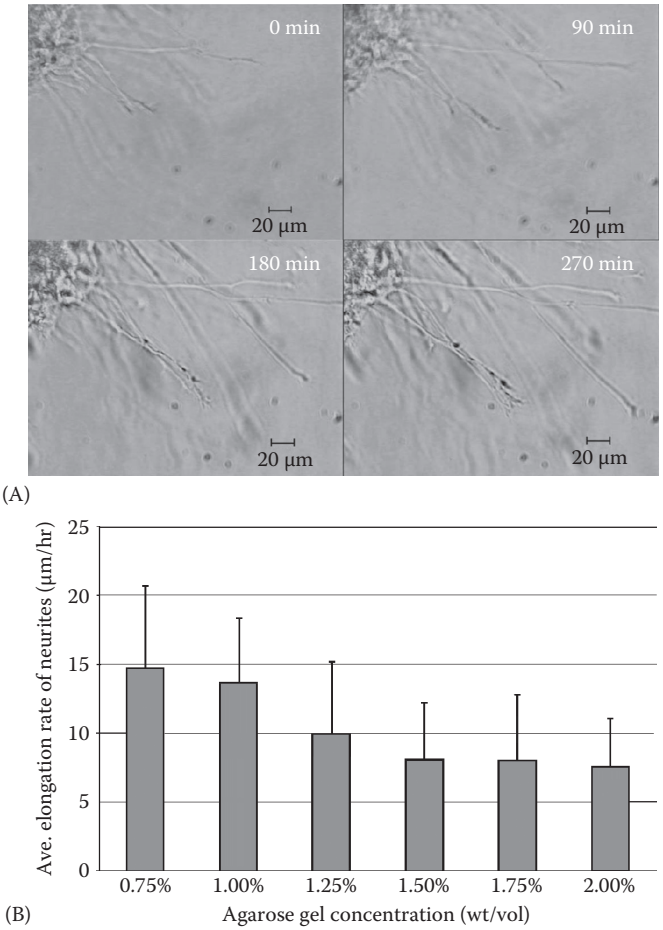


FIGURE 18.2 Neurite extension is regulated by microenvironmental mechanics. (A) Phase-contrast images of dorsal root ganglion neurons extending neurites into a 1% agarose hydrogel. Images were acquired at 90 min intervals. (B) Effect of agarose concentration on neurite extension. The rate of neurite extension is modulated by agarose gel stiffness, controlled by varying the concentration of agarose from 0.75% to 2.0% w/v. (Reprinted from Balgude, A.P. et al., *Biomaterials*, 22, 1077, 2001. With permission.)

microenvironment often do not support robust neurite extension in vitro, although the details of this relationship appear to depend upon the cell source and substrate geometry and composition. This correlation may have implications for neuroregeneration and tumorigenesis; for example, it was recently proposed that the softening of reactive astrocytes following mechanical injury may provide a compliant, brain-like mechanical substrate that promotes neurite extension [50]. We recently showed that the potency with which retinoic acid can induce neurite extension, reduce proliferation and suppress N-Myc expression in neuroblastoma tumor cells all depend strongly on ECM stiffness [51]. Given the substantial mechanical stress exerted by neurites on adhesive substrates [52–54], this phenomenon is likely related to the capacity of the underlying substrate to support generation of contractile forces within elongating projections. Interestingly, neurites have been elicited in vitro by direct application of tensile forces with glass microneedles, where active elongation is observed when tension is maintained above a threshold value, and active retraction is observed when tension is released [53–56]. Follow-up studies using magnetic beads to apply external loads to elongating neurites showed that forces on the order of 1.5 nN are required to elicit neurites, and that force-induced neurite initiation and elongation appears to be a highly conserved property that is largely independent of cell age and synaptic phenotype [57,58].

The initiation, extension, and maintenance of neurites is key to the functional integrity of the CNS, and importantly, these processes are intimately related to the mechanical properties of neurons and their subcellular components. AFM characterization of individual neurons showed that retinal neurons display the rheological characteristics of elastic solids, with cell processes that are often softer than the cell body [59]. Individual neurites display simple elastic behavior under transient stretching (neurite length increases in proportion to applied tension) [54] and viscoelastic properties under sustained stretching [60]; specifically, the initial tension relaxes passively to a lower level on the timescale of minutes, and when resting tension is released, the neurite shortens slightly prior to active retraction. Interestingly, cytoskeletal elements within the cell are known to behave in similar fashion; actin stress fibers in living cells also retract as viscoelastic cables following incision with a femtosecond laser nanoscissor [61], and curved microtubules have been observed to straighten briefly following nanoscissor incision due to release of elastic energy prior to rapid depolymerization [62]. This is also consistent with the viscoelastic behavior that has been measured in a wide variety of cultured non-neuronal cell types by magnetic twisting cytometry [35,63–65], AFM [66–69], micropipette aspiration [70–72], optical trapping [73,74], cellular de-adhesion measurements [75], and a variety of other methods [76].

Biochemical modifications to cytoskeletal elements may play a key role in neurite mechanics as well; in particular, it has long been known that cytoskeletal dynamics play a central role in driving the directionality of axonal growth [77–79]. More recently, however, contributions of cytoskeletal networks to the shape and mechanics of mature axons have begun to emerge. For example, repulsive forces between phosphate groups on neurofilament sidearm domains contribute to the organization of neurofilament networks within axons (Figure 18.3) [80–82]. Indeed, enzymatic dephosphorylation of neurofilaments significantly alters the mechanical properties of both purified neurofilament gels and single neurofilament proteins [83,84]. Conversely, traumatic nerve injury is sometimes accompanied by altered posttranslational modification and organization of neurofilaments and other cytoskeletal proteins [85,86]. Similar mechanisms have been proposed for the organization of microtubule bundles, which are commonly found in both axons and dendrites, by microtubule-associated proteins [87]. Modulators of the actin cytoskeleton also play a prominent role in regulating axonal growth and guidance; for example, activation of myosin motors and Rho-family GTPases, (e.g., Rho, Rac, Cdc42) play vital roles in regulating axon growth dynamics, and dysfunctional Rho family GTPase signaling has been tied to a surprisingly wide variety of adult and congenital neurological disorders [88–90]. Mechanosensitive ion channels have also been implicated in neurite growth kinetics [91–93] in addition to their well-documented role in fast sensation of stretch and other forces [94–96]. For example, inhibition of Ca^{2+} influx through stretch-activated ion channels in neurons from explanted *Xenopus laevis* spinal cord tissue was found to dramatically accelerate

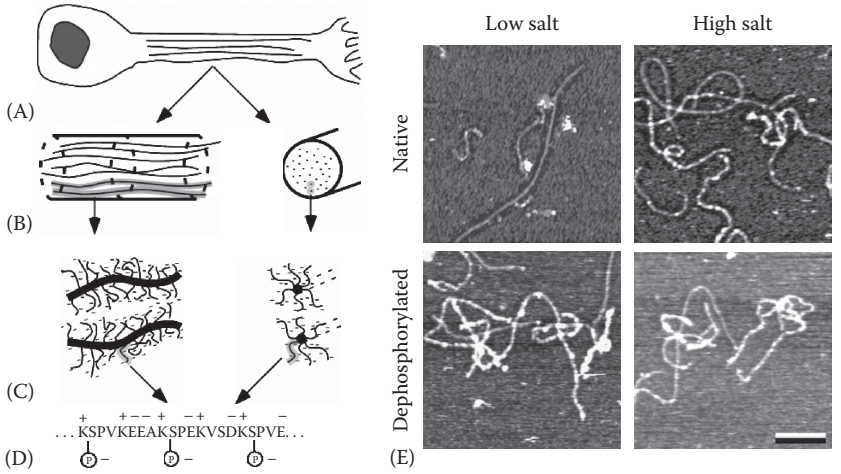


FIGURE 18.3 Axonal neurofilament (NF) networks. (A, B) Parallel orientation of NFs along the length of the axon. (C) Role of phosphorylated NF C-terminal sidearm domains in modulating interactions between adjacent NFs. (D) Representative amino acid sequence of sidearm domain of NF heavy chain showing cationic, anionic, and phosphorylated residues. (E) Effect of salt concentration and phosphorylation on structure of NF sidearms in vitro. When purified and imaged by AFM, native (i.e., phosphorylated) bovine NFs are surrounded by dark "exclusion zones" due to the thermal motion of the extended, unstructured sidearms. These zones persist whether the NFs are imaged in a low-salt buffer (top left) or a high-salt buffer (top right) in which long-range electrostatic interactions are screened. When the NFs are enzymatically dephosphorylated, the exclusion zones are no longer observed in either buffer, suggesting sidearm collapse (bottom left, right). (A–D: Reprinted from Kumar, S. et al., *Biophys. J.*, 82, 2360, 2002. With permission; E: Reprinted from Kumar, S. and Hoh, J.H., *Biochem. Biophys. Res. Commun.*, 324, 489, 2004. With permission.)

the rate of neurite extension, implying that calcium influx through mechanosensitive channels can inhibit outgrowth [92].

18.5 MECHANOBIOLOGY OF GLIAL CELLS IN THE CNS

Neurons have traditionally commanded the vast majority of attention in neurobiology research due to their propagation of action potentials, electrical impulses which travel along axons to facilitate quick and efficient transmission of signals over distances that can be greater than a meter. Non-neuronal cells within the CNS, known as glia (derived from the Greek word for "glue"), have historically been relegated to the accessory role of "support cells," with known functions ranging from the secretion and cilia-driven circulation of CSF by ependymal cells, to the myelination of axons by oligodendrocytes, to the structural support presumably provided by astrocytes, the most abundant cell type in the CNS. Over the last 20 years, new discoveries have challenged these old stereotypes and have stimulated renewed interest in the biology of glial cells, particularly astrocytes. Far from serving simply as the "glue of the nervous system," astrocytes are now thought to regulate adult neurogenesis [97–99], learning, and synaptic plasticity [100–105], and orchestration of the host response to injury in addition to protecting the CNS via maintenance of homeostasis and induction and maintenance of the blood–brain barrier [106].

The first direct viscoelastic characterization of individual glia and neurons, published in 2006, directly challenged the dogma of glia as structural support cells. These AFM indentation measurements revealed that glial cells are softer than neurons (and would therefore provide poor structural support) and that elastic forces dominate viscous forces in glial mechanics (therefore making them a poor glue) [59]. Yet, in 2008, direct probing of this hypothesis in situ via comparative tensile testing of spinal cord explants with an intact or disrupted glial matrix demonstrated that glia do provide

significant mechanical support to spinal cord tissue under uniaxial tension [107]. While this is still an active area of debate, it is possible that the high compliance of individual glial cells allows them to protect neurons by cushioning them during trauma, whereas the architectural arrangement of star-shaped astrocytes into a cellular scaffold that physically couples blood vessels, neurons, and other glia may be critical in providing mechanical support to the tissue, especially given the absence of a robust ECM.

The idea that astrocytes may be ideally situated to sense and resist mechanical disruption in the brain through their unique scaffold architecture is not new; in fact, it has been over two decades since Alen Mathewson and Martin Berry first hypothesized that "architectural disruption" in the brain may be responsible for the phenomenon of astrocyte activation [108]. This activation, also referred to as reactive gliosis or astrogliosis, involves both astrocyte hypertrophy (abnormal enlargement of cell size) and hyperplasia (increase in cell number) in response to CNS pathologies ranging from neurodegenerative diseases to direct trauma, and often results in the formation of a glial scar [109]. Because astrocytes function as a syncytium of interconnected cells, mechanical deformation in one area of the brain due to primary stress (e.g., the mass effect of a tumor or direct stress due to trauma) or secondary stress (e.g., increasing pressure due to edema, the buildup of fluid following tissue insult) could quickly be biochemically and mechanically communicated to distant astrocytes, allowing rapid induction of reactive gliosis and other host response mechanisms.

Mathewson and Berry's architectural disruption hypothesis has been supported by in vitro observations of strain rate-dependent gliosis in three-dimensional (3D) cell culture models [110] and also by growing evidence that the mechanosensory machinery of astrocytes is particularly robust. For example, astrocytes directly convert mechanical stimuli into chemical signals using stretch-activated or stretch-inactivated ion channels. Ostrow et al. used micropipette indentation to directly demonstrate this phenomenon, showing that deformation of a single astrocyte induces transmembrane flux of Ca^{2+}, which propagates as a transcellular wave through gap junctions to neighboring astrocytes in confluent culture [111]. Chemical stimuli such as glutamate can similarly trigger the initiation and propagation of calcium waves in cultured astrocytes [112,113]; these calcium waves are believed to constitute a key signaling mechanism to orchestrate astrocytic functions ranging from guidance of CNS growth cones [114] to alterations in cell structure, gene expression, and proliferation [115,116]. Importantly, mechanical induction of intracellular second-messengers (e.g., inositol triphosphate and release of intracellular calcium) have been observed in cultured astrocytes as well [111].

Mounting evidence suggests that the components of the cellular contractile machinery regulate calcium signaling via gap junctions in addition to traditional mechanotransductive pathways. A relationship between a functional cytoskeleton and active calcium signaling in astrocytes was clearly established in 1998 by Cotrina et al., who demonstrated that neonatal astrocytes are unable to propagate calcium waves until the actin cytoskeleton is fully developed (a process that takes several hours), despite the existence of extensive gap junctional coupling shortly after subculture [117]. Furthermore, the radius of propagated calcium waves increases in direct proportion to the percentage of cells exhibiting a well-organized actin cytoskeleton (measured as the fraction of cells with visible actin stress fibers). Importantly, associated pharmacologic inhibition experiments revealed that calcium wave propagation is significantly attenuated by inhibition of myosin light chain kinase activity or actin polymerization but does not require microtubule organization.

In addition to these specialized mechanotransductive mechanisms, glia also exhibit many of the mechanobiological properties that are typical of cell types outside of the CNS. For example, mechanical stress induces rapid reorganization of both the intermediate filament network and the actin cytoskeleton [118,119]. The unique coupling of cytoskeletal elements across the astrocyte syncytium (e.g., organization of stress fibers into parallel bundles spanning multiple cells), however, suggests that transmission of mechanical signals in the brain may act across an unexpectedly long range [120]. This is potentially important as a mechanism for the dynamic production and regulation of coordinated responses to CNS injury and disease, as we will explore next.

18.6 CNS MECHANOBIOLOGY IN INJURY AND DISEASE

Many CNS diseases are intimately associated with structural changes that would be expected to alter the mechanical properties of the ECM and resident cells. For example, Alzheimer's Disease involves the gradual buildup of amyloid plaques and neurofibrillary tangles in the brain [121], a process which may affect both macroscopic and microenvironmental mechanics. Enhanced cell proliferation, de novo secretion of ECM proteins, and increased interstitial pressure within tumors increase the mechanical rigidity of tumor tissues relative to normal brain, as visualized by MRI and ultrasound elastography imaging of CNS malignancies [17,122–125]. It has even been hypothesized that some diseases may actually be *caused* by changes in the brain's mechanical properties; for example, it is thought that loss of tissue tensile strength following infarction can lead to physical obstruction of CSF flow, resulting in normal pressure hydrocephalus [126,127].

On the cellular level, the innate physiological response to CNS trauma or disease is related to mechanobiological phenomena in a number of interesting ways. For example, one of the hallmarks of astrocyte activation is increased expression of cytoskeletal intermediate filaments, including glial fibrillary associated protein, vimentin, and nestin, in addition to upregulation of focal adhesion proteins, such as vinculin, talin, and paxillin, and the actin-crosslinking protein alpha-actinin, implying that activated astrocytes should express a highly contractile phenotype [118,119,128]. The increase in tissue volume accompanying astrocyte hypertrophy and hyperplasia increases the stress on surrounding cells, as does secretion of additional ECM proteins, such as collagen IV and laminin [129], which subsequently form a scar of collagenous basement membrane that is thought to be one of the major impediments to axonal regeneration [130]. This local increase in stress can produce positive feedback to initiate further pathological changes, including enhanced expression of endothelin, a potent vasoconstrictor and astrocytic mitogen that is also associated with astrocyte activation in response to a variety of pathologies [116].

There are few other organ systems in which changes in local stress or tissue volume have the same implications as in the brain, where the maximum tissue volume is resolutely fixed by the encasing skull and local mechanical disturbances can be rapidly transmitted over long distances by the unique architecture of the astrocyte syncytium. As a result, secondary increases in mass or pressure that accompany many CNS diseases and injuries (e.g., from edema or the mass effects of tumor growth) can often contribute more to morbidity and mortality than the primary insult. The distinction between primary and secondary mechanical effects is especially significant in the case of traumatic brain injury, where direct trauma often imparts large, transient forces to CNS soft tissues, resulting in a complex cascade of secondary mechanical and biochemical responses. Investigations of tissue and cell-scale responses to traumatic CNS injury have broadly sought to understand not only how the transient forces present during the injury are directly transmitted to cells and tissues, but also what acute and long-term host response mechanisms are subsequently activated, and how these mechanisms can be exploited to enhance repair and the return of function [131].

Having reviewed general concepts in CNS mechanobiology, we now turn to a specific CNS pathology for which we and others have recently begun to elucidate the role of mechanobiology: the growth and invasion of the brain tumor glioblastoma multiforme (GBM).

CASE STUDY Mechanobiology of Glioblastoma Multiforme

Primary brain tumors are abnormal masses of tissue that originate in the brain; they can be malignant (cancerous) or benign (noncancerous, i.e., not recurrent or progressive). Tumors arising from glia or their progenitors are called gliomas and are clinically divided into four grades according to the level of malignancy at diagnosis [132]. Grade IV gliomas, also known as GBM, represent the most common, aggressive, and neurologically destructive primary brain tumors. As the name would imply, GBM tumors are grossly heterogeneous (both inter- and

intratumorally and at all levels, from tissue to cell to molecular and genetic), which may help explain why they are remarkably refractory to therapy. It has been over 80 years since the *Journal of the American Medical Association* (JAMA) published neurosurgeon Walter Dandy's report of what is perhaps the most radical GBM therapy to date: surgical hemispherectomy—literally, removal of an entire hemisphere of brain cortex [133]. Yet, Dandy concluded that even this radical procedure is ineffective at preventing rapid recurrence of the tumor, and sadly, an editorial published in JAMA almost 80 years later still cites a brain tumor diagnosis as one of the most feared by patients, physicians, and oncologists alike [134]. This fearsome reputation is well-deserved: despite extensive clinical and biological research efforts over the past several decades, there are still few proven risk factors for the development of GBM and little hope for long-term survival [134,135]. Even with the best available surgical care, chemotherapy, and radiation therapy, the average life expectancy at diagnosis is 12–15 months [136].

Clearly, the factors driving GBM progression are tightly woven into a complex network that has not yet been adequately dissected from either a basic science or therapeutic perspective. Tremendous effort has been devoted to elucidating the genetic and biochemical underpinnings of GBM over the last several decades; however, poor translation of candidate therapies from animal models to human patients has only increased the sense of urgency for the development of new approaches in both the laboratory and the clinic. Using words that could as easily have been written by Walter Dandy in 1928, the aforementioned 2005 JAMA editorial ended with a warning: "Advancements for patients with malignant glioma have been negligible, and there is a real risk of going nowhere by simply continuing to travel the same path" [134]. Indeed, the survival time for GBM has increased only incrementally over the past 25 years, with the most substantial recent advance (due to the alkylating agent temozolomide) improving survival by only an additional few months.

One novel path that has recently proved promising is investigation of GBM tumor cell mechanobiology. It has been known for decades that glioma cells retain many of the mechanosensory abilities of their nonmalignant counterparts, including stretch-activated ion channels and the ability to communicate transcellularly via gap junction-mediated calcium signaling [137–140]. Increased expression of connexins, the molecular building blocks of gap junctions, is correlated with enhanced calcium signaling between glioma cells and host astrocytes in rat xenograft models, and results in glioma cell invasion through a greater volume of brain parenchyma [141]. This implies that functional integration into the astrocytic syncytium, which itself is ideally posed to sense and transmit mechanical and biochemical signals over long distances in the brain, may constitute a significant support system for invasive GBM cells as they migrate away from the tumor mass into the surrounding tissue.

The remarkable capacity of single GBM tumor cells to diffusely infiltrate the surrounding brain parenchyma prior to diagnosis and following treatment is often cited as one of the key factors driving the uncommon aggressiveness of GBM. This infiltration ultimately renders surgical debulking and tumor bed irradiation ineffective, as was powerfully demonstrated by the failure of even Walter Dandy's hemispherectomies to prevent tumor recurrence. A central therapeutic goal in GBM has therefore been to develop new strategies to limit tumor cell invasion, thereby rendering the tumor more susceptible to anatomically directed therapies. While it is clear that biochemical signaling from the ECM is an important regulator of GBM tumor cell invasion, the biophysical components of this crosstalk have received comparatively little attention to date.

Hints that glioma invasion may be partly regulated by cell and tissue biomechanics have been inconspicuously scattered throughout the academic literature for over half a century. For example, neuropathologist Hans Scherer published extensive observations in 1940 describing the organization of invasive glioma cells into distinctive and predictable patterns that radiate away from the main tumor mass into the surrounding brain parenchyma [142]. Importantly, he noted that invasive cells spread preferentially along the surface of anatomical structures in the brain, including the basement membrane of blood vessels, white matter tracts, and the pia mater.

These infiltrative patterns are now commonly known as the "secondary structures of Scherer," and their association with biomechanically distinct components of the brain architecture may be especially informative from a mechanobiological perspective.

Several recent studies have highlighted the importance of microenvironmental mechanics to glioma cell physiology and invasion. For example, we recently cultured a panel of GBM cell lines on biochemically identical polymeric ECM substrates of defined mechanical rigidity (ranging from 0.08 kPa to 119.0 kPa). Our studies revealed stark rigidity-dependent differences in cell structure, motility, and proliferation (Figure 18.4) [143]. Specifically, tumor cells cultured on highly rigid ECMs spread extensively, formed prominent actomyosin stress fibers and mature focal adhesions, and migrated rapidly, whereas cells cultured on the most compliant ECMs (with rigidities comparable to normal brain tissue) appeared uniformly rounded and failed to productively migrate. We have subsequently explored the role of the focal adhesion protein α-actinin in glioma cell mechanobiology, motivated by the observations that α-actinin structurally couples the cellular adhesive and contractile machineries [144] and is significantly upregulated in high-grade astrocytomas [145]. We found that suppression of either α-actinin isoform (1 and 4) in human glioma reduces cell motility and traction forces and compromises the ability of cells to mechanically adapt to changes in ECM stiffness [146]. Importantly, glioma

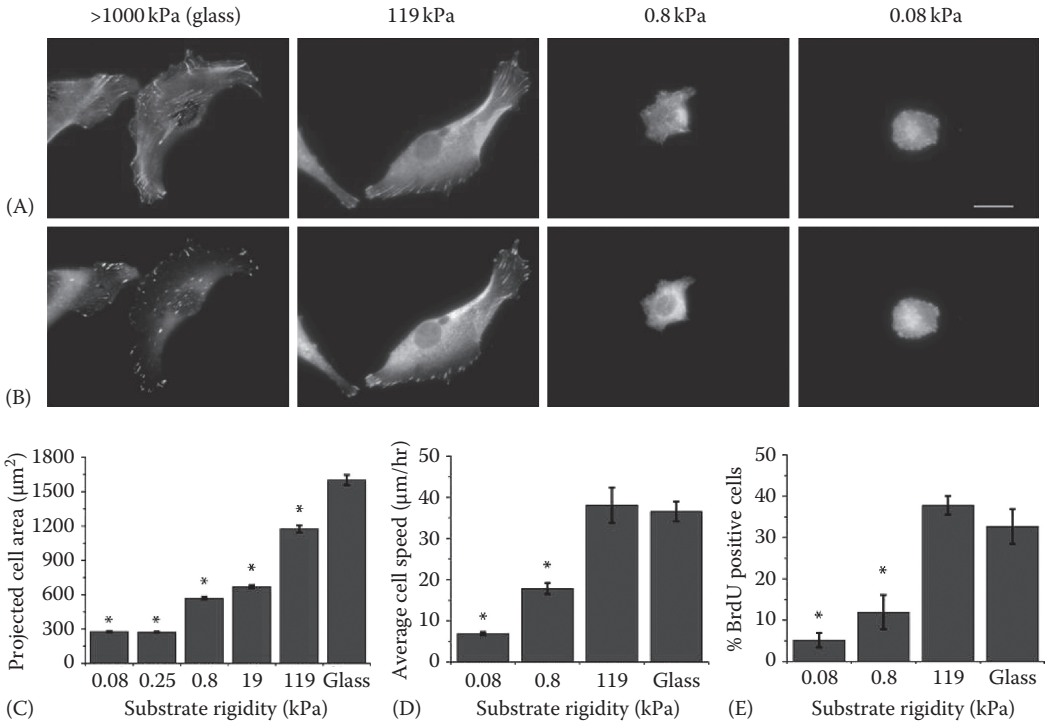


FIGURE 18.4 (See color insert.) The mechanical rigidity of the ECM regulates glioblastoma multiforme (GBM) tumor cell structure, motility, and proliferation. (A) Cell shape and cytoarchitecture. Human glioma cells cultured on fibronectin-conjugated glass and polyacrylamide gels of three different stiffnesses were fixed and stained for F-actin (green), nuclear DNA (blue), and the focal adhesion protein vinculin (red). Cells on glass and 119 kPa substrates exhibit robust focal adhesions and a well-defined cytoskeletal architecture, whereas cells on 0.80 and 0.08 kPa polyacrylamide gels are rounded with cortical rings of F-actin and small, punctate vinculin-positive focal complexes. Bar is 25 μm. (B) Isolated view of vinculin signal only, showing structure and distributions of cell-ECM adhesions. Effect of ECM mechanical rigidity on (C) Cell spreading area; (D) Migration rate; and (E) Cell proliferation, as measured by bromodeoxyuridine (BrdU) incorporation. (Adapted from Ulrich, T.A. et al., *Cancer Res.*, 69, 4167, 2009.)

cell rigidity-sensitivity can be blunted by direct or indirect pharmacologic inhibition of myosin-based contractility, providing support for a model in which ECM rigidity provides a transformative, microenvironmental cue that acts through actomyosin contractility to regulate the invasive properties of GBM tumor cells.

This model is consistent with the results of previous 3D in vitro studies of GBM invasion. While microenvironmental mechanics are more difficult to control in 3D cell culture models without altering integrin ligand density or microstructure, studies in which the stiffness of collagen I matrices was increased by increasing the concentration of collagen suggest that biophysical and biomolecular factors are both crucial regulators of glioma invasiveness [147,148]. We recently investigated 3D motility in collagen I matrices stiffened through the progressive addition of agarose, which we found restricted invasion by increasing steric barriers to motility and reducing the ability of tumor cells to bundle and remodel the collagen fibers [149]. Consistent with this finding, Kaufman et al. manipulated pore size in collagen I matrices by controlling the temperature of gelation and showed that pore sizes below 4–6 μm strongly limited glioma cell invasion speed [150]. To more carefully analyze microenvironmental mechanics during tumor invasion, Gordon et al. embedded 1 μm latex beads within Matrigel-based in vitro spheroid invasion assays, utilizing particle-tracking methods to analyze the spatial displacement of the tumor microenvironment at all stages of spheroid growth and invasion [151]. Their studies yielded a surprising juxtaposition of forces within the matrix surrounding the spheroid: volumetric expansion of the main tumor spheroid pushes the bulk of the gel outward, even as the matrix at the invasive front is pulled inward due to the localized generation of cell traction forces.

Importantly, there is evidence that the mechanobiological machinery of glioma cells differs from that of their nonmalignant counterparts, a crucial prerequisite for the development of mechanobiologically inspired therapeutics. For example, glioma cells exhibit reduced expression of cadherins (calcium-dependent transmembrane glycoproteins that facilitate cell–cell adhesion), enhanced expression of matrix metalloproteinases, increased expression of focal adhesion proteins, such as focal adhesion kinase (FAK), and altered expression of integrins compared to normal astrocytes [4,152–156]. These differences are potentially significant from the standpoints of both basic pathophysiology and therapeutics. For example, integrin-mediated adhesion of tumor cells to ECM proteins has been associated with greater resistance to ionizing radiation and chemotherapies, a phenomenon known as cell adhesion-mediated radioresistance/drug resistance (CAM-RR/CAM-DR) [157]. Recent studies have linked β1 integrin signaling in particular with inhibition of drug-induced apoptosis [158] and promotion of radioresistance [159]. Enhanced expression of integrins α2, α3, α5, and β1 in drug-resistant glioma cells has been correlated with enhanced adhesivity to ECM proteins such as fibronectin and collagen as well [160]; these proteins are more commonly found in tumor tissue and basement membrane than normal brain parenchyma, suggesting that CAM-DR may also promote tumor progression and invasiveness. Surprisingly, a recent study showed that pharmacologic inhibition of fibronectin assembly in the ECM can enhance sensitivity of GBM cells to nitrosourea chemotherapy in vitro and in vivo [161]; however, the dynamics of this relationship and the mechanisms driving ECM-derived chemosensitivity are not yet understood. Nevertheless, promising new chemotherapeutics are already beginning to target components of the contractility and adhesion machinery, including the potent integrin antagonists Cilengitide and SJ749 [162,163], radioiodinated antibodies directed against tumor-secreted ECM proteins [164,165], drugs that inhibit Rho GTPase-based signaling [166], and small-molecule inhibitors of FAK and other focal adhesion proteins [167].

The dynamic range of glioma cell mechanosensitivity may differ from that of their nonmalignant counterparts as well; for example, while highly compliant substrates (~100–500 Pa) have been found to select against the survival and proliferation of astrocytes in both explant studies and aNSC differentiation studies [42,49], cultured glioma cells survive and proliferate even on 80 Pa polyacrylamide gels [143]. Interestingly, however, the rate of incorporation of

bromodeoxyuridine (BrdU), a labeled nucleotide taken up only by dividing cells, on these soft gels is reduced approximately fivefold over BrdU incorporation on a stiff (119 kPa) polyacrylamide gel or glass. While the mechanism governing this effect is not yet known, ECM rigidity has been previously observed to modulate cell growth in other systems, including cultured fibroblasts [168], hepatocytes [169], and a variety of adult stem cells [40,42].

One potential explanation for the correlation between microenvironmental rigidity and cell proliferation is that changes in ECM rigidity might alter the speed of progression through the cell cycle by altering mechanochemical feedback during mitosis. Indeed, direct application of mechanical force has been observed to slow cytokinesis and induce shape asymmetries in *Dictyostelium discoideum* cells, which is actively corrected via mobilization of nonmuscle myosin II (NMMII) to produce a restoring force [170]. Second, ECM rigidity might regulate mitosis by synergistically triggering mechanotransductive and mitogenic signaling pathways, as has been suggested by recent studies from the breast cancer community, which reveal that modest increases in ECM rigidity can transform cultured breast epithelial cells from a benign, highly differentiated phenotype into a dysplastic and proliferative one [171,172]. Importantly, this matrix-driven transformation is accompanied by activation of extracellular signal-regulated kinase (ERK)-mediated proliferative signaling and activation of the contractile markers Rho GTPase and NMMII, which enables enhanced generation of contractile forces. Importantly for therapeutic applications, this rigidity-dependent phenotype can be reversed by pharmacological inhibition of Rho-associated kinase (ROCK) or ERK activity.

These contractility-mediating pathways are also intimately related to cell migration and invasion, which depend on actomyosin-generated contractile forces and involve a variety of dynamic and spatially regulated changes to both the cytoskeleton and the adhesion complexes that mediate interactions with the surrounding ECM. In an important study that has general implications for cell migration through 3D ECMs, Rosenfeld and coworkers recently demonstrated that NMMII is needed to deform the nucleus of glioma cells to enable amoeboid motion through ECM pores, and invading tumor cells in vivo significantly upregulate NMMII expression relative to endogenous brain cells (Figure 18.5) [173]. Other recent work has shown that pharmacologic inhibition of myosin light chain kinase results in dramatic inhibition of glioma cell motility [174] and that ROCK-dependent mechanisms are important in GBM cell migration and therapeutic sensitivity [175–182]. Rho/ROCK signaling is thought to be especially important in regulating cell survival and tumorigenesis as well; for example, the ROCK inhibitor Y-27632 and transfection with dominant negative RhoA and ROCK were each found to induce apoptosis in vitro and resulted in significantly smaller tumor mass following tumor inoculation in vivo [183].

In summary, the emerging field of GBM mechanobiology has begun to infuse an appreciation for mechanics into our overall understanding of GBM pathophysiology. These promising and surprising early results suggest that further exploration of the mechanobiological aspects of GBM tumor cells may constitute a new and valuable path toward the identification of novel therapeutic targets, and that these paradigms and approaches might be productively extended to other CNS pathologies.

18.7 CONCLUSION

The last several decades have seen an emerging appreciation for the complex and unexpected ways in which mechanobiology can regulate the CNS in health and disease. Here, we have reviewed this growing field, starting with the structure and mechanical microenvironment of the brain and the spinal cord, continuing with the mechanobiological characteristics of cells in the CNS, and concluding with the importance of mechanobiology to the progression of specific CNS disease states. Despite remarkable progress, many key challenges remain. In particular, it will be critical to determine how mechanobiological signaling in the CNS fits into the context of traditionally

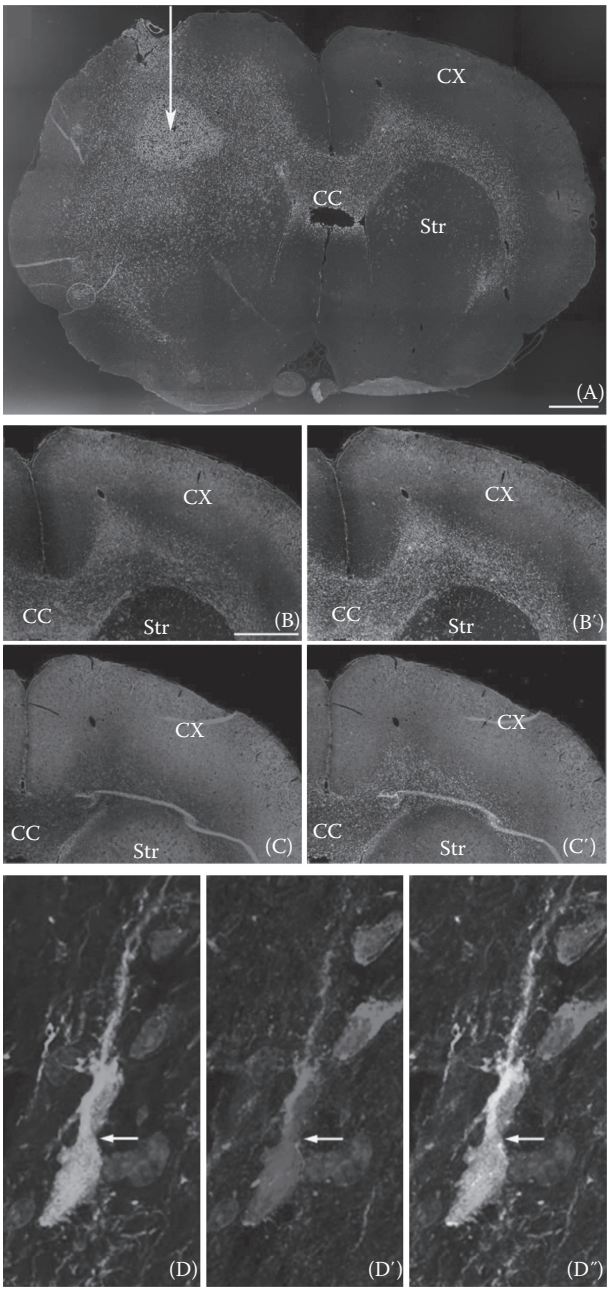


FIGURE 18.5 (See color insert.) Invasive glioma cells demonstrate enhanced myosin IIA expression. (A) Growth and spread of implanted tumor cells. Rat brain slice stained for human nuclear antigen (green) shows that implanted primary human glioma cells spread from the site of tumor inoculation (arrow) across the corpus collosum (CC) to the contralateral white matter, between the cortex (CX) and striatum (Str). Bar is 1 mm. (B) Enhanced immunofluorescence localization of myosin IIA in invasive cells (red). Bar is 1 mm. (B′) Colocalization of myosin IIA and human nuclear antigen. (C, C′) Corresponding immunofluorescence localization of myosin IIB (red), demonstrating equivalent or reduced expression in invasive glioma cells (green) relative to the surrounding normal brain tissue. (D) Nuclear deformation of invasive cells. A GFP-expressing human glioma cell (green) requires significant nuclear deformation (arrow; nucleus stained blue with DAPI) to infiltrate the surrounding normal brain tissue. (D′) Expression of myosin IIA (red) in infiltrative cells. (D″) Colocalization of myosin IIA and GFP. (Adapted from Beadle, C. et al., *Mol. Biol. Cell*, 19, 3357, 2008. With permission.)

understood genetic and biochemical control of neurobiology; cells in vivo are simultaneously subjected to space- and time-dependent mechanical and soluble/matrix-bound biochemical signals, and their response to this constellation of inputs undoubtedly depends on cell-intrinsic factors (e.g., regulation of gene expression) that may dramatically change from one cell to another in the same microenvironment. Understanding which signals dominate cell behavior in specific physiological settings is key to dissecting these cues' relative contributions to normal function and pathophysiology in the CNS. Related to this, it will be essential to extend the many elegant tools that have been developed for studying the mechanics and mechanobiology of single cells in culture to living tissues and organisms. As discussed earlier, noninvasive imaging methodologies that derive contrast from variations in tissue mechanics, such as ultrasound and MRE, hold great promise along these lines. One would also expect that an increased use of mechanosensitive optical probes and fluorescently labeled mechanosensory proteins in animal models may also enable in vivo visualization of CNS mechanotransduction. We anticipate that careful attention to these and other challenges will bring us closer to a day when both the CNS mechanical microenvironment and cellular mechanotransductory signaling systems can be exploited to regenerate nerve tissue, combat invasive brain tumors, and attack complex neuropathologies.

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