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Concise Review: Plasma and Nuclear Membranes Convey Mechanical Information to Regulate Mesenchymal Stem Cell Lineage

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ABSTRACT

Numerous factors including chemical, hormonal, spatial, and physical cues determine stem cell fate. While the regulation of stem cell differentiation by soluble factors is well-characterized, the role of mechanical force in the determination of lineage fate is just beginning to be understood. Investigation of the role of force on cell function has largely focused on "outside-in" signaling, initiated at the plasma membrane. When interfaced with the extracellular matrix, the cell uses integral membrane proteins, such as those found in focal adhesion complexes to translate force into biochemical signals. Akin to these outside-in connections, the internal cytoskeleton is physically linked to the nucleus, via proteins that span the nuclear membrane. Although structurally and biochemically distinct, these two forms of mechanical coupling influence stem cell lineage fate and, when disrupted, often lead to disease. Here we provide an overview of how mechanical coupling occurs at the plasma and nuclear membranes. We also discuss the role of force on stem cell differentiation, with focus on the biochemical signals generated at the cell membrane and the nucleus, and how those signals influence various diseases. While the interaction of stem cells with their physical environment and how they respond to force is complex, an understanding of the mechanical regulation of these cells is critical in the design of novel therapeutics to combat diseases associated with aging, cancer, and osteoporosis. STEM CELLS 2016;34:1455–1463

SIGNIFICANCE STATEMENT

This concise review details the ability of stem cells to regulate their lineage allocation based on mechanical forces exerted upon them. The mechanical qualities of both the external environment (the extracellular matrix) and the internal cytoskeleton of stem cells alter how they respond. Additionally, forces are translated into biochemical signals using different molecular machinery at the plasma membrane and nuclear membrane. These distinctions have been outlined in this paper and the consequent diseases have been described.

INTRODUCTION

Biological systems are fine-tuned to sense, respond, and adapt to physical stimuli [1]. In the case of stem cells, the external physical environment guides stem cell fate decisions [2]. As such, stem cells, as a prototype of multiple descendent lineages, possess mechanosensory machinery to translate mechanical signals into biochemical responses. Furthermore, cells adapt and respond to physical forces by remodeling their internal physical structures to regulate interactions with the external physical environment. Application of mechanical force initiates signaling cascades at the plasma membrane leading to the generation and remodeling of filamentous actin stress fibers, which enhance the cell's ability to perceive and transmit mechanical force intracellularly [3].

For bone marrow mesenchymal stem cells (MSCs), a more structured cytoskeleton leads to differentiation into lineages making up tissues with greater mechanical competence (i.e., bone, cartilage). It is important to note that MSCs form heterogeneous populations, distributed along a dynamic differentiation spectrum. While the question of whether the contribution of MSCs to different lineages stem from direct fate switching or a modular responsiveness of different subpopulations remains unresolved, it is reasonable to expect that up until some point the fate of MSC populations remain plastic, rendering them susceptible to incoming mechanical signals [4]. In this way, both "outside-in" and "inside-out" force

signaling determine MSC lineage fate. In the former, the stiffness of the substrate [5], or application of external mechanical force [6], induces intracellular effects. In inside-out signaling, scaffolding of intracellular proteins reinforces integrin-based attachments [7].

At the interface between the basal substrate and adjacent cells, the plasma membrane uses specific structural proteins to transduce force into biochemical signals. These structural proteins gather together to form focal adhesions (FAs), which span the cell membrane and connect the extracellular matrix to the actin cytoskeleton. FAs also serve as hubs for signaling molecules to congregate, where the combination of force transmission and signal transduction result in further cytoskeletal remodeling [8]. Recent work also identifies the nucleus, and its membrane, as mechanosensory organelles, where anchoring to the cytoskeleton via the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex enables transmission of mechanical force between the nucleus, the cytoskeleton outward to the external microenvironment [9]. In this way, mechanical signals have the potential to further regulate connections between the nucleus and the cell cytoskeleton, generating another level whereby mechanical input can control cell behavior. Thus, while it has become accepted that genetic elements within the nucleus respond to mechanical challenges indirectly through their transduction into intermediary biochemical cascades [1], it has only recently been considered that applied forces might also directly alter chromosomal conformations, thus influencing the accessibility of genetic information for binding of transcriptional enhancers or repressors [10].

As mechanical signals are critical for directing cellular responses, dysfunction of the mechanosensory machinery can lead to disease. One such example at the level of nuclear force control is Hutchinson-Gilford progeria syndrome, which manifests symptoms of aging at a very early age. This condition arises due to due a mutation in Lamin A/C, a structural protein at the inner nuclear membrane [11]. Thus, unlike diseases of "accelerated aging" including Werner syndrome, which are caused by defective DNA repair, this "laminopathy" results from inadequate mechanical support and abnormal nuclear structure [12]. Thus, the structural relationship of Lamin A/C and other nuclear membrane scaffolding proteins represents an interesting target to combat the degenerative effects of conditions associated with disuse and aging, especially since the structural relationship of these nuclear membrane scaffolds affects the accessibility and action of transcriptional regulators.

In this manuscript we focus on the example of the influence of mechanical force on bone marrow MSC differentiation, especially as it relates to the balance of bone and fat formation. Stem cells in other tissues, subject to unique mechanical environments, will have variations on this theme. The consequences of reduced mechanical loading on bone marrow MSC lineage allocation will be discussed, as will the role of various mechanosensory elements, with an emphasis on the contrasting role of force transduction at the plasma membrane with that at the nuclear envelope. Additionally, we will consider the role of mechanobiology in human disease and explore how disrupted mechanocoupling at differing subcellular locations can lead to pathology.

CELLULAR ARCHITECTURE INFLUENCES MSC DIFFERENTIATION

MSCs are abundantly found within the bone marrow and adipose depots and have the ability to develop into mesenchymal tissues including bone, cartilage, fat, and muscle [13]. MSC lineage fate is strongly dependent upon the context of the physical environment, both topographical and mechanical qualities of the surrounding matrix, as well as the dynamic mechanical environment modulate the allocation of MSCs [1].

Cellular structure is largely determined by its cytoskeleton, which is a dynamic structure primarily composed of three types of proteins: microfilaments (actin), microtubules (tubulin), and intermediate filaments [14]. While actin fibers and microtubules are made up of one type of monomer (actin and tubulin, respectively), intermediate filaments have a wide variety of different monomers that form the larger filamentous structures. Hundreds of other proteins associate with the cytoskeleton including crosslinkers, molecular motors, and signaling effectors. Cytoskeletal fibers, especially actin filaments, are acutely sensitive to both chemical and physical modulation. For instance, application of mechanical force enables globular actin monomers (G-actin) to aggregate and form structured filamentous fibers (F-actin). Formation of actin fibers, from monomers, is controlled in large part by Rho GTPases including RhoA, where exchange of GTP induces activation of RhoA, leading to actin stress fiber polymerization [15]. Thus, the structure of the cytoskeleton is extremely dynamic, enabling modification of cellular shape, intracellular signaling events, and thus cellular behavior and phenotype.

Mechanically, cell structure undergoes a continual balance of inner and outer forces which allow cellular components to experience both compression and tension, a process summed in the concept of tensegrity [16]. The tensegrity structure acts as a global sensory mechanism for mechanical cues, allowing for rapid structural responses to static and dynamic mechanical loads [17]. Such structural responses are mediated through the actin cytoskeleton, where FA connections facilitate the tensegrity balance, connecting the inner cytoskeleton with the matrix outside the cell.

Early studies showed that increased matrix stiffness enhanced the size of FA sites in fibroblasts, while softer substrates resulted in smaller, more mobile FAs [18]. As FAs are the physical "bridge" between the actin cytoskeleton and the extracellular matrix (Fig. 1), these studies highlight the ability of the matrix to signal internally, resulting in adaptation to the mechanical forces. Additional work using MSCs demonstrated that confining cell adhesion by plating cells on predetermined fibronectin substrate islands was able to control subsequent cell shape and the level of actomyosin contractility by regulating RhoA/ROCK signaling [19]. MSCs forced to attach on smaller fibronectin islands assumed a rounder shape, consistent with decreased cell stiffness, and showed increased adipogenic differentiation. In contrast, attachment to larger island areas resulted in greater cell spreading, increased RhoA/ROCK activity, and favored osteogenic lineage. Similarly, matrix elasticity regulates the internal cell tension and differentiation of MSCs. Soft substrates (0.1–10 kPa) induce neuronal and adipogenic differentiation [20, 21], while substrates with stiffness comparable to muscle tissue (∼10–17 kPa) supports allocation to myocytes [22]. Even stiffer substrates (>25 kPa), akin to hard bone surfaces, promote
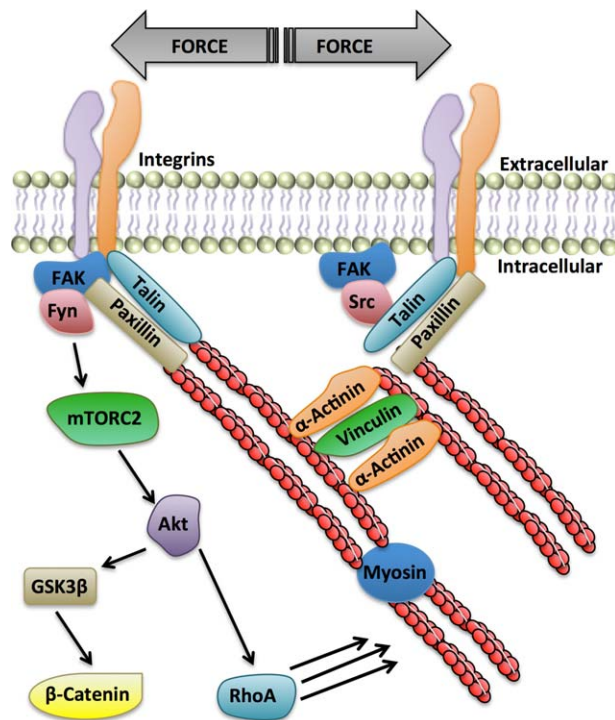
Figure 1. Force transmission across the plasma membrane uses membrane-spanning integrins, which connect to the actin cytoskeleton via talin and paxillin linker molecules. These focal adhesion sites also serve as signaling hubs for mechanosensitive kinases such as Fyn and FAK, which restrict mesenchymal stem cell adipogenesis by activating Akt which both enhances β-catenin availability and increases RhoA-mediated cytoskeletal assembly. Abbreviations: FAK, focal adhesion kinase; GSK3β, glycogen synthase kinase 3 beta; mTORC2, mammalian target of rapamycin 2.

osteogenic differentiation [23]. These studies highlight the influence of RhoA/ROCK pathway on cellular architecture, confirming that cellular tension generated by an enhanced cytoskeleton informs MSC lineage fate.

How might cellular architecture regulate lineage decisions? We are now learning that changes in cytoskeletal architecture not only modulate the physical qualities of the cell but also direct the reconfiguration of the mechanosensory signaling mechanisms within the cells. *Yorkie*-homologues YAP (Yes-associated protein) and TAZ (transcriptional coactivator with PDZ-binding motif) are examples of transcription factors that regulate MSC proliferation and differentiation [24] in response to mechanical cues [25]. Impaired YAP/TAZ activity was found when MSCs grown on softer matrices and following disruption of the actin cytoskeleton, by blocking RhoA activity [25]. These data indicate that MSC fate selection toward osteogenic or adipogenic lineages is modulated by YAP/TAZ, whose activity is modulated by both the mechanical properties of the extracellular matrix and the stiffness of the internal actin cytoskeleton. Furthermore, knockdown of YAP/TAZ resulted in loss of mechanical regulation of MSC differentiation, where depletion of YAP/TAZ not only inhibited osteoblastic differentiation but also promoted adipogenesis [26]. Similarly, adding adipogenic differentiation media down regulated RhoA/ROCK activity, leading to an increased monomeric G-actin to polymerized filamentous-actin (F-actin) ratio. This relative increase in monomeric actin is presumably the result of depolymeriza-

tion of F-actin, which indirectly results in export of the transcriptional coactivator MKL1 (megakaryoblastic leukemia 1, MAL) from the nuclear compartment [27]. Furthermore, the increase in cytoplasmic G-actin traps MKL1 outside the nucleus, releasing peroxisome proliferator-activated receptor gamma (PPARγ) repression, with consequent stimulation of adipogenic pathways. Actin dynamics also regulate the activity of the serum response factor (SRF), allowing this molecule to shuttle into the nucleus [28] where it interacts with MKL1, whose function is specifically dependent on intranuclear actin polymerization [29]. While F-actin assembly within the nucleus controls MKL1 function, the responses of F-actin structures to chemical (i.e., serum) and physical (i.e., integrin engagement) stimuli remain poorly understood [30].

To further highlight the importance of actin dynamics to MSC fate, Sen and colleagues recently used cytochalasin D, a mycotoxin that depolymerizes filamentous actin struts (F-actin) into globular-actin (G-actin) monomers. Reducing actin fibers to their monomeric components resulted in mass import of actin into the nucleus, and once inside the nucleus, actin monomers facilitated export of YAP, which normally suppresses Runt-related transcription factor 2 (Runx2) activity. The release of Runx2 repression contributes to accelerated osteogenesis [31]. These data suggest that the idea that F-actin depolymerization leads to adipogenesis, a dogmatic view in the field of MSC biology, should be amended. While formation of F-actin stress fibers pushes MSCs toward the osteogenic lineage by increasing the internal physical tension, full depolymerization of actin polymers leads to translocation of actin monomers into the nucleus. Localization of G-actin in the nucleus promotes osteogenic gene transcription, perhaps representing a secondary level of actin structural control within the nucleus itself. Importantly, injection of cytochalasin D into the tibial marrow space of live mice results in abundant bone formation [31]. Thus, both the structural filamentous actin fibers and the monomeric G-actin components have critical roles in regulating MSC lineage allocation, and their location in the cytoplasm or within the nucleus might provide differential control of transcriptional processes.

Mechanical Force Transmission at the Plasma Membrane

There are a variety of different mechanosensory structures capable of conveying mechanical information across the cell membrane including changes in lipid microdomains [32], G-protein coupled receptors [33], mechanosensitive ion channels [34, 35], and FA-based integrin attachments [36]. Integrins are perhaps the most widely recognized membrane-associated mechanosensory molecules. These heterodimeric molecules span the plasma membrane and use linker proteins, including talin [37] and vinculin [38], to attach to the internal actin cytoskeleton. Externally, connection of the actin stress fibers to the extracellular matrix (ECM) enables a functional attachment through which force can be distributed across the cell membrane to the matrix substrate (Fig. 1).

The physical attributes (i.e., stiffness, topography, and so on) of the ECM and the type of mechanical signals incurred (i.e., strain, fluid shear stress, and so on) influence integrin-mediated force transmission. A stiffer substrate induces osteogenic differentiation; whereas, a less stiff substrate leads to
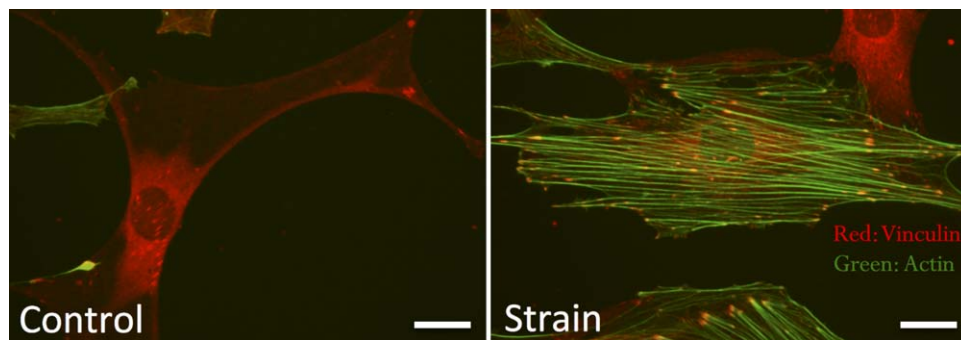
Figure 2. Substrate strain (2%, 100 cycles) induces cytoskeletal reorganization with a fivefold increase in focal adhesions (vinculin, red) and F-actin (green). Scale bars = 25 μm.

adipogenic lineage fate [5]. Similar changes in MSC differentiation can be achieved by applying dynamic mechanical loads. Externally applied substrate strain leads to an increase in FA formation and maturity of bone marrow MSCs [3]. The increase in matrix attachment contact points is not only correlated with increased actin stress fiber formation but also influences intracellular signaling. Interestingly, MSCs exposed to a 20-minute bout of mechanical strain (200 cycles at 2% deformation) resulted in a greater reduction of adipogenic commitment than 3,400 continuous strain cycles under the same deformation parameters [6]. Furthermore, providing a second bout of strain, following a 3-hour rest period, induced greater activation of antiadipogenic signals, suggesting that the initial application of force induces a priming effect, whereby intracellular signals are then positioned to react to the subsequent mechanical challenge.

The ability of mechanical signaling pathways to be amplified, depending on the timing of the mechanical regimen, demonstrates that FAs serve as signaling platforms. As such, applied forces induce recruitment of signaling effectors to FAs, where they are positioned to acutely respond to the next loading event (Fig. 2). It has been well-recognized that FAs serve as signaling hubs in many cell types, with molecules such as the Src family tyrosine kinases, extracellular signal-regulated-kinase (ERK), and focal adhesion kinase (FAK) functioning in association with FAs [39]. Following application of mechanical force, FAK is one of the first signals to arrive at the maturing FA, which is soon followed by Src. These kinases then induce activation of downstream signals including the growth-factor-receptor-bound protein 2 adaptor protein, Ras, and ERK [40].

In bone marrow MSCs, integrin-initiated signals are the genesis of a cascade that restricts adipogenic lineage fate in response to mechanical force. While some of the molecular mechanisms are conserved in the multiple cell types, MSCs appear to use several unique signaling mechanisms to respond to mechanical challenges. For example, mechanical activation of Src and FAK occur readily in fibroblasts [39]; however, in place of Src, MSCs recruit the Src-family member Fyn to FAs. Once located in the FA complex, Src and FAK cooperate to induce Akt phosphorylation at serine 473 (S473) via mammalian target of rapamycin 2 (mTORC2) [41]. ERK1/2 activation is another mechanically activated pathway in fibroblasts [39]. Some studies suggest that ERK signaling is important for mechanical control of MSC differentiation, by activating the osteogenic transcription factor Runx2 [42], while others indicate that ERK1/2 signaling is not involved in force-induced MSC

lineage fate decisions that prevent adipogenesis [41]. These contrasting results may be due to different types of force applied to the cells or simply convey the idea that there are multiple signaling pathways emanating from the FA complexes that are arbitrarily measured in response to time or input.

Mechanical activation of mTORC2, by Fyn and FAK, results in adipogenic repression of MSCs by acting through two distinct, but complementary pathways, one altering transcriptional regulation of adipogenic genes, while the other enhances cytoskeletal structure resulting in a more rigid cell. In both cases, recruitment of Fyn and FAK to FAs results in activation of mTORC2, which in turn phosphorylates Akt at S473 [41, 43]. To regulate gene expression, Akt phosphorylates glycogen synthase kinase 3 beta (GSK3β) at serine-9, resulting in GSK3β inactivation thus preventing proteasomal degradation of β-catenin (Fig. 1). Nuclear translocation of β-catenin then presumably enables transcriptional modification of lymphoid enhancer-binding factor (LEF) and T-cell specific, HMG-box (TCF) targeted transcription factors, or by directly repressing PPARγ activity, resulting in downregulation of adipogenic genes [44].

While control of β-catenin activity directly regulates adipogenic gene expression, mechanical activation of mTORC2 also impacts MSC lineage by enhancing formation of actin stress fibers. Similar to the way that a stiff substrate drives MSCs toward osteogenesis, by altering the physical environment of the cell, mechanical strain generates enhanced internal tension by formation of a filamentous actin network. Assembly of these actin fibers requires activity of RhoA, a GTPase that is acutely responsive to mechanical force. In MSCs, RhoA activation occurs after less than 100 cycles of 2% membrane deformation, followed by stress fiber assembly [3]. Applied substrate force is sensed at FA interfaces, where Fyn and FAK are recruited to the existing FA complex. Similar to the pathway resulting in preservation of β-catenin, Fyn activates mTORC2, resulting in Akt phosphorylation [41]. Recent work has shown that RhoA requires mechanically activated Akt to induce cytoskeletal remodeling and restrict entry of MSCs into the adipogenic lineage [41]. Thus, mechanical force leads to antiadipogenic pathways that begin at the FA and diverge to regulate both nuclear β-catenin localization and formation of actin stress fibers, through RhoA (Fig. 1).

While integrin-mediated attachments at FAs are a primary site of signal initiation to direct mechanical control of MSC fate, primary cilia provide another unique mechanosensory apparatus capable of influencing MSC differentiation. Each cell

contains only one cilium, a nonmotile microtubule-based organelle emerging from the distal centriole of the centrosome of many mammalian cells [45]. In contrast to the numerous and continually remodeling FAs needed to convey mechanical force to the cell, only a single hair-like cilia structure is required to induce biological responses. A compelling body of literature has shown that these structures mediate critical mechanosensory effects in various types of bone cells [46], including mesenchymal bone progenitors [47]. Knockdown of proteins that form the primary cilium in human MSCs results in repression of both Runx2 and PPARγ expression, suggesting that primary cilia also regulate osteogenic and adipogenic differentiation [48]. Additional work has recently shown that MSCs lacking primary cilia are deficient in several mechanosensory responses [49].

NUCLEUS AS A FORCE-RESPONSIVE ORGANELLE

The nucleus is poised to recognize physical signals from the environment as it is mechanically integrated into the cell structure through proteins that span the nuclear envelope enabling connections between intranuclear structures and the intracellular cytoskeleton. These nuclear to cytoplasmic connections not only provide spatial and structural integrity but also serve to transfer mechanical force across the nuclear envelope [50]. It is likely that the nucleoskeleton, and tension associated externally and internally, alter the structure of chromatin interfaces with the inner nuclear envelope and influence gene expression.

Lamin A/C scaffolding proteins inside the nuclear envelope are mechanically coupled to the cytoplasmic cytoskeletal elements via LINC complex proteins. A key LINC protein group providing actin connectivity are the actin-binding giant Nesprin isoforms of the spectrin repeat protein that spans the nuclear envelope. By using N and C terminal domains located at opposite ends of the protein, Nesprin attaches to actin fibers located in the cytoplasm via the calponin homology domain at the N-termini [51, 52] while the Klarsicht, ANC-1, Syne Homology domain at the C-termini binds to SUN proteins on the inner nuclear leaflet [53]. As such, Nesprin is part of a structural scaffold that connects the nucleus to the actin cytoskeleton providing a continuous connection that perceives and adapts to mechanical challenges [54, 55]. The ability of force to be transferred between actin fibers and the nucleus is evidenced by the development of organized structures, called transmembrane actin-associated nuclear lines [56] (also called the "actin cap"), which develop when cytoskeletal stress is increased [57]. Such nucleo-cytoskeletal connectivity not only provides a framework by which the nucleus is attached to the cytoskeleton but also the connectivity can itself adapt. As such, forces applied to the nucleus via the cytoskeleton will recruit LINC complex proteins and Lamin A/C to specific locales under stress fibers that press on the nuclear envelope [58]. The local accumulation of LINC proteins in response to local forces along the nuclear membrane is analogous to the dynamic maturation and proliferation of FAs on the plasma membrane in response to mechanical input at substrate attachment sites. Interestingly, the nuclear envelope, and by extension LINC complexes at the nuclear envelope, have been implicated as nucleation points for actin polymer-

ization at the cytoplasmic side of the nucleus [59]. This suggests that, just like FAs, initiation of actin polymerization at the nuclear envelope can attract other scaffolding proteins such as α-actinin [60] and zyxin [61]. This leads us to propose that LINC complexes are the nuclear equivalent of FAs. While force induces recruitment of proteins including talin, paxillin, and vinculin to form FAs at the plasma membrane, mechanical force similarly recruits Nesprin, SUN, and lamin proteins to create focal contacts at the nuclear membrane. As such, focal attachments, associated with the nuclear envelope play a direct mechanosensory role by enabling the transmission of externally or internally generated mechanical signals between nucleus and the actin cytoskeleton [54, 62, 63]. At this juncture, LINC complexes are being recognized for their contribution to the mechanosensory function of cells [64, 65]—including the sensation of sound [66] and mechanical vibrations [63]—but the mechanisms by which they regulate lineage allocation of MSCs remains unclear.

How might the changes in nuclear structure and connectivity modulate MSC mechanoresponses and differentiation? Recent evidence suggests that, similar to ECM stiffness, application of substrate strain increases the nuclear translocation of YAP [67]. This response is dependent on the level of cytoskeletal prestress and nuclear deformation but critically requires the structural connection between Nesprin-1 and actin; depletion of Nesprin results in greatly diminished nuclear YAP translocation [68]. These data suggest that nuclear transport of YAP requires Nesprin-mediated connections to the cytoskeleton, and this response is dependent on mechanical force. Other nuclear transport processes have been linked to Nesprin including that of β-catenin [69].

Another way in which mechanical force might regulate MSC fate via nuclear envelope connections is through LINC attachment to the inner nuclear Lamin A/C network. Lamin A/C binds to SUN proteins forming a scaffold on the inner leaflet of the nucleus [70]. In support of the role of LINC/nucleoskeleton control, adipogenic differentiation is associated with a decrease in Lamin A/C expression [71], and both partial or complete deletion of Lamin A/C accelerates adipogenic differentiation in MSCs [72–74]. In contrast, osteogenic differentiation leads to increased Lamin A/C expression [75], a change consistent with the increased cellular stiffness of osteoblasts [76, 77]. Additionally, overexpression of Lamin A/C promotes osteogenic differentiation [78]. These observations support the idea that Lamin A/C is associated with MSC lineage decisions, but it remains unclear whether the changes in Lamin A/C are the cause or effect of differentiation. As cells deficient in Lamin A/C and LINC complex proteins are more susceptible to mechanical damage [23, 79], it is intriguing to postulate that MSCs require full function of these structural proteins to properly respond to physiological forces and adapt to a changing mechanical microenvironment.

Direct evidence for LINC-mediated force transmission and regulation of nuclear stiffness has been demonstrated recently by Guilluy et al. [80], where Nesprin-bound bead motion by magnetic force caused stiffening of an isolated nucleus through phosphorylation of the LINC binding partner Emerin in a Src and Lamin A/C-dependent manner. This suggests that mechanical signals directly modulate the nucleoskeletal structure and in turn might influence MSC lineage fate. Recent work completed using multiple cell lines, demonstrated that

Lamin A/C polarization within the nucleus is controlled by cytoskeletal force, and depletion of cytoskeletal tension disrupted this Lamin A/C polarization [81]. Conversely, deletion or mutation of Lamin A/C alters mechanical actin dynamics and interferes with MKL1-mediated SRF activity [82]. Interestingly, MKL1 nuclear localization in Lamin A/C deficient cells was primarily dependent on the localization of Emerin at the nuclear envelope rather than on LINC-mediated nucleo-cytoskeletal connectivity [82]. This suggests that Emerin might also affect intranuclear actin dynamics to regulate MKL1 localization and functionality [83].

Consistent with the hypothesis that force can rearrange nucleoskeletal structure to modulate MSC fate, our lab and others, have shown that repeated application of mechanical input, such as substrate strain or high frequency vibration, sensitizes the cell to subsequent mechanical stimuli [84, 85]. As discussed above, this increased sensitivity to very low intensity mechanical signals is in part due to augmentation of FAs and RhoA signaling [3, 41]. Furthermore, increased RhoA activity has been correlated to enhanced force transfer into the nucleus [86]. Additional evidence demonstrates that the physical connections between the nucleus and cytoskeleton are capable of altering the structure and position of nuclear proteins. Recent findings show that the dynamic force application upon integrins, via substrate strain, directly controls the displacements of coilin and SMN proteins in Cajal bodies within the nucleus. Displacement of these proteins within the Cajal body nuclear suborganelles was directly proportional to the magnitude of the strain and was dependent on both cytoskeletal stress and the presence of Lamin A/C [87]. These findings support the idea that the nucleus participates directly in sensing forces from outside the cell. Thus it is likely that LINC-mediated mechanical coupling, between the nucleus and the cytoskeleton, impacts nuclear structure and function. In fact, we have shown that the LINC complex acts as a primary mechanosensory element that senses and initiates signaling in response to low intensity vibration, while substrate strain can initiate signaling independently of LINC connectivity [88]. As such, both low and high intensity mechanical forces, through the tensegrity continuum, are eventually transferred throughout the cell. With this in mind, it will be important to consider that alterations in nuclear-cytoskeletal connections may contribute to the decreased perception of mechanical input associated with aging, microgravity, or other musculoskeletal conditions. As such, a loss in LINC complex-dependent mechanosensitivity might even contribute to reported failure of musculoskeletal tissues by limiting the accessible spectrum of mechanical information.

DYSFUNCTIONAL MECHANOBIOLOGY

The nuclear envelope has been termed "the most important border in the eukaryotic cell" [75]. Mutations involving Lamin A/C, LINC complex proteins, and their binding partners are associated with a variety of musculoskeletal conditions, including Hutchinson-Gilford progeria [89], Emery-Dreifuss muscular dystrophy [90], and dilated cardiomyopathy [91]. Nuclear envelopathies in humans and mice are characterized by failure of mechanoresponsive tissues, including bone, skeletal muscle, and heart; all tissues of mesenchymal origin.
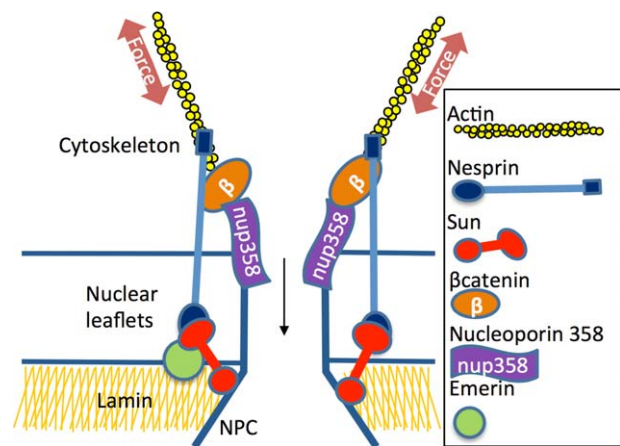

Figure 3. Model of LINC (Linker of Nucleoskeleton and Cytoskeleton)-mediated β-catenin availability in the vicinity of NPC. F-actin cytoskeleton associates with the calponin domain of Nesprin, which connects to Sun proteins through the KASH domain. Sun 1 and 2 link to the inner nuclear LaminA/C cytoskeleton. Known association between Nesprin and β-catenin may increase access of β-catenin to Nucleoporin Nup358, which facilitates nuclear import of β-catenin. Abbreviation: NPC, nuclear pore complex.

Transgenic mice with alterations in Lamin A have progeria with cardiomyopathy and sarcopenia, exhibit low bone mass, and have increased bone marrow adiposity [74]. Our group previously established that mechanical activation of β-catenin preserves MSC multipotentiality [92] and daily mechanical input effectively counteracts adipogenic stimuli, disrupting adipogenesis of MSCs [93, 94]. MSC lineage allocation, as noted above, is strongly influenced by nucleoskeletal structure and LINC function. The contribution of LINC complexes may be in regulating β-catenin compartmentalization, as nuclear translocation of β-catenin first requires binding to Nesprin on the outer nuclear membrane [69]. Indeed, limiting LINC function leads to accelerated MSC adipogenesis [88], a phenotype suggested in lamin A-deficient mice, which have musculoskeletal defects, fatty infiltration in the bone marrow, along with decreased β-catenin signaling [90]. In contrast, Emerin, another binding partner of the LINC complex [95], controls nuclear export of β-catenin; depletion of Emerin preserves nuclear β-catenin, thus limiting MSC adipogenesis [96]. Although the exact mechanisms through which LINC complexes control intracellular β-catenin compartmentalization remain poorly understood, it is known that β-catenin requires direct contact with the nuclear pore complex (NPC) for entry into the nucleus [97]. Furthermore, disruption of the nuclear pore protein, Nucleoporin 358 (Nup358), results in suppression of rapid nuclear β-catenin import [98]. As Nesprin forms a tight association with βcatenin [69], it is interesting to speculate that Nesprin, or other LINC proteins, interact with the NPC to regulate βcatenin availability and therein, MSC fate decisions (Fig. 3).

Dysregulation of Lamin A/C and the LINC complex each play an important role in aging. During the aging process, the Lamin A/C network is diminished [75] and mutated forms of Lamin A/C arise more frequently [99]. Mutations in Lamin A/C are detrimental to the formation and maintenance of LINC/nucleoskeleton connections [100] and may contribute to age-

related cell senescence [101]. In mice, aging not only results in decreased Lamin A/C expression [102] but partial knockdown of lamin A/C (Lmna$^{+/-}$) is associated with increased fat infiltration as well as impaired bone and muscle function [73]. Importantly, Lamin A/C mutant mice do not form bone in response to exercise, but instead lose bone, resulting in fractures [103].This suggests that physical connections between the cell cytoskeleton and the nucleus are important in MSC differentiation and are critical for mechanical competence of the cell. As such, one might propose that failure of musculoskeletal tissues during aging reflects an insufficient MSC response to the experienced mechanical environment; that is, an inability of MSCs to repair and reorganize those tissues subject to mechanical stress.

CONCLUSION

In the last decade considerable progress has been made in identifying the mechanisms by which cells sense and respond to both static and dynamic mechanical cues by initiating signaling events and remodeling cellular architecture. Mechanically induced structural changes modulate transmission of force within cells. Moreover, sensation of the mechanical qualities of the environment is critical in directing cellular function and, in the case of MSCs, regulating lineage selection. In this way, cells maintain a continuous flow of information—both mechanical and biochemical—between the nucleus, the cytoskeleton, and the outside environment. The importance of the physical connections between the nucleus and the cellular cytoskeleton have become increasingly apparent in recent years, especially in light of the propensity for disease accompanying the loss of structures such as Nesprin or the Lamin A/C network. As stem cells rely on mechanical cues from the environment to reorganize their structure and associated signaling mechanisms, it would be logical to expect the nucleus, as an adaptive and dynamic organelle, to participate in similar events including β-catenin, YAP, and MKL1-SRF signaling. To this end, future studies should consider the nuclear envelope and LINC complexes as an integral part of the cellular mechanosensory mechanisms that regulate bio-

chemical and physical coupling of the cell to its physical environment.

As both tissues, and the cells that comprise them, adapt to mechanical challenges, the type of mechanical input will dictate the adaptive cytoskeletal architecture and associated signaling responses. While there remain numerous challenges within the field, such as understanding the intricate manner in which mechanical signals interact with hormones or pharmacological interventions, mechanical input has the potential to regulate cell and tissue functions in both healthy and diseased states. As such, these and future studies will provide insight into how mechanical force guides growth and repair of skeletal tissues. Many questions remain including why aged cells may be resistant to physical signals, how force can be integrated into tissue engineering applications, and which exercise regimens provide optimal responses for specific disease states. In summary, it is critical that both scientists and clinicians understand the potential of mechanical forces to alter cellular and tissue response, with the ultimate goal of harnessing these signals for the repair and regeneration of injured or diseased tissues.

AUTHOR CONTRIBUTIONS

G.U. and R.F.: concept/design, manuscript editing, and final approval of manuscript; J.R.: concept/design, financial support, manuscript writing and editing, and final approval of manuscript; W.T.: concept/design, manuscript writing and editing, and final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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