Integrin signaling and mechanotransduction in regulation of somatic stem cells

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Abstract: Somatic stem cells are characterized by their capacity for self-renewal and differentiation, making them integral for normal tissue homeostasis. Different stem cell functions are strongly affected by the specialized microenvironment surrounding the cells. Consisting of soluble signaling factors, extracellular matrix (ECM) ligands and other cells, but also biomechanical cues such as the viscoelasticity and topography of the ECM, these factors are collectively known as the niche. Cell-ECM interactions are mediated largely by integrins, a class of heterodimeric cell adhesion molecules. Integrins bind their ligands in the extracellular space and associate with the cytoskeleton inside the cell, forming a direct mechanical link between the cells and their surroundings. Indeed, recent findings have highlighted the importance of integrins in translating biophysical cues into changes in cell signaling and function, a multistep process known as mechanotransduction. The mechanical properties of the stem cell niche are important, yet the underlying molecular details of integrin-mediated mechanotransduction in stem cells, especially the roles of the different integrin heterodimers, remain elusive. Here, we introduce the reader to the concept of integrin-mediated mechanotransduction, summarize current knowledge on the role of integrin signaling and mechanotransduction in regulation of somatic stem cell functions, and discuss open questions in the field.

Keywords: extracellular matrix, integrin, mechanotransduction, niche, somatic stem cell

Abbreviations: CAM, cell adhesion molecule; CAP, c-CBL-associated protein; ECM, extracellular matrix; FAK, focal adhesion kinase; FERM, four-point-one, ezrin, radixin, moesin; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; HSC, hematopoietic stem cell; ISC, intestinal epithelial stem cell; LARG, leukemia-associated RhoGEF; LIMK1, LIM domain kinase 1; LINC, linker of nucleoskeleton and cytoskeleton; MaSC, mammary epithelial stem cell; MRTF, myocardin-related transcription factor; MSC, mesenchymal stem cell; MuSC, muscle stem cell; NSC, neural stem cell; ROCK, Rho-associated protein kinase; SRF, serum response factor; TAZ, transcriptional coactivator with PDZ-binding motif; YAP, yes-associated protein 1

Introduction

Unlike most cells in the adult body, multipotent somatic stem cells, colloquially known as adult stem cells, have the capacity for self-renewal and ability to generate progeny of several distinct cell types. As such, they are integral for normal tissue maintenance and repair. Moreover, subpopulations of cancer cells exhibiting distinctly stem-like features have been suggested to contribute to treatment resistance and tumor recurrence in human cancer [1]. In all these cases, both self-renewal and differentiation are strongly affected by the specialized microenvironment surrounding the cells, known as the niche. The concept of stem cell niche comprises soluble signaling factors, extracellular matrix (ECM) ligands and neighboring cells, but also biomechanical properties, such as the elasticity, viscosity and nanotopography of the ECM (Fig. 1a) [2, 3]. Moreover, different niches can be organized in very different ways; figure 1 highlights this by presenting an overview of selected human epithelial and stromal stem cell niches (Fig. 1b) [4-6].

Cell-cell and cell-ECM interactions in the niche are mediated by different cell adhesion molecules (CAMs). Integrin heterodimers, composed of distinct alpha and beta subunits, are one of the main classes of CAMs and responsible almost exclusively for cell-ECM contacts [7]. The 24 known integrin heterodimers, or subtypes, all display high degrees of selectivity toward specific ECM components like collagens, fibronectin, vitronectin and laminins [8]. The ability of integrins to bind their targets in the extracellular space and associate with the cellular cytoskeleton via their cytoplasmic tails and a number of adaptor proteins provides a direct physical link between cells and their environment. Besides probing the mechanical qualities of the surrounding matrix, such interaction allows the cell to exert traction forces and, for example, migrate in an integrin-dependent fashion. The process by which cells sense mechanical stimuli and convert them into biochemical signals is termed mechanotransduction [9] and will be discussed in more detail below.

Several studies have addressed the niche regulatory mechanisms for different somatic stem or progenitor cells, including mesenchymal stem cells (MSCs), mammary epithelial stem cells (MaSCs), muscle stem cells (MuSCs), neural stem cells (NSCs) and intestinal epithelial stem cells (ISCs), and investigated how mechanical cues help regulate stem cell self-renewal and differentiation [3, 10, 11]. For example, MaSCs favor basal epithelial differentiation when substrate stiffness increases, while aging decreases their mechanosensitivity [12, 13]. In MSCs, elastic environments favor differentiation into adipocytes, while stiffer substrates promote osteogenesis [14]. While these and other studies demonstrate clearly that the mechanical properties of the stem cell niche are important, the underlying molecular details of mechanosensing remain elusive. Specifically, little is currently known about how the ECM composition and biomechanics, as well as the expression of specific integrin subtypes in different niches cooperate to properly support stem cell functions. This short review first introduces the

reader to the concept of integrin-mediated mechanotransduction, then summarizes current knowledge on the role of integrin signaling and mechanotransduction in regulation of somatic stem cells, and addresses open questions in the field.

Regulation of integrin activity, signaling and force transmission

Most integrin activity is thought to take place at the plasma membrane in specific integrin-mediated adhesion complexes (Fig. 2), even though integrin-mediated signaling can also occur in endocytic compartments [15, 16]. Integrins bind ECM at the leading edge of the cell and rapidly recruit additional adaptor and signaling molecules to their cytoplasmic tails, forming nascent adhesions (outside-in signaling). Alternatively, adaptor proteins like talin and kindlin can bind previously inactive integrins to increase their affinity for ECM ligands (inside-out signaling) [17]. All these interactions are intrinsically transient, and many nascent adhesions disperse soon after they are established. Others persist and get caught in the retrograde actin flow that stems from a combination of actin polymerization, membrane tension at the leading edge and contractile forces exerted by myosin II motors. Thus, adhesions and integrins link the rearward-flowing actin cytoskeleton directly to the extracellular environment, enabling cells to experience and exert mechanical forces through integrin-mediated adhesions. This assembly has become known as the molecular clutch, in an analogy for its mechanical counterpart [18, 19]. Integrin-ligand bond lifetime is finite and affected by forces exerted on the molecules [20]. Since the elasticity of the substrate affects the rate at which forces are built up in adhesions, this can have profound effects on adhesion assembly and turnover, and even cell-level properties like migration [21, 22].

The tensile stress resulting from actin flow and integrins bound to the ECM causes conformational changes in a set of mechanosensitive adhesion components, revealing cryptic binding and phosphorylation sites. This enables the recruitment and activation of additional proteins that further regulate downstream signaling pathways [23]. Talin and vinculin, two mechanosensitive proteins that mediate the connection between integrins and actin, are of special interest. Talin directly binds the β-integrin tail membrane-proximal NPxY motif via its N-terminal FERM (four-point-one, ezrin, radixin, moesin) domain. After subsequent binding to F-actin, talin undergoes a stretch-induced conformational change that exposes its first cryptic vinculin binding site in the rod R3 domain [24]. Interactions with both talin and actin allow vinculin to unfold from its closed, autoinhibited conformation [25], and a stepwise cascade follows: talin and vinculin stabilize each other's extended conformations and vinculin further links talin to actin, allowing more force to be exerted on talin. This, in turn, reveals additional binding sites for vinculin [24, 26]. Synergistic 'clutch reinforcement' by talin and vinculin strengthens the adhesion to ECM and decreases the likelihood of rupture under mechanical loading [27]. The initial step in talin unfolding can only take place if sufficient tension is reached before the integrin-

ligand bonds dissociate, thus providing a mechanism for differentiating between rigid and more elastic substrates and an additional layer of adhesion-mediated mechanotransduction [27].

As adhesions mature, they can either disassemble or undergo further force-dependent changes to their molecular composition and signaling activity [28, 29]. A considerable number of different proteins and signaling networks have been linked to adhesions: sixty proteins have appeared in most experimental studies and make up the core of the known adhesome on fibronectin [30]. However, the respective 'meta-adhesome' contains more than 2000 molecules with incidental evidence for interactions. While some of these may be explained by non-specific isolation of targets in the original studies, others most likely reflect real context-dependent differences in adhesion structure and function. Finally, although most of the data concerning adhesion dynamics and integrin signaling at adhesions originate from studies conducted on adherent 2D cultures, and care should be taken when translating these results to complex tissue environments, similar adhesive structures and clutch mechanics have recently been reported in 3D ECM [31].

Heterodimer-specific differences in integrin signaling

Integrin-mediated mechanotransduction is influenced by ECM composition, but also by the expression of particular subsets of integrin heterodimers. The subtype-specific features in integrin function are linked to 1) differential patterns of endocytic trafficking, 2) variation in integrin-ligand bond strength, and 3) unique cytoplasmic interactions with other adhesion proteins, leading to differential signaling [32]. Integrin trafficking has been shown to occur via two main intracellular pathways regulated by different Rab family proteins: shorter Rab4/Rab5-mediated recycling from early endosomes, used by integrins $\alpha_v\beta_3$, $\alpha_2\beta_1$ and $\alpha_3\beta_1$, and trafficking through the perinuclear recycling compartment, characterized by Rab11 and used mainly by integrin $\alpha_5\beta_1$ [33]. These differences in integrin recycling dynamics can have an impact on integrin availability and adhesion turnover and, by extension, signaling and cell migration. The regulation of integrin activity is also subject to variation. Unlike integrin $\alpha_5\beta_1$, whose activity is primarily regulated by inside-out and outside-in signaling, the activity of collagen-binding integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$ appears to be regulated at the level of heterodimer formation [34].

The distinct roles of integrin $\alpha_V\beta_3$ in structural adaptation to forces, and integrin $\alpha_5\beta_1$ in traction force generation are related to the weaker $\alpha_V\beta_3$-fibronectin bond strength compared to $\alpha_5\beta_1$-fibronectin bonds [35, 36]. Integrin $\alpha_2\beta_1$ bound to collagen can withstand even higher mechanical forces [37]. Thus, variation in bond strengths allows cells to execute tasks that require either strong bonds (maintenance of adhesion) or weaker but more dynamic bonds (mechanosensing) [36]. Different integrins also activate different signaling cascades upon ligand engagement and reside in specific adhesive structures (e.g. force-dependent, mature focal adhesions or ECM-

remodeling fibrillar adhesions on fibronectin), illustrating their distinct but cooperative functions in rigidity sensing [38].

Although the laminin-binding integrins $\alpha_3\beta_1$ and $\alpha_6\beta_1$ can functionally compensate for each other to promote epithelial cell adhesion, $\alpha_3\beta_1$ also antagonizes $\alpha_6\beta_1$-CD151-mediated focal adhesion kinase (FAK) signaling on laminin-111 [39]. Similarly, the collagen-binding integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$ have opposite effects in response to glomerular injury, inhibiting or promoting fibrotic collagen production, respectively [40]. They also differentially regulate crosstalk with growth factor receptors in response to collagen [41, 42]. Finally, β_3-integrin is needed in CHO cells for Rho activity and stress fiber assembly on fibronectin, whereas β_1 overexpression in β_3–lacking cells promotes Rac/JNK activity and lamellipodia formation [43]. This demonstrates how the choice of integrin heterodimer(s) used by a cell to adhere to the ECM can have a tremendous effect on cytoskeletal organization.

Currently, the majority of studies on integrin-mediated mechanotransduction have focused on fibronectin-binding integrins $\alpha_5\beta_1$ and $\alpha_V\beta_3$. However, emerging data from collagen- and laminin-binding integrins suggest that cellular responses to the physical properties of the ECM are under complex crosstalk and depend on the distinct integrin subtypes expressed by the cell. This will be an important area of investigation in the future. Characterization of integrin expression patterns and the biomechanical properties of each heterodimer can provide clues to the overarching regulation of integrin-mediated mechanotransduction, especially in complex ECM environments such as the niche in vivo.

Signaling between adhesions, actin cytoskeleton and the nucleus

Despite their obvious complexity, adhesions constitute only a part of the molecular machinery responsible for integrin-mediated mechanotransduction. Dynamics of the actin cytoskeleton, the mechanical link actin provides between adhesions and the nucleus, and nuclear mechanoresponses that finally convert biophysical cues into changes in gene expression are equally important (Fig. 2).

Stress fibers are contractile actomyosin bundles found in many non-muscle cells, including stem cells [44]. Different actin fibers have partially distinct roles in regulating adhesions and mechanotransduction [45]: for example, myosin-lacking dorsal stress fibers associate with developing adhesions near the leading edge of the cell, while so-called transverse arcs bind the proximal ends of the dorsal stress fibers. By contracting, the stress fibers in transverse arcs transmit forces all the way to the adhesions and ECM. Ventral stress fibers are connected to focal adhesions on both ends and facilitate cell movement by contracting and pulling the trailing edge of the cell. The perinuclear actin cap consists of stress fibers positioned above the nucleus and is of special interest in regards to mechanotransduction: its actin structures are directly linked to

the nuclear envelope, enabling the propagation of forces between the cyto- and nucleoskeletons as discussed below [45]. Indeed, recent results indicate that the perinuclear stress fibers can be highly contractile, terminating at integrin β_1- and zyxin-rich adhesions in the perinuclear region [46].

Despite their variable functions, the molecular components that make up different stress fibers are very similar. Accessory proteins like α-actinin link polarized F-actin filaments together. Non-muscle myosin II forms bipolar bundles of 15-20 molecules with motor heads at both ends, and the bundles are bound to actin filaments with the help of tropomyosin. Myosin slides actin filaments in opposite directions within the actomyosin bundle by using energy from ATP hydrolysis, generating force for actomyosin contraction [47].

The contractility of the actomyosin network changes in response to intra- and extracellular forces [47]. While adhesion maturation and mechanosensing depend on interactions with F-actin, the opposite is also true, and adhesions play a critical role in regulating actomyosin organization and myosin II activity. [48]. One key regulator of myosin II is the Rho-ROCK (Rho-associated protein kinase)-pathway, which promotes the phosphorylation of myosin II regulatory light chain and consequently facilitates myosin bundle assembly, myosin kinetics and actomyosin contraction (Fig. 2b). ROCK also inactivates Cofilin-1 through LIM domain kinase 1 (LIMK1), stabilizing F-actin filaments, whereas formin mDia1 promotes actin polymerization directly downstream of RhoA [45].

Mechanically activated ion channels, such as the Piezo family ion channels, have recently been linked to integrin-mediated mechanotransduction. Piezo1 and Piezo2 are expressed on the plasma membrane of a wide variety of cell types [49]. The channels respond to membrane tension and can be activated by external forces, as well as intracellular actomyosin contraction. Piezo1 activates β_1-integrins [50] and Piezo2 actuation, in response to metastatic cancer cells probing their extracellular environment, activates RhoA to control stress fiber and adhesion formation [51]. Thus, integrin-mediated adhesions, actin dynamics and Piezo activation can synergize to regulate cell adhesion through positive biomechanical feedback. The different functions of mechanosensitive ion channels are discussed in full detail elsewhere [52].

An integral part of cellular mechanotransduction takes place when information about biophysical cues reaches the nucleus. It is the change in gene expression that controls the cells' long-term adaptation to their environment and, in the case of stem cells, their capacity for self-renewal and differentiation [53, 54]. The different cytoskeletal systems of a eukaryotic cell are connected directly to the nuclear envelope and nucleoskeleton via linker of nucleoskeleton and cytoskeleton (LINC) complex, comprising nesprin proteins on the outer nuclear membrane and SUN domain-containing proteins on the inner nuclear membrane. The SUN proteins, in turn, are bound to a filamentous protein network just inside the nuclear envelope, known as the nuclear lamina.

Consequently, integrin-mediated mechanical forces can strain chromatin and nuclear components directly, altering nuclear rheology and causing structural changes and mechanical adaptations in the lamina. All of these processes have the potential, or have been proposed to alter transcription [55].

Additionally, direct nuclear mechanoresponses converge with other, indirect signals from adhesions and actin cytoskeleton to regulate the localization and activity of various mechanosensitive transcription factors, including e.g. Hippo pathway transcriptional coregulators yes-associated protein 1 (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), and myocardin-related transcription factors (MRTFs). YAP/TAZ, in particular, have recently emerged as a vital mechanotransducing hub that helps integrate cellular and tissue mechanics with metabolic and developmental signaling, allowing context-dependent transcriptional responses [56]. Specific mechanical cues that promote YAP/TAZ nuclear translocation and transcriptional activity include rigid environments, lack of spatial constraints and tensile loading [57]. MRTFs are integral components of the serum response factor (SRF) pathway. They translocate to the nucleus following biophysical cues that closely resemble those needed for YAP/TAZ activation, which enables transcriptional regulation by SRF. Mechanistically, MRTFs are responsive to the G/F-actin ratio, as G-actin binds MRTFs to promote their nuclear export and keeps the proteins sequestered in the cytoplasm (Fig. 2b) [58].

Integrin-mediated mechanotransduction is a complex, multistep process that reaches well beyond the molecular assembly of the bona fide adhesion. In the following chapters, we will discuss how integrin signaling and mechanotransduction can contribute to the regulation of stem cells.

Integrin expression and functions in stem cells

Integrin interactions with the niche ECM, along with other mechanical signals mediated by cell-cell contacts and other ECM receptors like syndecans, are crucial for establishing a balance between stem cell self-renewal and differentiation. Owing to this, different integrins such as α_6, β_1, β_3 and β_4 have been used as cell surface markers for stem cells in various normal and malignant tissues [59-61]. Integrin-mediated polarity and compartmentalization of signals regulate cellular responses to different cues on a cell-by-cell basis [62]. Such precise control of signaling is important for the process of asymmetric division, a fundamental characteristic of self-renewing cells [63].

Various integrin subtypes are crucial for somatic stem cell maintenance in different tissues [64]. Integrin-mediated anchorage to basement membrane components, including laminin, fibronectin, and collagen IV, promotes asymmetric cell division in many types of stem cells, thereby coordinating tissue homeostasis [63]. The laminin-binding integrin α_6 is widely expressed

in multiple stem cells types, including MaSCs, MuSCs, NSCs and different cancer stem cells [65, 66]. High expression of integrins α_6 and β_1 is used as a marker for the stem cell-enriched epithelial population of the mammary gland [59, 60], and adhesion to laminin-111 supports the functional differentiation of mammary epithelium [67]. Furthermore, conditional depletion of integrin β_1 in basal mouse mammary stem cells perturbs the asymmetric pattern of cell division that is necessary for maintaining the niche [68]. On the other hand, increased integrin β_3 expression distinguishes basal cells and mammary luminal progenitors from mature, differentiated luminal cells. Integrin $\alpha_v\beta_3$ mediates mammary gland remodeling events during mid-pregnancy by promoting MaSC expansion, clonogenicity, and expression of the master stem cell regulator slug [69].

Muscle regeneration initiates a remodeling event mediated by matrix metalloproteinases, leading to the deposition of laminins in the MuSC niche. Laminin α1-chain, deposited in the basement membrane covering activated stem cells, maintains MuSC polarity and asymmetric cell division via integrin $\alpha_6\beta_1$, and supports self-renewal [70]. On the other hand, low integrin β_1 expression in NSCs compared to actively dividing neural precursor cells, and thus limited interaction of NSCs with the laminin-rich microenvironment, has been suggested to contribute to the cells' relatively quiescent phenotype [71]. Interestingly, the optimal stiffness for culturing embryonic cortical progenitors and adult NSCs on bifunctionalized gels with laminin peptide IKVAV and polylysine varies by one order of magnitude (E = 2 kPa and 20 kPa, respectively), suggesting adaptation to different ECM conditions [72].

The intestinal crypt ECM adjacent to ISCs is rich in fibronectin, but as the ECM composition changes gradually toward the lumen, integrin expression patterns are concordantly altered from fibronectin-binding integrin $\alpha_5\beta_1$ in the crypt to laminin-binding integrins $\alpha_3\beta_1$ and $\alpha_6\beta_4$ in the villus (Fig. 1b) [73]. Similarly, the differentiation of bipotential pancreatic progenitors to either ductal or endocrine lineage has been attributed to the cells' access to fibronectin during development. Cells that encounter more laminins downregulate their fibronectin-binding $\alpha_5\beta_1$ integrins, leading to NGN3 activation and eventual endocrine differentiation [74]. Together, these observations demonstrate the variety of temporal, spatial and activation-induced plasticity occurring in the basement membrane-associated stem cell niches.

The bone marrow stem cell niche supports hematopoietic stem cell (HSC) maintenance via integrin $\alpha_v\beta_3$-mediated adhesion [75], whereas the survival of MSCs, derived from the same niche, is mediated by integrins $\alpha_2\beta_1$ and $\alpha_{11}\beta_1$ on collagen matrix (Fig. 1b) [76]. Substrate biomechanics, including elasticity [14, 77] and nanotopography of the ECM ligand [78], strongly influence the fate of MSCs: their stiffness-dependent osteogenic differentiation is driven by integrin $\alpha_1\beta_1$ or, to a lesser degree, $\alpha_2\beta_1$ signaling when the cells are cultured in/on collagen, and $\alpha_5\beta_1$ signaling when fibronectin is used [77-79]. In this way, the MSCs appear quite plastic, possibly reflecting their origin in a relatively complex stromal niche (Fig. 1b).

Taken together, integrins provide biomechanical cues to many types of somatic stem cells. How the binding dynamics and downstream signaling cascades of different integrin heterodimers contribute to mechanotransduction in stem cells, and ultimately translate into decisions of quiescence, cell division or differentiation, remains to be systematically investigated. Mechanisms that most likely contribute to these differential signaling responses are discussed below.

Adhesion signaling and stem cells: beyond integrins

Mechanotransduction via integrin-mediated adhesions has been studied extensively, however, many of the results have not been replicated in stem cells. Even though detailed studies on stem cell adhesion dynamics are lacking, more is known about the roles of individual adhesion components, or signaling cascades downstream of integrins, and how they relate to stem cell mechanotransduction. In this regard, the MSCs and their progeny are especially well characterized.

Increased osteogenic differentiation of MSCs on rigid (≥40 kPa) collagen substrates correlates with an increased expression of integrin α_2, phospho-FAK and phospho-ERK1/2 [80]. Knockdown of integrin α_2 or inhibition of FAK downregulates osteoblast-related genes *COL1A1* (type I collagen) and *BGLAP* (osteocalcin), inhibits ERK1/2 phosphorylation and ultimately decreases osteogenesis, indicating that the stiffness of the collagen matrix regulates MSC osteogenic differentiation through an integrin α_2-FAK-ERK1/2-dependent pathway. Additionally, MSC osteogenesis is dependent on substrate stiffness-induced adhesion reinforcement: on stiff matrices, vinculin depletion promotes the usually suppressed adipogenic differentiation [81]. This is linked to vinculin function in integrin-mediated adhesions, as knockdown of c-CBL-associated protein (CAP), a vinculin-binding protein that immobilizes vinculin in adhesions, is enough to recapitulate the phenotype [82].

Normal MSC osteogenesis also requires kindlin-2, an integrin- and actin-binding protein that supports integrin activation [83]. Depletion of kindlin-2 in human MSCs induces spontaneous adipogenic differentiation and decreases cell viability. Even though kindlin-2 can bind myosin light-chain kinase directly to regulate myosin II phosphorylation and actomyosin contractility, re-expressing integrin-binding defective kindlin-2 in MSCs only modestly increases integrin β_1 activation and osteogenesis. This indicates that the adipogenic effect depends on kindlin-2 integrin-modulating function. Finally, in concordance with its positive reciprocal relationship with integrin mechanosignaling, Piezo1 promotes BMP2 expression and osteogenic lineage commitment in MSCs [84]. Taken together, these results underline the importance of integrin-mediated mechanotransduction for MSC differentiation.

Besides MSCs, the importance of fully functional adhesions has been demonstrated in HSCs. While vinculin is dispensable for HSC migration, adhesion and spreading on fibronectin in vitro,

loss of the protein severely impairs the ability of HSCs to support reconstitution of hematopoiesis after competitive transplantation into lethally irradiated mice [85].

Actomyosin regulates stem cell lineage commitment

Integrin-mediated adhesions and actomyosin dynamics and contractility are fundamentally interconnected. It is not surprising, then, that several components and regulators of the actin cytoskeleton are involved in the mechanosensitive maintenance and differentiation of stem cells.

Rho-ROCK signaling seems especially important for differentiation and lineage commitment. In MSCs, inhibition of RhoA or low RhoA expression leads to adipogenic differentiation via Cofilin-1 activation, actin filament depolymerization and Smad2/ERK signaling. In contrast, activation of RhoA is needed for osteogenesis and involves Cofilin-1 inactivation, actin filament stabilization and p38, JNK and FAK signaling [86].

Guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) modulate RhoA activity downstream of adhesions. Moreover, emerging evidence points to a role of specific GEFs and GAPs in MSC differentiation. GEF-H1 is one such protein, promoting Rho-ROCK signaling by switching RhoA into its active, GTP-bound state. Interestingly, GEF-H1 also links adhesions to the cytoskeleton in MSCs by recruiting non-muscle myosin heavy chain II-B to adhesions, leading to stress fiber polarization, increased adhesion formation and osteogenesis [87]. Another GEF, leukemia-associated RhoGEF (LARG), is needed for the mechanical activation of RhoA and suppression of adipogenesis in MSCs. In contrast, ARHGAP18, a Rho GAP, is necessary for normal adipogenic differentiation, suggesting that the protein is responsible for persistent downregulation of RhoA activity and cytoskeletal assembly in MSCs [88].

Actin dynamics have also been linked to lineage commitment in non-mesenchymal somatic stem cells. A stiff matrix increases RhoA activity in adult NSCs, leading to increased cellular contractility and astrocytic differentiation, at the cost of decreased potential for neuronal differentiation [89]. Another actin modulator, mDia1, facilitates force-dependent myofibroblast differentiation [90].

Perhaps the most striking example of actin modulation in stem cells, however, comes from human pluripotent stem cells. When these cells are cultured in rigid 2D environments, actin forms a strong contractile ring around the compacted colony, exerting extensive Rho-ROCK-myosin-dependent forces and promoting pluripotency. During differentiation the cells shift from ventral to dorsal stress fiber organization, concordant with reduced overall mechanical stress [91].

Regulation of adhesion dynamics, actomyosin assembly and contractility are critical in biomechanically induced stem cell differentiation. Most studies to date have focused on MSCs, while the same mechanotransduction processes in other somatic stem cell types are still poorly understood. Furthermore, while the role of Rho-ROCK signaling in the mechanical regulation of

stem cells has been studied extensively, for other (direct) actomyosin regulators like tropomyosins this is not the case.

Nuclear mechanoresponses integrate mechanical cues to regulate stem cell functions

Downstream of integrin-mediated adhesions and force generation and propagation by actomyosin, information about the cell's biophysical environment is transmitted to the nucleus. There, changes in gene expression are often mediated by mechanosensitive transcription factors and coregulators.

The core mechanotransducers YAP/TAZ have increasingly been associated with cell plasticity and stemness in numerous physiological and malignant contexts. In addition to mediating the kindlin-2- and vinculin-dependent osteogenesis of MSCs [81, 83], YAP/TAZ have been shown to contribute to self-renewal or differentiation in e.g. lung basal stem cells, ISCs and adult NSCs [92-94]. In mouse cartilaginous airways, basal stem cells exist in an Fgf10-expressing niche that is dependent on YAP-Wnt7b signaling. Interestingly, integrin-linked kinase (ILK) positively regulates the Hippo pathway to suppress YAP outside the niche, and its inactivation allows the airway epithelium to respond to injury [92]. In mouse colitis, the colonic epithelium during tissue repair is reversibly reprogrammed into a primitive state with fetal-like properties. This requires YAP/TAZ and is preceded by collagen I deposition and increases in the levels of integrin β_1, FAK and phospho-Src [93]. Together with β-catenin, YAP/TAZ also regulates mechanical memory in neuronal lineage commitment. NSCs are maximally receptive to ECM elasticity within the first 12-36 hours of exposure, during which a soft 0.7 kPa substrate decreases YAP levels and promotes neuronal differentiation relative to the stiffer 75 kPa substrate. Mechanistically, YAP antagonizes the neurogenic effects of β-catenin [94]. Transient ectopic expression of YAP may even turn primary differentiated cells into progenitor cells of the same lineage: reported examples include MaSCs and pancreatic duct-like progenitors, both of which can be expanded in long-term organoid cultures [95].

YAP/TAZ can regulate stem-like features also in cancer. For example, breast cancer stem cells deposit laminin-511, which supports self-renewal and tumor initiation in an integrin $\alpha_{6B}\beta_1$-dependent manner [96]. As an example of positive mechanobiological feedback, $\alpha_{6B}\beta_1$ activates TAZ, which then promotes the transcription of laminin-511 α5 subunit and formation of a laminin-511 matrix. All these results indicate that YAP/TAZ-mediated transcriptional programs are tightly linked to stemness and niche maintenance in health and disease.

Besides YAP/TAZ, other integrin-regulated, mechanosensitive transcription factors have also been indicated in stem cell functions. For example, MRTF-SRF signaling is indispensable for bone marrow colonization with HSCs during development (Fig. 1b) [97]. SRF is known to regulate the

388 expression of different cytoskeletal target genes; interestingly, mouse *Srf* KO HSCs also exhibit
389 reduced levels of integrins α_2, β_1, and β_2 [97].

390 Importantly, some of the transcriptional responses to integrin-mediated mechanosensing may
391 require direct strain propagation and/or structural changes to the nucleus itself (Fig. 2b). While
392 some mechanosensitive signaling pathways, like ERK signaling, may be activated in the absence
393 of nuclear strain, cytoskeletal strain transfer to the nucleus has been shown to be necessary for
394 the stretch-induced YAP/TAZ activation in bovine bone marrow MSCs [98]. Indeed, recent work
395 has demonstrated how adhesion-mediated forces can trigger the nuclear entry of YAP by
396 regulating transport through nuclear pores, and the same mechanism may contribute at least
397 partially to the translocation and activity of other mechanosensitive transcription factors [99].
398 Moreover, forces propagated by actomyosin on a rigid substrate stabilize A-type lamins by
399 decreasing their phosphorylation and turnover [100]. In other words, cells can tune the
400 mechanical properties of their nuclei to match the requirements of their environment, which in
401 turn feeds back to transcriptional programs. In MSCs, lamin-A knockout leads to suppressed SRF,
402 but not YAP signaling, which is enough to promote adipogenic differentiation. Conversely, lamin-
403 A overexpression supports MSC osteogenesis [100].

404 In somatic stem cells, like other adult cell types, direct force-induced responses and modulation
405 of mechanosensitive transcription factors converge in the nucleus. Together with additional
406 biochemical and metabolic signals, these mechanisms determine the cellular response to
407 integrin-mediated physical cues. YAP/TAZ have recently emerged as regulators of stem-like
408 capabilities in several contexts, presenting an intriguing possibility: the varied integrin expression
409 profiles in different stem cell niches may be adaptations for biomechanically distinct ECM
410 compositions and architectures, with a universal aim of regulating a relatively conserved,
411 mechanosensitive transcriptional program. However, this remains to be formally investigated.

412 ***Conclusions and future outlook***

413 Different somatic stem cells, residing in their respective niches, are exposed to a variety of cell-
414 ECM interactions during quiescence, cell division and differentiation. How integrin-mediated
415 mechanotransduction helps regulate these states, and promotes or suppresses transitions
416 between them, is not yet fully understood. However, it is clear that the molecular composition
417 and biophysical features of the ECM, as well as the expression patterns of individual integrin
418 subtypes, additional mechanosensitive adhesion proteins, and actomyosin dynamics and
419 contractility all play key roles in the process. The specific functions of different integrin
420 heterodimers may be attributed to their differential trafficking, unique cytoplasmic interactions
421 and downstream signaling cascades, and biophysical qualities (e.g. different integrin-ligand bond
422 strengths) that affect mechanotransduction directly.

Recent advances in technology [101] have enabled the dissection of integrin signaling and transmitted forces in fine detail, down to the single-molecule level. However, the full variability between different integrin subtypes has only begun to be appreciated. Additionally, in order to advance our understanding of stem cell mechanobiology, many of the current reductionist models will eventually need to be complemented by experimentation in more physiologically relevant settings. Ideally, this would mean in vivo characterization of tissue biomechanics, or at least better biomimetic culture systems to help overcome the limitations of conventional 2D assays. Technology imposes limitations here, yet the first instances of stress measurements in vivo using deformable inserts of known mechanical properties have already been reported [102].

So far, key mechanosensitive transcriptional regulators like YAP/TAZ offer the most promising examples of overarching integration of mechanical and biochemical signals. These mechanisms appear relatively conserved across different cell types, and importantly, many recent studies have highlighted the role of YAP/TAZ in niche maintenance and regulation of the stem cell phenotype. Nevertheless, simply knowing the outcome at the transcriptional level may not be enough for medical interventions or bioengineering; what is ultimately needed is a systems-level understanding of mechanotransduction from the plasma membrane to the nucleus, and the means to target specific parts of the network in a meaningful way. Whether common integrin-based mechanisms regulating stem cell fate in health and disease can be found, and whether the different integrin profiles in various somatic stem cell niches represent adaptations to biomechanical differences above all, remain exciting topics for future research.

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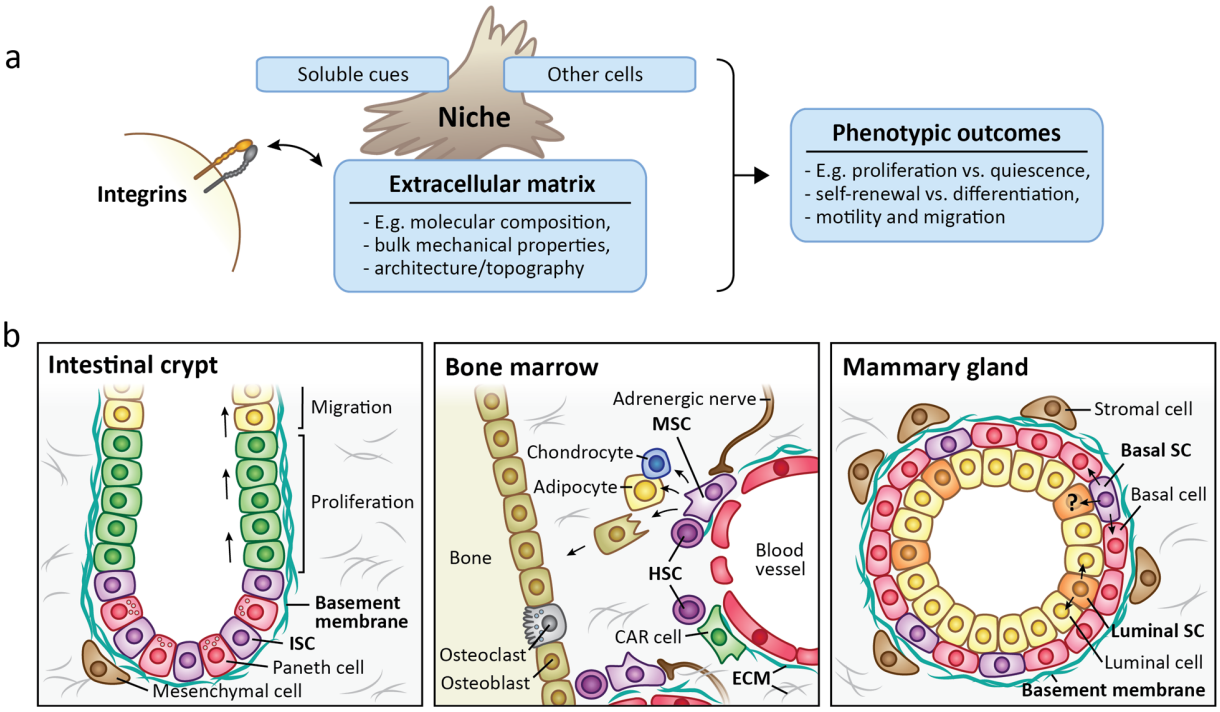
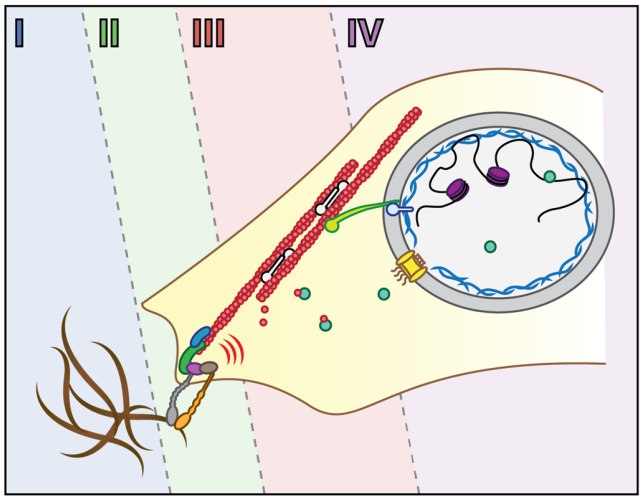
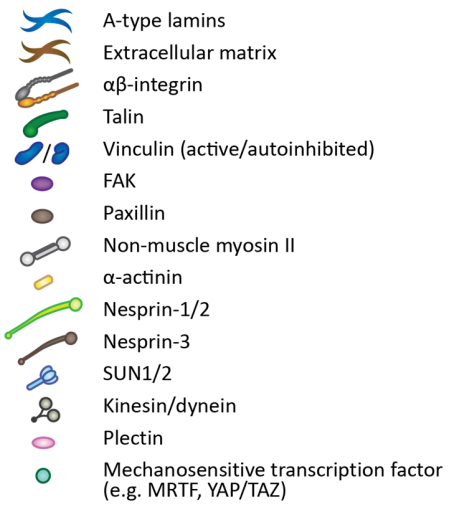


Figure 1. Stem cell functions are regulated by carefully coordinated biochemical and mechanical signals. (**a**) Different aspects of the somatic stem cell niche. In multicellular organisms, the vast majority of cells are in continuous contact with proteinaceous extracellular matrix; different stem cells and progenitors are not an exception. Together with cell-cell contacts and gradients of soluble signaling molecules, integrin-mediated cell-ECM interactions contribute to the homeostasis of the stem cell niche. (**b**) Schematic overviews highlighting the differential organization of three adult stem cell niches: intestinal [4], bone marrow [5], and mammary gland [6]. CAR, CXCL12-abundant reticular; SC, stem cell.

a


Integrin-mediated mechanotransduction
I
II
III
IV


Key:
A-type lamins
Extracellular matrix
αβ-integrin
Talin
Vinculin (active/autoinhibited)
FAK
Paxillin
Non-muscle myosin II
α-actinin
Nesprin-1/2
Nesprin-3
SUN1/2
Kinesin/dynein
Plectin
Mechanosensitive transcription factor
(e.g. MRTF, YAP/TAZ)

b

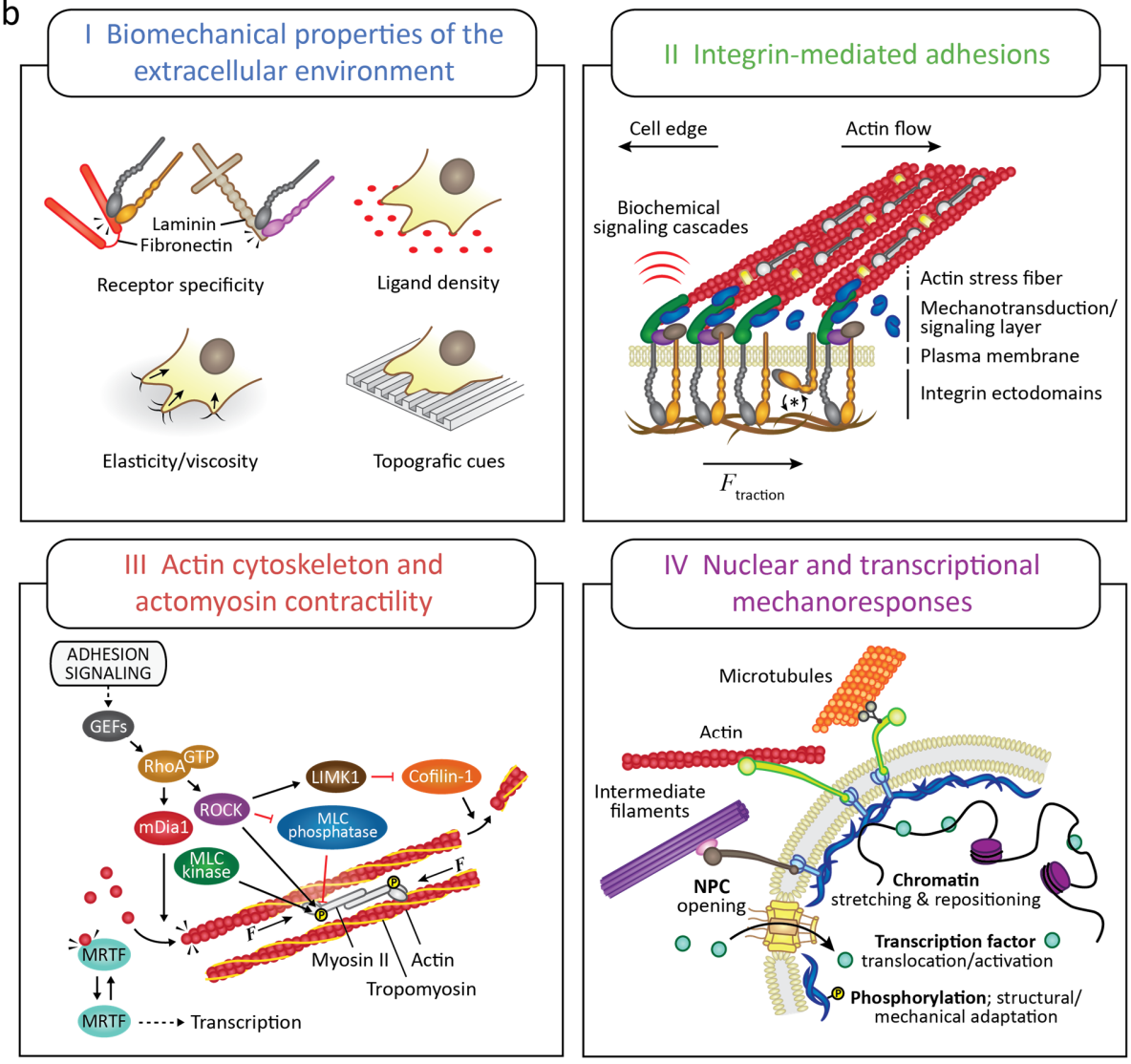

I Biomechanical properties of the extracellular environment
Laminin
Fibronectin
Receptor specificity
Ligand density
Elasticity/viscosity
Topografic cues
II Integrin-mediated adhesions
Cell edge
Actin flow
Biochemical signaling cascades
Actin stress fiber
Mechanotransduction/ signaling layer
Plasma membrane
Integrin ectodomains
F traction
III Actin cytoskeleton and actomyosin contractility
ADHESION SIGNALING
GEFs
RhoA
GTP
ROCK
LIMK1
Cofilin-1
mDia1
MLC phosphatase
MLC kinase
MRTF
Myosin II
Actin
Tropomyosin
MRTF
Transcription
IV Nuclear and transcriptional mechanoresponses
Microtubules
Actin
Intermediate filaments
NPC opening
Chromatin stretching & repositioning
Transcription factor translocation/activation
Phosphorylation; structural/ mechanical adaptation

Figure 2. Integrin-mediated mechanotransduction and its constituent processes. (**a**) Adhesion dynamics and signaling, actomyosin assembly and force generation, as well as nuclear mechanoresponses all contribute to the cell's functional and transcriptional responses to external biomechanical cues. (**b**) Detailed overview of the different aspects of integrin-related mechanotransduction. (i) Different ECM components are recognized by defined integrin subtypes, which can lead to differential binding dynamics and downstream signaling. Besides the bulk elasticity and viscosity of the substrate, the distribution of available ligands and micro-/nanotopographic features all affect the assembly and function of integrin-mediated adhesions. External tensile forces can also be propagated via integrins, but are not shown here. (ii) Integrin-mediated cell-ECM adhesions are intrinsically dynamic. Integrins and a plethora of adaptor and signaling proteins link filamentous actin to the underlying substrate, slowing down actin retrograde flow and transmitting traction forces to the environment through a 'molecular clutch'. The binding and unbinding rates of individual integrins (*) are affected by forces exerted on these molecules; together with other force-sensitive adhesion proteins (e.g. talin, vinculin) that help regulate adhesion reinforcement and downstream signaling, this allows the cells to fine tune their response to substrate mechanics. (iii) Actomyosin plays a dual role in integrin-mediated mechanotransduction. On one hand, it forms a direct mechanical link between integrins and the nuclear envelope, enabling many of the downstream nuclear mechanoresponses. In addition, myosin II motors allow the cells to exert contractile forces of their own. This is crucial for processes that require motility, e.g. cell migration and ECM remodeling, but it also allows differentiating between mechanically distinct substrates in the absence of external forces. Finally, kinetics of actin assembly and disassembly regulate the intracellular G/F-actin ratio. This can have a profound effect on gene expression, for example by modulating the localization and activity of the SRF pathway transcriptional coregulators, MRTFs. (iv) Biophysical cues may reach the nucleus in different ways. In the context of integrin-mediated mechanotransduction, this can mean direct strain propagation via actin and the LINC complexes, leading to e.g. chromatin stretching, changes in nuclear rheology, or structural and mechanical alterations in the nuclear lamina, all of which may have an effect on gene expression. Tension can also help open nuclear pore complexes (NPCs), promoting the nuclear translocation of transcriptionally active proteins like YAP/TAZ. However, many mechanosensitive transcription factors are also regulated directly by signals from adhesions or actomyosin, via post-translational modifications and/or interactions that control the protein's localization and activity. The schematics presented here are not exhaustive, and several adhesion and nuclear components, as well as proposed nuclear mechanoresponses have been omitted for clarity. For an in-depth discussion on these topics, the reader is directed to other recent reviews [19, 30, 55].