Role of integrin adhesions in cellular mechanotransduction

Balcıoğlu, H.E.

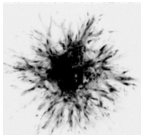
Chapter 1

Introduction: Cell matrix adhesions - feeling the force [1]

[1] This chapter is partially based on: Karin A Jansen; Dominique M Donato; Hayri E Balcıoğlu; Thomas Schmidt; Erik HJ Danen; Gijsje H Koenderink, A guide to mechanobiology: Where biology and physics meet., *Biochimica et biophysica acta. 2015*

Abstract

In their natural context, cells are in contact with the extracellular matrix (ECM) that provides cells with chemical and physical cues. The physical properties of the ECM control cell survival, proliferation, and differentiation, and its deregulation can contribute to pathologies such as fibrosis and cancer. Transmembrane receptors of the integrin family couple the ECM network to the intracellular cytoskeletal network. Integrins sense and transmit biophysical cues in both directions, providing mechanical homeostasis between cells and ECM. Here, we discuss recent advances in our understanding of the integrin-associated mechanotransduction complex within cell-matrix adhesions and how this, in concert with chemosensory signaling pathways, controls cell fate.



Cells are able to sense and respond to physical as well as chemical aspects of their surrounding extracellular matrix (ECM) to maintain homeostasis with their environment. The physical aspect of this interaction determines normal cell function, stem cell differentiation and tissue homeostasis [1, 2], while deregulation can contribute to onset and progression of cancer [3]. Forces also play a crucial role in embryogenesis [4, 5] and cells in our body are constantly under force; e.g. cell-cell forces in epithelial tissues, compression and tension due to muscle contraction, shear forces in vasculature, lung epithelium and intestines. Therefore, in addition to its importance in cancer research, manipulating the mechanical properties of the ECM has become a powerful tool in stem cell research and tissue engineering.

1.1 Mechanics of mechanosensing

Several signal transducers have been implicated in the ability of cells to sense and respond to extracellular forces, including ion channels, cell matrix adhesion complexes and membrane-associated phospholipases [6, 7]. In any case, a force-transmitting cytoskeleton is essential for cells to sense the mechanical properties of the environment. The microtubules (MT) [8], actin cytoskeleton [9] and intermediate filaments (IF) [10] have all been implicated in cellular mechanotransduction. Indeed, Rho GTPases, the enzymes in control of cytoskeletal organization [11], play important roles in cellular sensing of- and responding to force [12, 13].

1.2 The mechanical scaffolds: the cytoskeleton and the ECM

Cytoskeletal networks, enable cells to maintain their shape and mechanical strength [14]. Of the three cytoskeletal systems; MTs, IFs and actin cytoskeleton, the emphasis has been on actin cytoskeleton that is responsible for traction force generation [15]. The actin cytoskeleton forms a continuous network between the nucleus and, via the adhesion complex, the ECM [16] (Figure 1.1). Cells, prominently on 2D substrates, form long contractile actomyosin structures termed stress fibers that apply traction forces via myosin molecular motors pulling on polarized actin filaments [15]. Formation and organization of such stress fibers

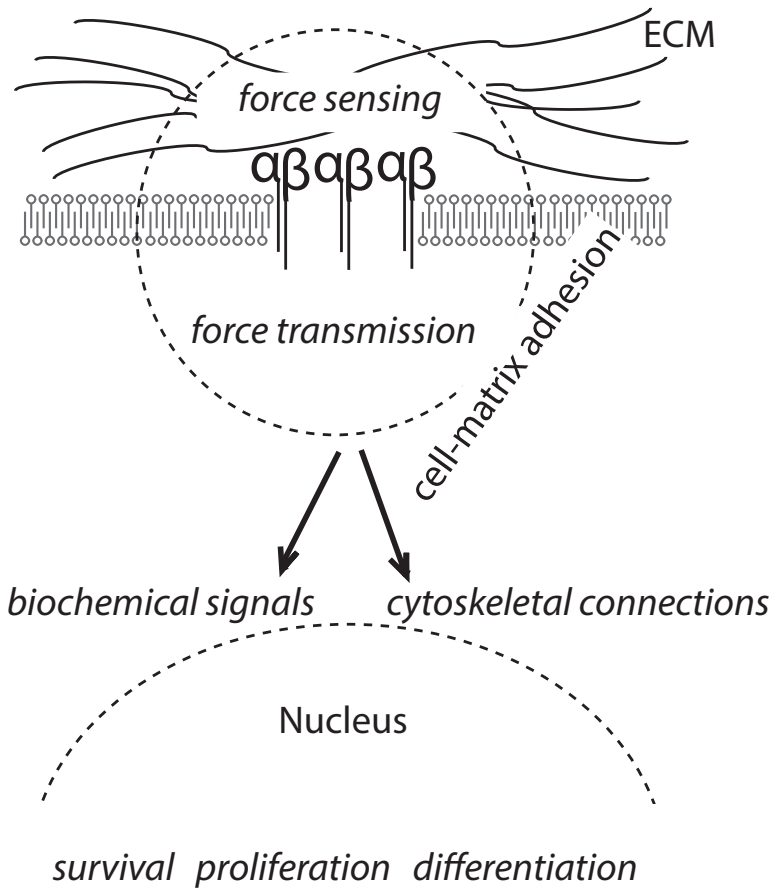
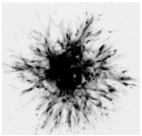


Figure 1.1
Mechanical cues from the environment dictate cell fate decisions. *Cartoon depicting force sensing, transmission, and translation into biological response through cell matrix adhesions.*



are stiffness dependent [17, 18]; and the formation of adhesion complexes is dependent on the actin cytoskeleton [19].

Purified networks of actin and intermediate filaments increase their stiffness under the influence of force. In other words, these networks strain-stiffen in response to mechanical shear or stretch [20–22]. This phenomenon allows cells to actively stiffen their actin cytoskeleton on hard substrates [18]. Moreover, strain-stiffening of IFs is thought to prevent excess deformation of cells and epithelial tissues [21, 22].

Microtubules (MTs) are not so widely studied in the context of mechanotransduction but MTs also influence cell-matrix adhesions by regulating traction forces via crosstalk with the actomyosin machinery and it has been shown that both on 2D substrates [23, 24] and in 3D collagen gels [25, 26], MT depolymerization causes increased traction forces and thereby adhesion maturation [27].

ECM properties play an important role in mechanosensing. Cells can sense the global (i.e. macroscopic) and local (e.g. fiber) matrix stiffness, matrix topography [28], the porosity [29] and dimensionality as well as actively change the physical properties of the ECM [30, 31]. In fibrous collagen or fibrin networks, cells can sense to a length scale of $\sim$200 µm [32, 33], whereas on 2D flexible gels, this distance is reduced to a few tens of microns [34, 35]. The organization of ECM network is tailored to the function of each tissue, for instance collagen fibers are thick and aligned in stiff tissues like tendon to ensure tensile strength, whereas they are thin and organized in a meshwork in cornea to ensure optical transparency. During disease progression and aging the physiological organization of the ECM is subject to changes and ECM is increasingly recognized as an active player and potential therapeutic target in diseases such as fibrosis, atherosclerosis and cancer [36–40].

The ECM forms a scaffold for cells to adhere to and acts as a reservoir for growth factors, cytokines and proteolytic enzymes. ECM structures can be 2D (e.g. basement membrane) as well as 3D (connective tissue) and cell matrix adhesion proteins regulate cell motility on both of these ECM environments [41].

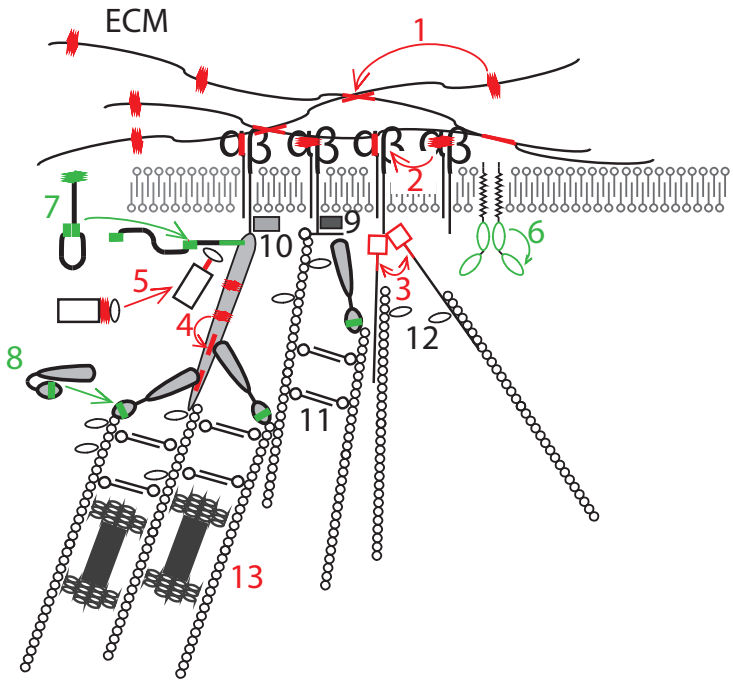
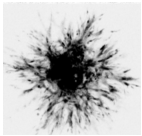


Figure 1.2

Cell matrix adhesions as hotspots for bidirectional mechanotransduction. *Elements associated with cell matrix adhesions that act as force sensors; e.g. change conformation/interactions in response to force are indicated in red. Elements that are implicated in force transmission but have not (yet) been directly implicated as force sensors are indicated in green. 1) When cytoskeletal contractility, through integrins, stretches fibronectin fibers; cryptic sites are exposed that cause enhanced cross-linking; 2) intracellular or extracellular forces cause conformational changes in the integrin head domain of some integrins driving strengthening of a catch bond with ECM; 3) kinetics of filamin dimerization change under force, which affects its actin and integrin binding; 4) stretching of flexible domains in talin exposes cryptic vinculin (8)-binding sites; 5) stretching of the linker domain in p130Cas may expose phosphorylation sites, which can trigger new protein-protein interactions; 6) force-dependent unfolding of the zipper-like autoinhibitory domains in RPTPalpha may underlie its role in rigidity sensing; 7) force-dependent breaking of an autoinhibitory intramolecular interaction involving the FERM domain and/or stretching of its adhesion targeting domain may trigger FAK activation and explain its role in force transmission; 8) myosin contractility-dependent interaction of vinculin head and tail domains is important for its role in mechanotransduction; 9-12) ILK, paxillin, alpha-actinin and zyxin have been implicated in rigidity sensing but it is not known whether they undergo conformational change in response to physiological force; 13) extracellular forces, through cell matrix adhesions enhance actomyosin contractility thereby balancing intra- and extracellular forces in the cell matrix adhesions and coupling through physical linkage to the nuclear envelope.*



1.3 Cell matrix adhesions at the heart of force sensing

The regions where cells are in close physical contact with their environment and connect to the actin cytoskeleton - the "cell matrix adhesions" - appear to be hotspots for mechanotransduction [42] (Figure 1.2). Within cell matrix adhesions, clustered integrin transmembrane receptors bind ECM components with their globular head domains and connect to the actin cytoskeleton through their short cytoplasmic tails [43–45]. Coupling to the cytoskeleton is indirect, involving a large, regulated protein complex that connects the integrin tails to f-actin fibers [43]. Cell matrix adhesions are mechanosensitive structures [46–48] that may also be centers for protein synthesis through force dependent recruitment of ribosomes [49]. The activity of Rho GTPases is regulated by force responsive signaling cascades in cell matrix adhesions [50]. In turn, Rho GTPase-mediated alterations in cytoskeletal tension affect growth and turnover of cell-matrix adhesions [51, 52].

Cell matrix adhesions have a well-preserved nanoarchitecture [53], their size correlates with cell migration speed in 2D [54] and the presence of cell matrix adhesions in 3D ECM environments has been established [55–57]. The tight connection between force and cell matrix adhesions has been studied using laser tweezers [58], traction force microscopy on deformable gels [59], micropillar arrays [60, 61] and bead displacement maps in 3D ECM networks [62].

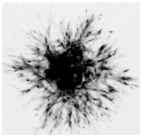
Integrins recruit more than 150 different proteins to the cell-ECM adhesion complex, many of which are Lin11, Isi-1, Mec-3 (LIM) domain proteins that were recruited to the adhesion in a force responsive manner [63]. Integrins as well as several integrin-associated proteins that reside in cell matrix adhesions have also been shown to act as mechanotransducers [64]: they change conformation and/or expose new protein-binding sites when stretched by force. This allows cell matrix adhesions to alter intermolecular interactions that affect signaling pathways and connections to the actin cytoskeleton in response to force, thereby ensuring a balance between extracellular (ECM) and intracellular (cytoskeletal) forces. Indeed, the molecular architecture and size of cell matrix adhesions depend on myosin-derived cellular contractility [65, 66]. Key force sensors associated with cell matrix adhesions are described in Figure 1.2. Notably, for several additional cell matrix adhesion-associated proteins,

despite their important role in adhesion and migration, such as tensin, parvins, kindlins; neither conformational changes in response to force nor direct implication in force transmission has been demonstrated thus far.

Integrins are bi-directional transmembrane signaling receptors. Intracellular proteins bind to the tail region of integrins, thus causing conformational changes in the head region that increases affinity for its extracellular ligands (inside-out signaling) and ligand binding triggers conformational changes that activate intracellular signaling cascades (outside-in signaling). Integrins are heterodimers of an α and a β subunit and so far 24 different heterodimers formed by combinations of 18 different α subunits and 8 β subunits have been identified [45]. Most integrins recognize multiple ligands, for instance, integrin αvβ3 can bind to vitronectin, fibronectin and fibrinogen through the RGD-binding motif [67]. Additionally, in 3D environments, integrins are required for the fibrillogenesis of various ECM proteins [68, 69].

Integrins play a central role in environment sensing: integrin binding to the ECM promotes integrin clustering and recruitment of additional proteins into cell matrix adhesions [70], and through cytoplasmic linker proteins integrins connect to the actin cytoskeleton, which in turn is physically connected to the nucleus [71]. The spacing and pattern of integrin ligands controls cell spreading and cell matrix adhesion maturation [72]. It has been shown that clustering of integrins to form adhesion complexes requires a certain minimum ligand density [72–76] and that forces supported by individual integrin-RGD pair increases with reduced ligand spacing [77]. In addition, integrins go through force dependent binding/unbinding cycles, which regulate the activity of Rho GTPases, cell matrix adhesion formation, and integrin turnover [78, 79].

Mechanical loading has been shown to influence the lifetime of some integrin-ECM bonds. For instance αIIbβ3, exhibits slip-bond behavior characterized by a decreased lifetime with increasing load [80], whereas the integrin α5β1 heterodimer forms catch bonds with the ECM protein, fibronectin: the bonds are strengthened in response to external (ECM-driven) or internal (cytoskeleton-derived) force application [81, 82]. This force-dependent strengthening of catch bonds between α5β1 and fibronectin is necessary to create downstream signaling cascades [83] and theoretical modeling has shown that catch bond clusters can act as autonomous mechanosensors [84, 85].



Different integrin heterodimers that bind to the same ECM protein have been shown to respond differently to applied force. Cells adhering to fibronectin substrates through αvβ3 versus α5β1 integrins, for instance, differ in traction force generation [86, 87], dynamics [88], and adhesion [88, 89]. These integrins activate different intracellular signaling cascades [87, 90] and interchanging the ligand binding domains reverses the signaling phenotype [91, 92]. Similarly, expression of αvβ6 integrins in the presence or absence of α5β1 changes traction force generation [84]. Different splice variants of α6β1 also give rise to different phenotypes due to the two distinct cytoplasmic domains [93]. Thus cells can regulate their mechanosensitivity by modifying the integrin expression profile.

ILK (integrin linked kinase) is a pseudokinase that is part of the ILK-Pinch-Parvin (IPP) complex that plays critical roles in coupling integrins to the f-actin cytoskeleton in cell matrix adhesions [94]. ILK is directly recruited to integrin beta1 and beta3 cytoplasmic domains and is crucial for actin rearrangement, cell polarization, spreading, migration, proliferation, survival and tumor metastasis [95]. The ILK protein itself has not been shown to directly respond to force but it is recruited to cell matrix adhesions in a myosin II activity-dependent manner [96].

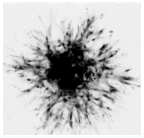
Talin and ***vinculin*** are adaptor proteins located in the cell matrix adhesions that have a mechanosensitive interaction. The talin head domain activates integrin through binding to its beta tail causing dissociation of the alpha and beta cytoplasmic domains [97]. Talin also directly connects integrins to the actin cytoskeleton. Talin is important for force-induced adhesion strengthening through interactions with integrin alphavbeta3 [88]. Vinculin is recruited to cell matrix adhesions in a force dependent manner [66, 98] and mediates cell matrix adhesion growth through binding to talin and f-actin [99]. Vinculin is required for force-induced cell matrix adhesion stabilization [100] and overall cell responses to environment stiffness [101, 102] possibly through Src-mediated phosphorylation at residues Y100 and Y1065 [103]. Despite enhancing cellular traction forces, vinculin is not required for force transmission at cell matrix adhesions but myosin contractility-dependent interaction of the vinculin head and tail domains is important for cellular mechanotransduction [102, 104]. Experiments with isolated talin and vinculin molecules showed that application of physiological forces to talin molecules leads to exposure of cryptic vinculin-binding sites [105]. This unfolding of talin has also been observed in isolated cells [106]. Possibly through this in-

teraction, vinculin stabilizes talin in an unfolded conformation and its localization shifts from integrin proximal to actin proximal region with increasing force [107]. Notably, vinculin interacts with several other cell matrix adhesion proteins and can be recruited to cell matrix adhesions upon force application in a talin-independent manner as well (see paxillin section).

Filamin and ***alpha-actinin*** are f-actin crosslinking proteins that can also directly bind integrin to actin filaments [108]. The filamin A-integrin interaction requires force [109], can stimulate activation of Rho GTPases in a force dependent manner [110], and is necessary for cells to induce collagen gel contraction [111]. Filamin A can unfold and change actin-binding dynamics under force [112]. Filamin A and talin bind to the same region in the integrin cytoplasmic tail, which might suggest a competition between filamin A and talin for integrin binding [113]. However knockdown of filamin A causes, in addition to an increased number of force-induced apoptotic cells, a reduction in force-induced beta1 integrin activation and a reduction in recruitment of talin and vinculin molecules to the adhesion [114]. Alpha-actinin, competes with talin for integrin beta3 tail binding but cooperates with talin when binding the integrin beta1 tail [115]. Alpha-actinin is not required for cell matrix adhesion force generation but it controls cell matrix adhesion maturation through its role in generating an actin network [9] and in connecting this network to the integrin mediated adhesions [115].

Zyxin recruits actin polymerizing proteins to integrin-mediated adhesions [116]. It changes binding kinetics and induces actin polymerization at cell matrix adhesions under force [117, 118]. Zyxin is also known to mobilize from cell matrix adhesions to actin fibers upon stretch [119] in a force-dependent manner [120]. Upon force-dependent relocalization to actin fibers, zyxin, together with alpha-actinin plays a role in actin stress fiber maintenance [121, 122].

p130Cas is a member of the Cas (Crk-associated substrate) family of proteins that is localized to cell matrix adhesions. p130Cas plays a role in migration, cell cycle control, apoptosis, differentiation and cancer development [123, 124]. Stretching the p130Cas protein in vitro increases its tyrosine phosphorylation, which is known to influence adhesion formation and actin dynamics [125, 126]. p130Cas phosphorylation is also important in cellular reorientation upon cyclic stretch [127] and coupling of the cytoskeleton to the adhesion during migration [128]. Studies of



vinculin knockout cells and vinculin mutants unable to bind to p130Cas, have shown that vinculin is necessary for p130Cas to respond to changes in substrate rigidity [129].

FAK (Focal Adhesion Kinase) is a protein-tyrosine kinase that is present in cell matrix adhesions. FAK regulates the activity of Rho GTPases and its kinase activity increases in response to extracellular forces [130]. Modification of an autoinhibitory intramolecular interaction involving the FAK four-point-one, ezrin, radixin, moesin (FERM) domain may be involved in this regulatory mode [7]. Direct evidence from *in vitro* studies demonstrating that FAK is a mechanoresponsive protein is not available but computer simulations have predicted that the cell matrix adhesion targeting (FAT) domain of FAK protein will extend under physiological force and this might regulate its interaction with paxillin [131]. There is evidence that FAK can be activated in a tension dependent or independent manner through its interaction with different integrins [132]. Indeed, FAK is recruited to cell matrix adhesions in a myosin contractility-dependent manner [66].

Paxillin is a multidomain adaptor protein that is essential for cell matrix adhesion formation, plays an important role for cell migration in 2D and 3D [131, 133] and mediates force induced Rho GTPase activity [134]. Paxillin phosphorylation, but not its localization to cell matrix adhesions, depends on myosin II activity [66]. This force dependent phosphorylation of paxillin is regulated by FAK activity, which in turn regulates vinculin recruitment to cell matrix adhesions, adhesion assembly and turnover, and cellular response to changes in ECM stiffness [135, 136].

RPTP-alpha (receptor-like protein tyrosine kinase alpha) is a transmembrane protein that co-localizes with alphav integrins at the leading edge of migrating cells and takes part in force-dependent formation and strengthening of cell matrix adhesions [137, 138]. RPTP-alpha might be able to respond directly to mechanical stimuli through force-dependent unfolding of its zipper-like autoinhibitory domains [139]. RPTP-alpha-dependent rigidity sensing influences neuronal migration [140] and is required for cells to exert forces on the ECM [61].

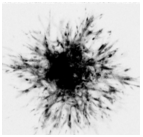
ECM proteins, similar to intracellular cell matrix adhesion proteins discussed above, can be stretched when force is applied and expose cryptic binding sites or growth factors [141]. The ***fibronectin*** matrix is an example of an ECM that is modified as force is applied to it. Fi-

bronectin is a globular ECM protein that is highly abundant in plasma and produced by cells during active processes such as tissue regeneration, angiogenesis and tumor invasion. Fibronectin is assembled into a fibrillar network via interactions with integrins and syndecan receptors [69]. Rho GTPase activity is required to generate the contractile force for fibronectin fibrilogenesis and assembly of a fibronectin matrix. The fibronectin fibrillar network stiffens with applied force [142]. This stiffening is probably due to cryptic binding sites for intramolecular interactions within the network that become exposed under force [143, 144].

Taken together, integrins and several associated cell matrix adhesion proteins undergo conformational changes in response to force. This leads to new protein-protein interactions within cell matrix adhesions, strengthened interaction with the cytoskeleton, and cytoskeletal network stiffening when extracellular force is applied. Vice versa, enhanced cytoskeletal tension - likely through the same complex of proteins - exerts forces on ECM proteins (such as fibronectin), which induces ECM reorganization through enhanced protein unfolding and protein-protein interactions, causing ECM stiffening. Thus, integrin-containing cell matrix adhesions act as key protein complexes that mediate bidirectional force transduction across the plasma membrane to ensure physical homeostasis between cells and ECM.

1.4 Cell matrix adhesions in cell fate decisions

Cell survival and proliferation is supported by ECM attachment in a manner that requires an intact actomyosin network and the ability of cells to spread [145, 146]. Crucial determinants of cell cycle progression, including mitogen-activated protein(MAP) kinase activity, cyclin D expression, and cyclin-dependent kinase (cdk) inhibitor levels are not properly regulated when cells attach to soft, rather than stiff collagen matrices leading these cells into quiescence [147]. Integrin signaling through FAK is one mechanosensitive mechanism involved: on rigid but not soft ECM substrates FAK is activated causing Rac-mediated cyclin D1 gene induction, cyclin D1-dependent phosphorylation of the retinoblastoma(Rb) protein, and passage through the restriction point into synthesis(S) phase [148]. ECM stiffness also controls endothelial cell proliferation during angiogenesis *in vitro* and *in vivo*: in this case Rho-dependent regulation of the balance between two mutually antagonistic



transcription factors that influence expression of the vascular endothelial growth factor receptor (VEGFR) is the mechanoresponsive switch [149].

Stem cell differentiation is one of the processes where mechanotransduction has been shown to have a major impact [150]. Cellular mechanosensing drives mesenchymal stem cell differentiation, with soft substrates promoting neuronal- and stiff substrates promoting osteoblast lineage specification [1]. Traction forces and integrin signaling regulate cell stemness [151]. On the one hand sensing of global rigidity was hypothesized to be involved [1]. On the other hand, the underlying mechanism was reported to involve differences in ligand anchoring density: stiffer hydrogels provide a denser network of ECM protein anchorage points and the resulting larger resistance to integrin-mediated cellular pulling force is sensed by the cells and controls cell fate decisions [152]. Similarly, in 3D environments, the cell-mediated degradation of the ECM resulted in larger traction forces and higher osteogenesis [153]. The spacing and patterning of integrin ligands, through its regulation of cytoarchitecture, controls mechanical properties of mesenchymal stem cells that would be expected to affect differentiation [72, 154]. In embryonic stem cells, substrate stretching has provided somewhat confusing results with evidence for stretch supporting either differentiation or stemness [155–157]. In agreement with a need to balance cellular and extracellular forces, the ability to stimulate actomyosin contractility through RhoA signaling is important for *in vivo* differentiation of lung epithelium [158]. Given the importance for these findings to the field of tissue engineering and stem cell therapeutics, cell culture techniques have been developed where substrate rigidity can be fine-tuned to control the balance between pluripotency, differentiation, and lineage specification. This includes patterned substrates [159], 2D and 3D substrates with different rigidities [160–162], or substrates with dynamically controlled rigidity [163].

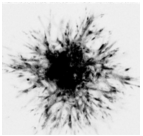
Tumor progression is another aspect in which integrin-mediated mechanotransduction plays a critical role [164]. Tumor malignancy is affected by ECM stiffness with increasing ECM rigidity promoting invasive growth through force-induced integrin- [39], FAK- [165, 166], Rho- and extracellular signal-regulated kinases(ERK)-signaling [40] and actomyosin contractility [167]. RPTP-alpha-dependent rigidity sensing also supports cancer cell invasion [168]. Integrin antagonists, which would disrupt the ability of cell matrix adhesion to act as mechanotransduc-

ing units, are considered to be promising anticancer therapeutics [169]. Interfering with integrin-mediated adhesions can reduce the ability of metastatic tumor cells to proteolytically degrade ECM during invasion [170] and it can increase tumor cell sensitivity to radiotherapy [171]. However, the role of integrins in different cancer types / oncogenic backgrounds is complex [172] and blocking integrin adhesions have been shown to both induce [173–175] and block [176] cancer progression.

Other biological processes - In the zebrafish, mutations in ILK interfere with the ability of cardiomyocytes to sense mechanical stretch and respond to it by upregulating crucial factors that regulate calcium waves [177]. Silencing beta-parvin phenocopied the ILK mutation, together providing genetic evidence that the integrin-IPP complex is important in heart function. This interaction is also important in the development and functionality of the mammalian heart [178] and has been implicated in cardiomyopathy in humans [179]. Integrin-mediated mechanosensing also plays an important role in normal vascular physiology and atherosclerosis. Changes in fluid shear stress affect endothelial cell biology in developing and adult bloodvessels. It has been proposed that the glycocalyx, receptors, and ion channels at the luminal surface all participate in shear stress sensing and the resulting tension is transmitted (i.e. via the cytoskeleton) to integrin-mediated cell-matrix adhesions at the basal cell surface. These adhesions subsequently act as mechanotransducers and activate signaling pathways to adapt to the altered blood flow [180].

1.5 Concluding remarks

It has become evident over the past years that mechanical cues from the ECM control physiology and pathology in a wide range of biological settings. It is clear that integrin-mediated adhesion sites are important mediators of bidirectional force transmission that connect the ECM and cytoskeleton. The force-regulated conformations and associations within cell matrix adhesions are partly resolved and many more molecular interactions that are subject to force modulation are expected to be discovered. Another aspect that is only partially understood is how mechanical signaling in cell matrix adhesions is coupled to cell fate decisions. The cytoskeleton connects integrins to LINC (linker of nucleoskeleton and cytoskeleton) complexes in the nuclear envelope. There, nesprin proteins



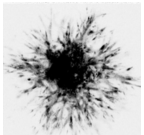
in the outer membrane connect to microtubules, actin fibers, and intermediate filaments while Sad1 and UNC84 (SUN) domain proteins in the inner membrane bind the nuclear lamina [181]. Since chromatin-binding proteins and DNA are attached to the nuclear lamina, extracellular mechanical stress may be propagated into the chromatin and affect gene expression through conformational regulation of DNA and associated proteins. However, although extracellular forces, through integrins are mechanically linked to changes in nuclear orientation and shape [71], direct evidence for such purely mechanical coupling between ECM and gene expression is lacking.

Mechanical perturbations are translated into biochemical signaling in cell matrix adhesions. Therefore the molecular composition of the adhesion is important for cellular mechanosensing. Studies relating myosin activity to protein localization and turnover rates have shown that the adhesion structure itself is force dependent [66, 117, 118]. Additionally, super resolution microscopy has also allowed the study of force dependent nanoscale architecture of adhesion protein vinculin [107]. However force-molecular recruitment relation in cell-matrix adhesions is unknown. This relation can be unraveled through a reliable method that addresses the abundance of adhesion molecules. Cell-matrix adhesions that are coupled to the ECM via different integrins have differential mechanoresponse [88]. Cellular expression profile of integrins also dictate activated signaling pathways and regulate cellular force application [86, 87]. However how different integrins regulate cellular response to mechanical cues remains to be addressed.

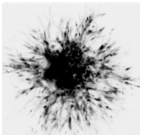
Integrin expression profile and role of mechanical cues have also been addressed in relation to cancer [39, 40]. ECM-tumor cell interaction as well as the ECM itself is deregulated in cancer and such changes affect cancer progression [3]. Understanding the altered mechanoresponse in cancer may help develop new therapeutic interventions. Extensive crosstalk with various other signaling pathways further complicates the concerted effect of physical and chemical stimuli. Therefore it is necessary to isolate the effect of mechanosensing that is cell type and protein expression independent to understand how the physical tumor-ECM communication might affect hallmarks of cancer such as activation of invasion, metastasis and angiogenesis.

1.6 Aim and outline of this thesis

With the studies described in this thesis, I aimed to further the understanding of the mechanism and importance of cellular mechanotransduction. The focus was on integrin mediated adhesions and their roles in inside-out and outside-in mechanosensing. In chapter 2, the role of physical signaling in tumor progression is studied. Using automated sequential microprinting of tumor and endothelial cells in 3D collagen gels in combination with reflection microscopy it is shown that; i) tumor expansion and tumor induced collagen organization are highly correlated, ii) this relation is dependent on cellular force generation but is resistant to depletion of collagen-binding integrins, iii) the remote organization of collagen induced by the tumor steers directional migration of endothelial cells, iv) this directional migration is impaired upon severing the physical connection between the tumor and endothelial cells. The physical signaling by the tumor is thus shown to influence tumor expansion and angiogenesis. Chapter 3 focusses on fibronectin binding integrins $\alpha 5\beta 1$ and $\alpha v\beta 3$ and describes their differential role in outside-in and inside-out cellular mechanosensing. It shows that cells expressing either of these integrins are able to reorganize their cytoskeleton upon cyclic stretch and induce ECM stiffness driven cellular spreading with similar efficiencies. Likewise, these integrins are shown to support similar magnitudes of cellular traction force generation and stiffness dependent regulation of cellular traction forces. However, cells that express $\alpha v\beta 3$ are identified to form adhesions on softer substrates and to be able to better organize their actin cytoskeleton upon cyclic stretch and maintain this organization at higher strain rates. In contrast, cells that express $\alpha 5\beta 1$ are shown to support more centripetally oriented traction forces in a ROCK/myosin activity dependent manner that also supports generation of longer actin fibers. Therefore it is shown that differential expression of fibronectin binding integrins regulate cellular plasticity by fine tuning sensing-force application capacities through differential regulation of ROCK/myosin signaling and actin cytoskeleton. In chapter 4 the relation between molecular composition of the adhesion, the force generation and environment stiffness is shown. Using a new approach to quantify the number of molecules in a cellular structure, the recruitment of adhesion proteins talin, paxillin, vinculin and FAK is studied in relation to force application and environment stiffness. Chapter 5 studies the cellular mechanotransduction in context of cancer cell migration and adhesion



structure. Genes that are identified as regulators of cell-matrix adhesion and cancer cell migration are shown to regulate cellular traction force generation mechanisms. Formation of larger adhesions, reduced cellular migration, higher traction force generation and slow force turnover rates are identified to be interrelated. Lastly, in chapter 6 the overall conclusions of the studies in this thesis and future perspectives are described.



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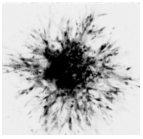

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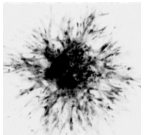

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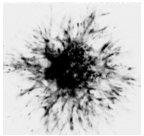

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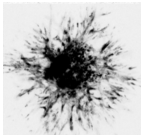

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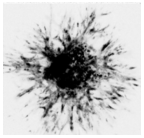

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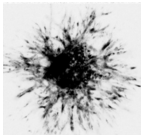

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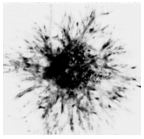

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