Mechanical signaling through the discoidin domain receptor 1 plays a central role in tissue fibrosis

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ABSTRACT
The preservation of tissue and organ architecture and function depends on tightly regulated interactions of cells with the extracellular matrix (ECM). These interactions are maintained in a dynamic equilibrium that balances intracellular, myosin-generated tension with extracellular resistance conferred by the mechanical properties of the extracellular matrix. Disturbances of this equilibrium can lead to the development of fibrotic lesions that are associated with a wide repertoire of high prevalence diseases including obstructive cardiovascular diseases, muscular dystrophy and cancer. Mechanotransduction is the process by which mechanical cues are converted into biochemical signals. At the core of mechanotransduction are sensory systems, which are frequently located at sites of cell-ECM and cell-cell contacts. As integrins (cell-ECM junctions) and cadherins (cell-cell contacts) have been extensively studied, we focus here on the properties of the discoidin domain receptor 1 (DDR1), a tyrosine kinase that mediates cell adhesion to collagen. DDR1 expression is positively associated with fibrotic lesions of heart, kidney, liver, lung and perivascular tissues. As the most common end-point of all fibrotic disorders is dysregulated collagen remodeling, we consider here the mechanical signaling functions of DDR1 in processing of fibrillar collagen that lead to tissue fibrosis.

I. Introduction
The development, maintenance and repair of tissues and organs depends on interactions of cells with their adjacent neighbors and with the extracellular matrix (ECM). These interactions are influenced not only by the chemical composition and structural organization of the ECM but also by the mechanical properties of the proteins, proteoglycans and glycosaminoglycans that comprise the matrix. Cells use ATP-dependent molecular motors to generate forces that shape and remodel the ECM and a repertoire of matrix receptors to interrogate the mechanical properties of the ECM. In tissues with high cell densities, the physical proximity of cells to one another enables the formation of intercellular junctions, which provide mechanical continuity for cell networks. But intercellular junctions also contribute to the adaptations of cells in response to local alterations of the ECM, including the mechanical properties of the ECM [1–5].

It is now recognized that mechanical forces play important roles in many physiological processes that are fundamental to life. These processes extend from early embryonic development, to normal tissue homeostasis to late-stage aging processes. For example, the morphology of cardiovascular tissues and many of the pathological processes that affect the cardiac system, are strongly influenced by the pressure and shear stress associated with the circulation of blood. In mineralized tissue biology, the forces arising from muscle contraction and gravity influence bone shape, density, compressive and tensile strengths. Neural responses to pressure stimuli enable hearing and touch while respiration-related forces regulate lung development and physiology. Deregulation of the mechanical processes that are critically involved in myriad biological functions can result in loss of hearing, cardiovascular diseases, muscular dystrophy, cancer and a wide repertoire of fibrotic diseases [1,2,6].

Mechanotransduction involves the conversion of information (in the form of mechanical cues, either extrinsic to the cell microenvironment or intrinsic, such as cytoskeletal structures) into biochemical signals. Mechanotransduction involves a large array of proteins, glycosaminoglycans and proteoglycans distributed through the ECM, as well as cell membrane proteins, the cytoskeleton, the nuclear membrane and nuclear...
chromatin. At the functional core of the systems that convert mechanical signals into biological responses are a broad repertoire of sensory systems, typically clustered at discrete regions of cell-ECM and cell-cell contacts, which we designate here as mechanoreceptors [1–3, 5].

The integrins (at cell-ECM junctions) and cadherins (at intercellular contacts) are the most extensively studied cellular mechanoreceptors. But other mechanoreceptors include some of the membrane-bound receptor tyrosine kinases (RTKs), several G-protein coupled receptors, a large repertoire of ion channels and the glycosylated domain receptor 1 (DDR1), a RTK collagen adhesion mechanoreceptor. DDR1 is fundamental for proper embryonic development and organogenesis but its role in the homeostatic processes that regulate mature tissues, and particularly in disease states, are not as well defined.

The expression of DDR1 is associated with fibrotic lesions of kidney [7], liver [8], lung [9] and perivascular tissues [10–12]. The common end-point of all fibrotic disorders is dysregulated collagen remodeling. DDR1 and DDR2 are the only tyrosine kinases that interact directly with collagen. DDR1 also interacts through its C-terminal kinase domain with non-muscle myosin IIA [13, 14]. Because collagen fibers contribute to the physical properties of connective tissues, and because actomyosin contractility enables cells to apply traction forces to the ECM through DDR1, it seems likely that this unusual adhesion protein plays a central role in the conversion of mechanical signals into biological responses. We and others have reported on the roles of DDR1 in collagen processing events that contribute to tissue fibrosis and cancer progression [13, 15–19]. Here we consider general concepts that link mechanical forces to fibrotic disease. We then review the mechanoreceptor functions of DDR1 in collagen processing events that can lead to tissue fibrosis.

II. Mechanotransduction and fibrosis

Homeostasis of connective tissues and the organs which they invest is a dynamic process that is reliant on the ability of resident cells to sense and respond to local changes in the chemical composition, structural organization and mechanical properties of their microenvironment. For preservation of tissue homeostasis, the production, deposition and organization of ECM molecules is balanced by the degradation and remodeling of existing ECM. After injury or chronic infection, tissues frequently initiate a wound healing response, which is intended to create a new ECM and restore tissue structure and function. If the wound healing response is dysregulated or if the tissue injury or infection is severe or long-lasting, the tissue repair process may evolve into fibrosis [1, 4, 6, 17, 20–22]. Fibrosis is thought to contribute to about 45% of deaths in the industrialized world [23]. It is manifest in several high prevalence diseases including cardiovascular diseases, pulmonary fibrosis, liver cirrhosis, chronic kidney disease and cancer. Although many studies have identified several cellular and biomolecular mediators of fibrosis, the developmental mechanisms and the mediators of progression of this disorder at the organ level remain poorly understood [24–28].

Fibrosis is characterized by the excessive deposition of collagens and other matrix proteins in the interstitium, which leads to distortions of tissue architecture and function. These abnormalities can ultimately lead to organ failure. Fibrosis arises from a normal repair process that has run amok and involves a wide spectrum of cells, signaling pathways and regulatory systems. The nature of fibrotic repair is highly influenced by mechanical forces. Indeed, the disproportionate deposition of collagens and other matrix proteins continuously modify the local mechanical properties of tissues. This pathological accumulation of collagen and other matrix proteins is in part attributable to excessive recruitment of fibroblasts to the injury site and/or to their transformation into contractile myofibroblasts [17, 29–31].

Fibroblasts sense physical alterations of their microenvironment and translate these alterations into chemical signals that inform gene expression and cell fate decisions through a process known as mechanotransduction. The impact of the cellular environment on cell fate decisions emerged in the early 1980s and arose from several research groups including the work of Bissell [32] and Ingber [33] showing that the “cellular context” was a key determinant of cell behavior in general and the malignant phenotype in particular. More recent studies have shown that tissue stiffness is associated with the localized differentiation of fibroblasts into myofibroblasts [34, 35]. Notably, after attachment to stiff substrates, myofibroblasts maintain their phenotype, even when re-plated on soft matrices [27]. Myofibroblasts exhibit some properties of fibroblasts (e.g. secretion of collagen) but they also express alpha smooth muscle actin, a marker of smooth muscle cells, which is consistent with their marked contractility. Fibroblasts and myofibroblasts respond to local increases in the ECM by increasing actomyosin-generated tensile forces on collagen fibrils, which are applied through collagen adhesive receptors. Increased contractility also involves activation of Rho and Rho-associated coiled-coil containing protein kinase (ROCK), and mechanically-associated Ca²⁺ signaling [4, 34, 36].
The physical properties of the microenvironment also impact the behavior of mesenchymal stem cell differentiation and cell fate [37]. When stretched, human mesenchymal stem cells adopt an osteogenic phenotype but when maintained in low mechanical stress environments, they differentiate into adipocytes. However, if the actomyosin contractile machinery is perturbed, osteogenesis is blocked and adipogenesis is enhanced [38]. These examples highlight the notion that mechanotransduction is a feedback-regulated system. Cells must apply myosin-based contraction forces to their microenvironment in order to sense and respond to local variations of ECM stiffness. Further, cells match their intracellular tension with local ECM mechanical properties by modulating their contractile forces with continuous cytoskeletal remodeling and myosin-related signal transduction.

The key element in mechanotransduction is a molecule (or molecular complex) that is characterized by the presence of a structure and/or activity that is regulated by force. This sensory complex is also known as a mechanoreceptor. The most widely studied mechanoreceptors include integrin adhesive complexes, cadherin complexes, receptor tyrosine kinases, G protein-coupled receptors, ion channels and the glycocalyx. A brief description of the different functions of these mechanoreceptors is provided later in this review to set the stage and to focus on identifying the mechanoreceptor functions of DDR1 in tissue fibrosis.

Dysregulated collagen remodeling is a common endpoint of all fibrotic disorders and leads to local alterations of the mechanical properties of tissues. One of the rate-limiting steps of collagen remodeling is the binding of cells to collagen fibrils by specific cell adhesion receptors, which sense alterations in the structure and arrangement of collagen molecules and the generation of specific cellular responses. As this review focuses on the mechanoreceptor functions of a collagen adhesive receptor (i.e. DDR1), we will first consider the multistep processes of collagen remodeling and then focus on the contribution of DDR1 in collagen processing events that can lead to fibrosis.

III. Collagen remodeling: The fine line between health and fibrotic disease

The mechanical properties of the ECM depend largely on collagen, the most abundant protein of vertebrates [39]. There are more than 25 types of collagen but the fibrillar type I and III collagens are the most abundant. The type I:III collagen content ratio is typically tissue-specific, but in general, the ratio is 80:20 in mature healthy connective tissues, while in early stages of wound healing the ratio might be lower, due to increases of type III collagen secretion [17].

Fibrillar collagens are responsible for providing tissues with stiffness and strength but as collagens have a relatively short half-life [40] they are continuously remodeled by resident cells throughout life. The processes by which collagen fibrils are renewed is termed remodeling and the rate of remodeling is tissue-specific. Collagen remodeling is central to maintain tissue homeostasis and is a critical metabolic process in the health of mammals [21]. Collagen remodeling comprises distinct, well-orchestrated processes involving synthesis, degradation, reorientation and crosslinking of collagen within the ECM [41–48]. Connective tissue homeostasis depends largely on the balance between collagen production, rates of removal, and cell-mediated traction forces transmitted to the newly deposited collagen molecules. Traction force has the effect of condensing collagen fibrils into more dense arrays, which is a characteristic feature of fibrosis [17,22].

 Fibroblasts are the major architects of the ECM as they secrete collagen and organize the structure of ECM constituents. In some tissues, smooth muscle cells, pericytes, and cells derived from epithelial to mesenchymal transformation can also contribute to collagen synthesis and deposition in the ECM. On balance however, it is thought that in soft connective tissues, fibroblasts play the most fundamental role in maintaining collagen homeostasis because they not only synthesize and mechanically organize collagen in the ECM but also because they secrete proteases that are involved in collagen degradation. Further, they degrade collagen through internalization in phagosomes [49,50].

An isotropic arrangement of fibrillar collagen is often indicative of healthy tissue in which degradation, de novo synthesis and reorganization of collagen are perfectly balanced. Anisotropic, highly aligned collagen fibers are the hallmark of a pathological ECM, which is frequently observed in fibrotic lesions or in the stroma associated with epithelial tumors. In fibrotic lesions, the de novo synthesized collagen is also heavily cross-linked and often forms extensive accumulations of matrix with abnormalities of tissue architecture and mechanical properties [24,51–53]. This abnormal matrix affects also the availability of cytokines and growth factors that are normally trapped in the ECM, which can have implications for maintenance of tissue homeostasis.

All these alterations in collagen remodeling and the consequent implications in ECM signaling lead to increased stiffening of the ECM, which in turn affects both normal and pathological tissue responses. While the mechanisms by which ECM adhesion receptors contribute to ECM stiffening are not defined, it is known...
that the rate-limiting steps of collagen remodeling is the binding of specific adhesion receptors to collagen fibrils and the generation and application of actomyosin-independent contractile forces [41,54–56]. This insight suggests that collagen adhesion mechanoreceptors are at the center of ECM homeostasis that is affected by mechanical processes.

IV. Mechanoreceptors in fibrosis

Cell-ECM interactions are dynamic processes as cells continuously sense the surrounding microenvironment and respond to changes not only in temperature, oxygen and nutrient content but also in the context of physical properties such as osmotic pressure, shear force, compression loading, tissue architecture and rigidity. As described above, cells transform mechanical cues into biological programs through a process termed mechanotransduction. In order to activate these signal transduction pathways, cells need to be able to detect changes in force through mechanoreceptors. The molecules in these sensory complexes then amplify and propagate these mechanical signals and promote a change in cell behavior.

A. Ion channels

One of the key elements for the mechanotransduction processes that regulate migration, proliferation and differentiation of different cell types is the activation of ion channels. The bacterial stretch activated ion channels, MSC-L and MSC-S, were the first mechanically-activated channels to be described. They regulate cell responses to changes of osmotic pressure and affect cell growth by modulating ion flux from the extracellular environment in response to membrane stretching [57,58]. The Piezos [59], the transient receptor potential (TRP) ion channels and members of the degenerin/epithelial sodium channel (DEG/ENaC) superfamily in mammals are involved in somatosensation (touch perception, proprioception and pulmonary respiration, red blood cell volume regulation and vascular physiology). Upon stimulation of these ion channels, which can impact the activation of effector kinases and small GTPases, signals are propagated and ultimately modify cellular biological programs such as migration [60,61]. Dysregulation of mechanical activated ion channels is associated with mechanotransduction-related pathologies such as cardiovascular diseases, kidney diseases and cancer [62–64].

B. Integrins

The most extensively studied mechanoreceptors are integrins, which are transmembrane glycoproteins that act as major cell surface receptors for many ECM proteins. Integrins are heterodimeric proteins with non-covalently associated α and β integrin subunits. Their large extracellular domains enable binding to specific amino acid sequences in many ECM proteins. The C-terminal cytoplasmic domain binds a large number of cytoskeletal, adhesion-related proteins and/or peripheral membrane proteins. Upon binding to an ECM ligand such as collagen, integrins undergo a conformational change and become allosterically activate. After aggregation of integrins in the plane of the plasma membrane, integrins interact with ECM proteins to form adhesion clusters which in cultured cells are denoted as focal complexes. Contraction forces generated by the cell contributes to the maturation of focal complexes into the more mature, focal adhesions. Integrins play a central role in the maintenance and repair of various tissues which arises from their ability to provide anchorage and to trigger signals that direct cell survival, migration and differentiation [1,65–68].

Integrins play fundamental roles in tissue fibrosis not only because they bind ECM molecules and modulate the organization of the ECM, but also because they regulate the signaling cascades elicited by several growth factors (e.g. IL-1, TGF-β) either by direct action on the activity and release of growth factors or by their clustering and activation of cytokine or growth factor receptors [69–73].

The duration of a mechanotransduction event strongly impacts the ultimate biochemical response. This phenomenon is related to force-induced conformational changes that regulate protein-protein interactions or that alter enzymatic reactions involving key signaling molecules. For example, the maturation of focal adhesion complexes is associated with the resistance of ECM molecules to applied, cell-generated contraction forces. The maturation of focal adhesion complexes involves integrin clustering, autophosphorylation of focal adhesion kinase (FAK) and the consequent phosphorylation and activation of mechanosensitive signaling events such as mitogen activated protein kinases and RhoA. Talin, vinculin are actin binding proteins that are enriched in focal adhesions and provide a physical link between the integrins and the actin cytoskeleton. Activation and clustering of integrins upon ligand binding recruits talin to the cytoplasmic domain of integrins while vinculin and talin provide linkages of integrins to the actin cytoskeleton, processes that promote cell adhesion and spreading. The biological significance of these processes is manifest in the observations that force transduction and focal adhesion function are compromised if vinculin is not expressed [74]. Talin and vinculin are good examples of actin binding proteins in focal adhesions that are directly
involved in the sensing of force-generated signals arising from the ECM and their contribution arises in part because they undergo tension-dependent conformational changes for activation [75, 76].

RhoA is involved in the cytoskeletal response to the sensing and response to ECM mechanics as it activates ROCK, which in turn phosphorylates the myosin light chain in order to generate actomyosin contraction forces. When FAK and Src family kinases are activated upon mechanical stretching of integrins, there is a rapid stiffening of cells that is mediated through the Rho guanine nucleotide exchange factors, LARG and GEF-1 [77]. Stiff ECM induces non-transformed epithelial cells to proliferate and convert to an invasive phenotype [78]. The enhanced intracellular tension due to increased actomyosin contractility stimulates translocation of mechanosensitive transcription factors such as MRTFs and YAP/TAZ, which can thereby elicit cellular mechanical responses. YAP/TAZ activation is associated with variations of ECM stiffness and alterations of cell proliferation and differentiation because these transcription factors contribute to the increased expression of pro-survival genes [79, 80].

C. Cadherins

During development, remodeling and when cells undergo migration in morphogenesis, cell-cell adhesions experience compressive and tensile forces that are attributable to myosin motors. In addition, as a result of physical trauma, skeletal muscle contraction and flow in blood and lymphatic vessels, mechanical forces can induce remodeling and turnover of cell-cell coupling regions located at adherens junctions. The formation of adherens junctions arises from a precisely timed series of sequential molecular processes. Initially, the formation of cell protrusions initiates contacts between neighboring cells and gives rise to cadherin-catenin clusters that signal intracellularly to remodel the actin cytoskeleton and contribute to the formation of lamellipodia and filopodia through Rac, cdc42 and, downstream, Arp2/3 activation. Once contacts have formed, Rho acts through ROCK-mediated myosin contraction to expand intercellular contacts, align cadherin-catenin complexes, and form linear contractile actin bundles that lead to the matura-

D. G-protein coupled receptors (GPCRs)

These transmembrane receptors are involved in the regulation of myriad physiological processes including inflammation, cell growth, migration and differentiation. GPCRs are also responsible for mediating responses to a wide variety of stimuli including light, sound, taste and variations of hormone concentration [90, 91]. Several studies have identified the mechanoreceptor function of these receptors and some notable examples include the endothelial cells that upon laminar shear stress, increase mRNA and protein levels of the GPCRs, CXR1 and CXR2. Upon ligand binding, GPRs induce Rho and Rac signaling pathway activation, actin cytoskeleton reorganization and cell motility [63, 92]. When activated GPRs behave as mechanoreceptors that acquire different conformations depending on which agonist or mechanical stimuli is responsible for the activation [93].

E. Glycocalyx

The outer surface coating of cells or glycocalyx is a complex of membrane-bound macromolecules including glycosaminoglycans, proteoglycans, and glycoproteins which serve protective and mechanosensitive functions. The mechanoreceptor functions of the glycocalyx are associated with forces induced by blood flow and contribute to the maintenance of endothelial barrier function and the control of leukocyte adhesion and inflammation [94, 95]. When exposed to fluid flow, endothelial cells and vascular smooth muscle cells tend to align and elongate in the direction of the flow. However, if the glycocalyx of these cells is
degraded the cells no longer align. Upon application of shear stress, several core proteins of the glycocalyx become glycosylated, which reinforces its connection with the actin cytoskeleton [96]. Syndecans are a major component of the endothelial glycocalyx but also are associated with the organization of the cytoskeleton as a result of their binding through their cytoplasmic domain to Src and α-actinin. Syndecan 1 is also required for the activation of RhoA in the initial stages of endothelial responses to shear stress [94,97], which highlights the mechanoreceptor function of the glycocalyx.

**F. Receptor tyrosine kinases (RTK)**

These proteins phosphorylate tyrosine residues in target proteins and promote the formation of multiprotein associations that are critical in signal transduction. RTKs regulate a large variety of physiological processes including growth, differentiation and apoptosis. The majority of RTKs form di- or oligomers upon ligand binding, a process that leads to autophosphorylation of the intracellular kinase domain and typically, increased catalytic activity. Autophosphorylation induces the recruitment and phosphorylation of cytoplasmic signaling molecules directly or indirectly, as a result of the involvement of docking proteins that are activated by tyrosine kinases. Dysregulation of RTKs signaling is associated with neoplastic, cardiovascular, and fibrotic diseases. Several growth factor receptors that are members of the RTK family exhibit autophosphorylation in response to changes of substrate stiffness [98–100]. However, in this review we focus on identifying the mechanoreceptor functions of DDR1, the only RTK (along with its family member, DDR2) that is activated by an insoluble factor – collagen fibrils.

**V. Discoidin Domain Receptor (DDR) I**

These collagen adhesion receptors are a subfamily of receptor tyrosine kinases that are comprised of two members, DDR1 and DDR2. Both proteins exhibit an extracellular ectodomain composed of a globular discoidin domain (DS), a discoidin-like domain (DS-like) and an extracellular juxta-membrane (JM) region, which is linked by a transmembrane domain (TM) to the intracellular ectodomain. An unusually large JM region and a C-terminal kinase domain characterize the intracellular ectodomain (Figure 1). While DDR2 is expressed as a single protein, DDR1 is expressed as 5 isoforms that are generated by alternative splicing. DDR1a, DDR1b, and DDR1c are full-length functional receptors while DDR1d is truncated and lacks the whole kinase domain. DDR1e has an inactive kinase domain. DDR1a and DDR1b are the most common isoforms while DDR1c is the longest isoform (919 amino acids). DDR1c has a six-amino acid insert in the kinase domain with unknown biological significance while DDR1a lacks 37 amino acids in the intracellular JM region (Figure 1) [18,101–103].

Both DDR1 and DDR2 bind to GVMGFO motifs in collagen with a highly conserved region entirely contained in their DS domains [104,105]. Both DDRs bind fibrillar type I-III collagens; DDR1 also binds type IV and VII collagens while DDR2 can also bind type X collagen. DDR1 is expressed mainly by epithelial cells, but also by smooth muscle cells, fibroblasts, oligodendrocytes, and macrophages. DDR2 is more strongly expressed by mesenchymal cells including fibroblasts, myofibroblasts, smooth muscle cells and chondrocytes [17]. Therefore, DDR2 is associated with collagen remodeling that led to fibrotic lesions of the heart [106] and liver [107,108]. Further, DDR2 expression was reported to be either present or increased in many different human tumor samples. Previously our group reported that DDR2 does not associate with myosin [14] others reported that DDR2 expression is associated with the expression and activation of MMPs [108]. These findings point to the involvement of DDR2 in collagen processing events that lead to fibrotic lesions but more detailed discussion on the potential mechanoreceptor functions of DDR2 in fibrosis are beyond the scope of this review.

**Figure 1.** DDR1 isoforms. Discoidin domain (DS); discoidin-like domain (DS-like); transmembrane domain (TM); N terminal (NT); C terminal (CT).
A. DDR1 activation: A central regulator of collagen mechanical signaling

To identify the mechanosensitive functions of DDR1 we need to establish that: 1) efficient mechanisms of mechanotransduction involve receptor molecules that assemble into clusters to enhance signal sensitivity (e.g. integrins, cadherins); 2) mechanosensing receptor-cluster systems need to be reinforced at the inner cell membrane by the actomyosin machinery; 3) there should be a direct relationship between the mechanical stimulus and the magnitude of protein activation and/or conformational modification.

For the majority of RTKs, activation by phosphorylation of tyrosine residues occurs within seconds of ligand binding, which is followed by a rapid downregulation of activity, caused by either dephosphorylation (mediated by phosphatases) or by receptor/ligand internalization and subsequent degradation [109]. DDR1 is unusual in that, its phosphorylation occurs within minutes to hours after collagen binding and can last for days with no apparent attenuation. Accordingly, collagen binding to DDR1 is associated with sustained, slow responses instead of the acute, rapid responses that are typically associated with signaling through RTKs [103]. With the exception of the insulin receptor, that forms disulfide-linked dimers and is activated by conformational changes within the dimer, the majority of classical RTKs exist as monomers and the process of dimerization occurs upon ligand binding. Dimerization leads to conformational changes in the dimer that facilitates approximation of the kinase domains, thereby promoting phosphorylation of tyrosine residues [109].

DDR1 forms constitutive dimers in the absence of ligand [110–114], therefore dimerization cannot alone explain how binding to collagen leads to the activation of the DDR1 kinase domain. One of first reports of DDR1 dimerization indicated that only dimeric DDR1 ectodomains bind to collagen I while DDR1 monomeric ectodomains do not [113]. After several receptor-receptor contact sites were identified in the extracellular, transmembrane and cytosolic ectodomains [111,112], one leucine-based sequence motif in the transmembrane domains of DDR1 and DDR2 was evidently essential for receptor dimerization [111]. As mutations in cysteine residues in the extracellular juxta-membrane region resulted in covalent dimers independently of collagen binding, the authors suggested that dimerization occurs during DDR biosynthesis and is constitutive [111,114].

As DDR1 is a pre-existing dimer, the classical model of RTK activation by dimerization after ligand binding cannot be applied. Notably, other RTKs also form dimers (and oligomerize) in the absence of ligand. The insulin receptor is a constitutive dimer that after ligand binding shows enhanced tyrosine kinase activity and signals through structural changes within the dimers after insulin or IGF1 binding [109]. Previous structural and biochemical studies did not report collagen-induced conformational changes in DDR1 ectodomains or in the flexible juxta-membrane regions, which tends to exclude this mode of activation [104,114,115].

One proposed mechanism for activation involves collagen-induced DDR1 clustering [114,116] since DDR1 oligomerization is required for high affinity binding to collagen [117]. Recent work using cells expressing DDR1d, a kinase-deficient isoform, supports the involvement of the kinase domain in regulating receptor oligomerization, auto-phosphorylation and clustering at the cell surface [118]. We found that upon fibrillar collagen stimulation, activated DDR1 (phosphorylation at the kinase domain (Y792)) was mainly present as oligomers, which supports the notion that receptor oligomerization precedes receptor activation [13,117]. Cells expressing the kinase-depleted DDR1d and cells expressing a kinase-dead DDR1 isoform (DDR1e) formed clusters but cells that express kinase-active DDR1 (DDR1b) exhibited large increases in cluster size. Further, cells expressing DDR1b that were spread on non-activating fibronectin substrates also formed clusters, but the clusters were smaller then when cultured on collagen. Accordingly, DDR1 receptor clustering induces DDR1 activation, which reinforces DDR1 binding to collagen, most likely because of a feed-forward mechanism that increases receptor clustering (Figure 2) [13].

A recent report using signaling-incompetent DDR1 mutants (receiver) co-expressed with functional DDR1 (donor) further supported a clustering mechanism for DDR1 activation. The authors found that the lateral association between DDR1 molecules is the result of collagen binding and that it results in the phosphorylation in trans between dimers, as opposed to the normal, within-dimer phosphorylation [115].

DDR1 regulates adhesion and migration on collagen by association with myosin IIA [14]. When adhesion receptors bind ECM molecules, actomyosin contractility is increased, which establishes a positive feedback loop that reinforces cell adhesion. The contractility of actomyosin relies in part on the ATPase activity of the motor domain of myosin IIA and on phosphorylation of the myosin light chain (MLC), which also regulates myosin filament assembly. MLC can be phosphorylated either directly by MLC kinase (MLCK) or by ROCK, and indirectly by ROCK phosphorylation of the regulatory subunit of the MLC phosphatase [119,120]. Intracellular cytoskeletal tension, which is proportional to cell adhesion strength, ultimately plays an important role in
affecting cellular responses. We identified an integrin-independent mechanotransduction pathway that involves collagen, DDR1, and myosin IIA. Upon binding to collagen, the DDR1 C-terminal kinase domain associates with myosin IIA filaments. DDR1 clustering after collagen binding promotes the activation of the DDR1 kinase domain, which enhances the association of DDR1 with MLCK-activated myosin IIA filaments. This generates a positive feedback loop, which optimizes the transmission of myosin-dependent contractile forces to collagen (Figure 2) [13].

DDR1 can regulate adhesion to collagen by modifying integrin activation [121]. We found that independently of integrin function DDR1 activation (Y792) and myosin contraction increased significantly on stiff substrates. DDR1 activation also increased when cells were cultured on soft collagen substrates but intercellular tension was increased with lysophosphatidic acid (LPA) [13]. LPA increases intracellular tension by activating Rho and Rock and inhibiting MLC phosphatase. We can conclude that DDR1 clustering is reinforced at the inner cell membrane by the myosin IIA contractile machinery and that the magnitude of DDR1 activation is directly linked to collagen mechanical properties and intracellular tension [13].

The 3 key features of a mechanoreceptor described in the beginning of this section are summarized for DDR1 in Figure 2. Collagen binding induces initial DDR1 clustering, which promotes the activation of the DDR1 kinase domain and its association with myosin IIA filaments. In a feed-forward fashion, DDR1 activation increases receptor clustering and enhances the association of DDR1 with myosin IIA filaments. These processes optimize the transmission of myosin contractile forces to collagen (Figure 2). Finally, DDR1 activation and the consequent generation of mechanical signals is strongly influenced by substrate compliance.

**B. Mechanoreceptor functions of DDR1 that contribute to tissue fibrosis**

Independently of the trigger or of the initial events, a common feature of all fibrotic diseases is the activation of ECM-producing cells, which is one of the key processes in the tissue remodeling seen in fibrosis [22]. Fibroblasts and their subtypes are the archetypal ECM-producing cells and they deposit mainly fibrillar collagen I and III into the nascent matrix, although other types of matrix proteins are also secreted. DDR1 is mainly expressed by epithelial cells but keloid fibroblasts express DDR1 and treatments with anti-sense DDR1 in keloid wounds blocked collagen production [122]. We previously reported that human gingival fibroblasts [123] and periodontal ligament fibroblasts [13] also express DDR1. Recently we showed that DDR1 expression and activation were enhanced in mechanically restrained wounds, which were also populated with markedly more myofibroblasts and with more aligned collagen than non-restrained wounds. In mechanically constrained wounds, there was enhanced co-localization of DDR1 with α-SMA, indicating that a least a subset of fibroblasts present in the wounds express DDR1 [13].
The focal enrichment of highly aligned collagen fibers can contribute to increases in local tissue stiffening [124]. In turn, tissue stiffness perpetuates the differentiation of local fibroblasts into myofibroblasts [125]. A recent study using human dermal fibroblasts showed that combined treatment with ascorbic acid and TGF-β facilitates the adoption of a myofibroblast phenotype and induces the expression of DDR1, while treatment with TGF-β alone does not induce DDR1 expression [126]. TGF-β is a pro-fibrotic growth factor that plays a key role in fibrogenesis and tumorigenesis. Recently the impact of TGF-β on invadosome formation was associated with the expression of DDR1 and the membrane bound metalloproteinase, MT1-MMP, through nuclear translocation of Smad4. TGF-β1 treatment favored also collagen and lysyl oxidase-like2 (LOXL2) secretion, which underlines the linkages between TGF-β1-induced linear invadosome formation, matrix stiffness and DDR1 expression [16]. We showed in vivo and in vitro that DDR1 expression and activation are associated with collagen alignment and consequent tissue stiffening [13]. In smooth muscle cells DDR1 downregulates collagen production by restricting TGF-β1 release and resultant p38 activation [127]. In an atherosclerosis model, DDR1 expression was associated with the regulation of ECM homeostasis by limiting collagen synthesis and extracellular vesicle-derived micro-calciﬁcations [10,72,128]. In an in vitro study using mouse osteoblasts stably expressing DDR1 extracellular ectodomains, collagen ﬁbrogenesis was inhibited [129,130]. These data indicate that DDR1 might contribute to ﬁbrotic lesions not only by direct involvement in collagen remodeling processes but also by activating downstream pro-ﬁbrotic signaling pathways.

In renal fibrosis, DDR1 expression is associated with increased inﬂammation and collagen I and IV deposition [7,131,132]. In pulmonary ﬁbrosis, DDR1 null mice show diminished injury after bleomycin treatment, which was associated with reduced collagen deposition [9]. In a kidney injury model DDR1 expression and kinase activity were associated with the regulation of glomerular collagen I and IV deposition [15]. The mechanisms that link DDR1 to collagen production in these disease models are not well-deﬁned but can attribute at least in part, DDR1-dependent activation of downstream signaling pathways (p38 and ERK) that regulate collagen synthesis to the expression of pro-inﬂammatory cytokines such as TNF-α, MCP-1, and IL1β and TGF-β1 [133–136].

The involvement of DDR1 in the regulation of MMP expression and/or activation was one of the ﬁrst pieces of evidence linking DDR1 and matrix remodeling [18]. The rates of ECM synthesis are associated with changes in local mechanical properties [137]. Similarly, the rates of protease synthesis are linked with mechanical tension, which suggest that mechanical loading increases both the production and removal of ECM constituents [1]. DDR1 expression is also directly associated with enhanced mechanical tension in vivo and in vitro [13] and is possibly a link between DDR1 expression and protease production. DDR1 mediates the expression of MMP-2 and MMP-9 in mouse vascular smooth muscle cells [11,138] while in human vascular smooth muscle cells, DDR1 is associated with the expression of MMP-1 [139]. DDR1 is also associated with the expression of MMP-7 in epithelial cells [140] and MMP-2 and MMP-9 in several malignant cells [18,19].

One of the signatures of ﬁbrotic disorders is the enhanced alignment of collagen ﬁbrils. Indeed, highly aligned collagen ﬁbers alter local tissue mechanical properties and can contribute to the progression of ﬁbrosis by prolonging the activation of key ﬁbrogenic pathways. The transmission of actomyosin forces involved in mechanical alignment of collagen has been mainly attributed to integrins and the activation of the Rho-ROCK pathway [55,141]. Notably, over-expression of DDR1 is associated with increased levels of collagen compaction in vitro [121] and regulates collagen deposition and tissue architecture in the mouse auditory system, thereby maintaining tension and elasticity in the inner ear [142,143]. DDR1 associates with non-muscle myosin II A in cells adhering and migrating on collagen [14], and mechanical traction of collagen ﬁbers requires myosin compaction [144]. We showed that the C-terminal kinase domain of DDR1 associates with myosin IIA and enables the force-transmission involved in collagen alignment independently of integrin function [13]. Further, we showed that DDR1 activation (Y792) controls the functional interaction that links collagen ﬁbrils to myosin and determines the amplitude and kinetics of contractile force generation involved in collagen alignment [13].

While the major contribution of DDR1 to tissue ﬁbrosis is likely related to collagen processing events, DDR1 may also contribute to tissue ﬁbrosis by stimulating downstream pro-ﬁbrotic signaling pathways as previously described. DDR1 regulates cell adhesion and migration on collagen[14] but it also stabilizes cell-cell junctions by binding the cell polarity regulators Par3 and Par6. Intercellular junctions coordinate the localization of Rho E and moderate actomyosin contraction [145]. Epithelial to mesenchymal transfor-
mation (EMT) is a pathobiological process that enables polarized epithelial cells to acquire the mesenchymal phenotype, which enables cells to migrate faster and also to produce large amounts of
collagen. EMT leads to a switch of intercellular adhesion molecules: the epithelial adhesion molecule E-cadherin is replaced by N-cadherin. Notably, the DDR1b isoform, is responsible for collagen I-induced upregulation of N-cadherin and involves tyrosine 513 of DDR1 [146,147].

**C. Targeting DDR1 in anti-fibrotic therapies**

Fibrosis is associated with many chronic diseases but often, it takes many years of lesion development before tissue and organ function are affected. This slow rate of lesion development may be attributable in part to the relatively slow conversion of normal repair processes into pro-fibrotic and disordered repair processes [23,28,148,149]. Despite the overwhelming need for improved diagnostics and therapies, few effective treatments with robust anti-fibrotic effects have been applied to patient care. At least part of this shortfall can be attributed to the fact that fibrosis involves a wide spectrum of cells, signaling pathways and regulatory systems. Therefore, the detection of fibrosis, particularly in deeply situated organs like liver, contributes to analytical challenges that complicate identification of biomarkers, reduces diagnostic sensitivity and confounds the conduct of clinical trials for evaluating the efficacy of novel therapeutics.

The expression and activation of DDR1 have been linked to fibrotic disorders such as atherosclerosis, arthritis, and many types of cancer. These findings indicate that DDR1 is a potentially promising therapeutic target for treatment of fibrotic disorders. As DDR levels increase significantly during the development of fibrotic disease, one strategy may be to reduce DDR expression. In this context, anti-sense oligonucleotides reduced by 50% DDR1 expression in experimental glomerulonephritis, similar to DDR1-deficient mice [134,150]. In idiopathic pulmonary fibrosis, a recently developed DDR1 inhibitor reduced inflammation and collagen deposition in the bleomycin-induced rat pulmonary fibrosis model [151]. Another strategy to perturb DDR1 function is the use of inhibitors of receptor tyrosine kinases such as imatinib or nilotinib. Both have given mixed results so far, most likely due to their lack of selectivity and secondary effects [152]. *In vitro* studies showed that nilotinib reduces the association of DDR1 with myosin IIA and blocks collagen contraction [13]. *In vivo* delivery of nilotinib to mice was shown to inhibit colorectal cancer cell invasion and metastasis. DDR1 activation mediated the phosphorylation of BCR substrate, which is involved in maintaining the β-catenin transcriptional activity necessary for tumor cell invasion and nilotinib prevents this [153]. More selective DDR1 inhibitors have been developed recently and one of these, 7rh, reduces invasion by non-small cell lung carcinoma and blocks tumor initiation and progression in a murine model of KRAS-induced lung adenocarcinoma [154].

As collagen binding is a requirement for DDR1 activation, another strategy could involve the development of antibodies that bind either the discoidin domain or the DDR1 binding region of collagens and thereby block DDR1 function.

Considering the roles of DDR1 in collagen processing events described above, it is likely that DDR1-based therapies would be more effective if adopted during early stages of the progression of the disease. Treatment with DDR1 inhibitors in established tissue fibrosis could retard the continued progression of the disease but it is unlikely that lesions will regress.

**VI. Conclusion**

In the last decade, DDR1 has emerged as a possible biomarker and therapeutic target in fibrotic disorders. These advances are a reflection of an improved understanding that in adult tissues, high levels of DDR1 expression are strongly associated with fibrotic diseases, often of deeply situated organs. Although there is considerable interest in DDR1 biology, only recently has the unusual mode of activation of these receptors begun to be appreciated. But more research is needed to define the downstream signaling pathways that link DDR1 expression and activation to fibrotic disease. As is often true, a really deep, basic understanding of DDR1 biology will be needed to develop more effective therapies of fibrotic diseases that involve this receptor.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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References

[1] Humphrey JD, Dufresne ER, Schwartz MA. Mechanotransduction and extracellular matrix homeostasis. Nat Rev Mol Cell Biol. 2014;15(12):802–12.

[2] Ingber DE. Mechanobiology and diseases of mechanotransduction. Ann Med. 2003;35(8):564–77.

[3] Ingber DE. Cellular mechanotransduction: putting all the pieces together again. FASEB J. 2006;20(7):811–22.

[4] Janmey PA, McCulloch CA. Cell mechanics: Integrating cell responses to mechanical stimuli. Annu Rev Biomed Eng. 2007;9(1):1–34.

[5] Orr AW, Helmke BP, Blackman BR, et al. Mechanisms of Mechanotransduction. Dev Cell. 2006;10(1):11–20.

[6] Lu P, Takai K, Weaver VM, et al. Extracellular matrix degradation and remodeling in development and disease [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov’t Review]. Cold Spring Harb Perspect Biol. 2011;3(12).

[7] Guerrot D, Kerroch M, Placier S, et al. Discoidin domain receptor 1 is a major mediator of inflammation and fibrosis in obstructive nephropathy. Am J Pathol. 2011;179(1):83–91.

[8] Song S, Shackel NA, Wang XM, et al. Discoidin domain receptor 1: isoform expression and potential functions in cirrhotic human liver [Research Support, Non-U.S. Gov’t]. Am J Pathol. 2011;178(3):1134–44.

[9] Avivi-Green C, Singal M, Vogel WF. Discoidin domain receptor 1-deficient mice are resistant to bleomycin-induced lung fibrosis [Research Support, Non-U.S. Gov’t]. Am J Respir Crit Care Med. 2006;174(4):420–7.

[10] Franco C, Ahmad PJ, Hou G, et al. Increased cell and matrix accumulation during atherogenesis in mice with vessel wall-specific deletion of discoidin domain receptor 1. Circ Res. 2010;106(11):1775–83.

[11] Hou G, Vogel W, Bendek MP. The discoidin domain receptor tyrosine kinase DDR1 in arterial wound repair [Research Support, Non-U.S. Gov’t]. J Clin Invest. 2001;107(6):727–35.

[12] Krohn JB, Hutcheson JD, Martínez-Martínez E, et al. <span>hw_pid = "article-title-1" class = "article-title">Discoidin domain receptor-1 regulates calcific extracellular vesicle release in vascular smooth muscle cell fibrocalcific response via transforming growth factor-β signaling</span> <span>hw_pid = "article-title-46" class = "sub-article-title">Significance</span>. Arterioscler Thromb Vasc Biol. 2016;36(3):525–533.

[13] Coelho NM, Arora PD, van Putten S, et al. Discoidin domain receptor 1 mediates myosin-dependent collagen contraction. Cell Rep. 2017;18(7):1774–1790.

[14] Huang Y, Arora P, McCulloch CA, et al. The collagen receptor DDR1 regulates cell spreading and motility by associating with myosin IIA [Research Support, Non-U.S. Gov’t]. J Cell Sci. 2009;122(Pt 10):1637–46.

[15] Borza CM, Su Y, Tran TL, et al. Discoidin domain receptor 1 kinase activation is required for regulating collagen IV synthesis. Matrix Biol. 2017;57–58:258–71.

[16] Ezzoukhry Z, Henriet E, Piquet L, et al. TGF-β1 promotes linear invadosome formation in hepatocellular carcinoma cells, through DDR1 up-regulation and collagen I cross-linking. Eur J Cell Biol. 2016;95(11):503–512.

[17] Coelho NM, McCulloch CA. Contribution of collagen adhesion receptors to tissue fibrosis [journal article]. Cell Tissue Res. 2016;365(3):521–538.

[18] Leitinger B. Transmembrane collagen receptors [Review]. Annu Rev Cell Dev Biol. 2011;27:265–90.

[19] Valiathan RR, Marco M, Leitinger B, et al. Discoidin domain receptor tyrosine kinases: new players in cancer progression [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov’t, Non-P.H.S. Review]. Cancer Metastasis Rev. 2012;31(1–2):295–321.

[20] Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease [Review]. Nat Rev Mol Cell Biol. 2014;15(12):786–801.

[21] Cox TR, Erler JT. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer [Research Support, Non-U.S. Gov’t]. Dis Models Mech. 2011;4(2):165–78.

[22] Duscher D, Maan ZN, Wong VW, et al. Mechanotransduction and fibrosis. J Biomech. 2014;47(9):1997–2005.

[23] Friedman SL, Sheppard D, Dufﬁeld JS, et al. Therapy for ﬁbrotic diseases: nearing the starting line. Sci Transl Med. 2013;5(167):167sr1.

[24] Wynn TA. Cellular and molecular mechanisms of ﬁbrosis. J Pathol. 2008;214(2):199–210.

[25] Dufﬁeld JS, Lusher M, Thannickal VJ, et al. Host responses in tissue repair and ﬁbrosis. Annu Rev Pathol. 2013;8(1):241–76.

[26] Klingberg F, Hinz B, White ES. The myoﬁbroblast matrix: implications for tissue repair and ﬁbrosis. J Pathol. 2013;229(2):298–309.

[27] Wells RG. Tissue mechanics and ﬁbrosis. Biochim Biophys Acta. 2013;1832(7):884–90.

[28] Wynn TA, Ramalingam TR. Mechanisms of ﬁbrosis: therapeutic translation for ﬁbrotic disease. Nat Med. 2012;18(7):1028–40.

[29] Hinz B. Myoﬁbroblasts. Exp Eye Res. 2016;142:56–70.

[30] Kisseleva T, Brenner DA. Mechanisms of ﬁbrogenesis. Exp Biol Med (Maywood). 2008;233(1):109–22.

[31] Manon-Jensen T, Kjeld NG, Karsdal MA. Collagen-mediated hemostasis. J Thromb Haemost. 2016;14(3):438–48.

[32] Dolberg DS, Bissell MJ. Inability of Rous sarcoma virus to cause sarcomas in the avian embryo. Nature. 1984;309(5968):552–6.

[33] Ingber DE. Tensegrity: the architectural basis of cellular mechanotransduction. Annu Rev Physiol. 1997;59:575–99.

[34] Handorf AM, Zhou Y, Halanski MA, et al. Tissue stiffness dictates development, homeostasis, and disease progression. Organogenesis. 2015;11(1):1–15.

[35] van Putten S, Shaﬁeyan Y, Hinz B. Mechanical control of cardiac myoﬁbroblasts. J Mol Cell Cardiol. 2016;93:133–42.

[36] Schwartz MA. Integrins and extracellular matrix in mechanotransduction. Cold Spring Harb Perspect Biol. 2010;2(12):a005066.

[37] Vining KH, Mooney DJ. Mechanical forces direct stem cell behaviour in development and regeneration. Nat Rev Mol Cell Biol. 2017;18(12):728–742.

[38] Engler AJ, Sen S, Sweeney HL, et al. Matrix elasticity directs stem cell lineage specification [Research Support,
Non-U.S. Gov’t Research Support, U.S. Gov’t, Non-P.H. S.J. Cell. 2006;126(4):677–89.

[39] Perez-Tamayo R. Pathology of collagen degradation. A review. Am J Pathol. 1978;92(2):508–566.

[40] Sodek J. A comparison of the rates of synthesis and turnover of collagen and non-collagen proteins in adult rat periodontal tissues and skin using a microassay. Arch Oral Biol. 1977;22(12):655–665.

[41] Kirmse R, Otto H, Ludwig T. Interdependency of cell adhesion, force generation and extracellular proteolysis in matrix remodeling. J Cell Sci. 2011;124(11):1857–1866.

[42] Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior [Research Support, U.S. Gov’t, P. H.S. Review]. Annu Rev Cell Dev Biol. 2001;17:463–516.

[43] Cullen J. Intracellular collagen in experimental arthritis in rats. J Bone Joint Surg Br. 1972;54:351–359.

[44] Soames JV, Davies RM. Intracellular collagen fibrils in early gingivitis in the beagle dog. J Periodontal Res. 1977;12(5):378–386.

[45] Neurath MF. Detection of Luse bodies, spiralled collagen, dysplastic collagen, and intracellular collagen in rheumatoid connective tissues: an electron microscopic study [Research Support, Non-U.S. Gov’t]. Ann Rheum Dis. 1993;52(4):278–84.

[46] Madsen DH, Leonard D, Masedunskas A, et al. M2-like macrophages are responsible for collagen degradation through a mannose receptor-mediated pathway [Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov’t]. J Cell Biol. 2013;202(6):951–66.

[47] Arora PD, Manolson MF, Downey GP, et al. A novel model system for characterization of phagosomal maturation, acidification, and intracellular collagen degradation in fibroblasts [Research Support, Non-U.S. Gov’t]. J Biol Chem. 2000;275(45):35432–41.

[48] Groves E, Dart AE, Covarelli V, et al. Molecular mechanisms of phagocytic uptake in mammalian cells [Research Support, Non-U.S. Gov’t Review]. Cell Mol Life Sci. 2008;65(13):1957–76.

[49] Everts V, van der Zee E, Creemers L, et al. Phagocytosis and intracellular digestion of collagen, its role in turnover and remodelling [Review]. Histochem J. 1996;28(4):229–45.

[50] McCulloch CA, Coelho NM. Collagen processing and its role in fibrosis. In: Dixon IMC, Wigle J.T, editors. Cardiac fibrosis and heart failure: Cause or effect? Advances in biochemistry in health and disease. Vol. 13. Springer International Publishing; 2015. p. 261–278.

[51] Levental KR, Yu H, Kass L, et al. Matrix crossing forces tumor progression by enhancing integrin signaling. Cell. 2009;139(5):891–906.

[52] Malik R, Lelkes PI, Cukierman E. Biomechanical and biochemical remodeling of stromal extracellular matrix in cancer. Trends Biotechnol. 2015;33(4):230–236.

[53] Paszek MJ, Zahir N, Johnson KR, et al. Tensional homeostasis and the malignant phenotype. Cancer Cell. 2005;8(3):241–54.

[54] Wyckoff JB, Pinner SE, Gschmeissner S, et al. ROCK- and myosin-dependent matrix deformation enables protease-independent tumor-cell invasion in vivo. Curr Biol. 2006;16(15):1515–1523.

[55] Kural MH, Billiar KL. Regulating tension in three-dimensional culture environments [Research Support, N.I.H., Extramural Research Support, U.S. Gov’t, Non-P.H.S. Review]. Exp Cell Res. 2013;319(16):2447–59.

[56] Wolf K, Te Lindert M, Krause M, et al. Physical limits of cell migration: control by ECM space and nuclear deformation and tuning by proteolysis and traction force [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov’t]. J Cell Biol. 2013;201(7):1069–84.

[57] Kung C, Martinac B, Sukharev S. Mechanosensitive channels in microbes. Annu Rev Microbiol. 2010;64:313–29.

[58] Iida H, Nakamura H, Ono T, et al. MID1, a novel Saccharomyces cerevisiae gene encoding a plasma membrane protein, is required for Ca2+ influx and mating. Mol Cell Biol. 1994;14(12):8259–8271.

[59] Murthy SE, Dubin AE, Patapoutian A. Piezos thrive under pressure: mechanically activated ion channels in health and disease. Nat Rev Mol Cell Biol. 2017;18(12):771–783.

[60] Barnes JM, Przybyla L, Weaver VM. Tissue mechanics regulate brain development, homeostasis and disease. J Cell Sci. 2017;130(1):71–82.

[61] Orr AW, Helmeke BP, Blackman BR, et al. Mechanisms of mechanotransduction. Dev Cell. 2006;10(1):11–20.

[62] Lehen’kyi YY, Prevarskaya N. Oncogenic TRP Channels. In: Islam MS, editor. Transient Receptor Potential Channels. Dordrecht: Springer Netherlands; 2011. p. 929–945.

[63] Gasparski AN, Beningo KA. Mechanoreception at the cell membrane: More than the integrins. Arch Biochem Biophys. 2015;586:20–6.

[64] Vennekens R. Emerging concepts for the role of TRP channels in the cardiovascular system. J Physiol. 2011;589(7):1527–1534.

[65] Arnaout MA, Goodman SL, Xiong JP. Structure and mechanics of integrin-based cell adhesion [Research Support, N.I.H., Extramural Review]. Curr Opin Cell Biol. 2007;19(5):495–507.

[66] Barczyk M, Carracedo S, Gullberg D. Integrins [Review]. Cell Tissue Res. 2010;339(1):269–80.

[67] Hynes RO. Integrins: Bidirectional, allosteric signaling machines. Cell. 2002;110(6):673–687.

[68] Geiger B, Yamada KM. Molecular architecture and function of matrix adhesions [Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov’t Review]. Cold Spring Harb Perspect Biol. 2011;3(5).

[69] Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. J Cell Sci. 2010;123(Pt 24):4195–200.

[70] Margadant C, Sonnenberg A. Integrin-TGF-beta cross-talk in fibrosis, cancer and wound healing. EMBO Rep. 2010;11(2):97–105.

[71] Hinz B. The extracellular matrix and transforming growth factor-beta1: Tale of a strained relationship. Matrix Biol. 2015;47:54–65.

[72] Muller AK, Meyer M, Werner S. The roles of receptor tyrosine kinases and their ligands in the wound repair process. Semin Cell Dev Biol. 2012;23(9):963–70.

[73] McCulloch CA, Downey GP, El-Gabalawy H. Signalling platforms that modulate the inflammatory response:
new targets for drug development. Nat Rev Drug Discov. 2006;5(10):864–76.

[74] Dumbauld DW, Lee TT, Singh A, et al. How vinculin regulates force transmission. Proc Natl Acad Sci. 2013;110(24):9788–9793.

[75] del Río A, Perez-Jimenez R, Liu R, et al. Stretching single talin rod molecules activates vinculin binding. Science. 2009;323(5914):638–41.

[76] Atherton P, Stutchbury B, Wang D-Y, et al. Vinculin controls talin engagement with the actomyosin machinery [Article]. Nat Commun. 2015;6:10038. https://www.nature.com/articles/ncomms10038 – supplementary-information.

[77] Guilluy C, Swaminathan V, Garcia-Mata R, et al. The Rho GEFs LARG and GEF-H1 regulate the mechanical response to force on integrins. Nat Cell Biol. 2011;13(6):722–7.

[78] Paszek MJ, Zahir N, Johnson KR, et al. Tensional homeostasis and the malignant phenotype. Cancer Cell. 2005;8(3):241–254.

[79] Meng Z, Moroiishi T, Guan KL. Mechanisms of Hippo pathway regulation. Genes Dev. 2016;30(1):1–17.

[80] Sun Z, Guo SS, Fassler R. Integrin-mediated mechanotransduction. J Cell Biol. 2016;215(4):445–456.

[81] Baum B, Georgiou M. Dynamics of adherens junctions in epithelial establishment, maintenance, and remodeling. J Cell Biol. 2011;192(6):907–917.

[82] Takeichi M. Dynamic contacts: rearranging adherens junctions to drive epithelial remodelling. Nat Rev Mol Cell Biol. 2014;15(6):397–410.

[83] Harris TJ, Tepass U. Adherens junctions: from molecules to morphogenesis. Nat Rev Mol Cell Biol. 2010;11(7):502–14.

[84] Chu YS, Thomas WA, Eder O, et al. Force measurements in E-cadherin-mediated cell doublets reveal rapid adhesion strengthened by actin cytoskeleton remodeling through Rac and Cdc42. J Cell Biol. 2004;167(6):1183–94.

[85] Mege RM, Ishiyama N. Integration of cadherin adhesion and cytoskeleton at adherens junctions. Cold Spring Harbor Perspect Biol. 2017;9(5).

[86] Ladoux B, Mege RM. Mechanobiology of collective cell behaviours. Nat Rev Mol Cell Biol. 2017;18(12):747–757.

[87] Benham-Pyle BW, Pruitt BL, Nelson WJ. Cell adhesion. Mechanical strain induces E-cadherin-dependent Yap1 and beta-catenin activation to drive cell cycle entry. Science. 2015;348(6238):1024–7.

[88] De Pascalis C, Etienne-Manneville S. Single and collective cell migration: the mechanics of adhesions. Mol Biol Cell. 2017;28(14):1833–1846.

[89] Han MK, de Rosji J. Converging and unique mechanisms of mechanotransduction at adhesion sites. Trends Cell Biol. 2016;26(8):612–23.

[90] Rosenbaum DM, Rasmussen SG, Kobilka BK. The structure and function of G-protein-coupled receptors. Nature. 2009;459(7245):356–63.

[91] Venkataraman AJ, Deupi X, Lebon G, et al. Molecular signatures of G-protein-coupled receptors. Nature. 2013;494(7436):185–94.

[92] Chacisvili M, Zhang YL, Frangos JA. G protein-coupled receptors sense fluid shear stress in endothelial cells. Proc Natl Acad Sci U S A. 2006;103(42):15463–8.

[93] Storch U, Schnitzler MMM, Guder Mann T. G protein-mediated stretch reception. Am J Physiol–Heart Circ Physiol. 2012;302(6):H1241–H1249.

[94] Tarbell JM, Pahakis MY. Mechanotransduction and the glyocalyx. J Intern Med. 2006;259(4):339–50.

[95] Weinaum S, Tarbell JM, Damiano ER. The structure and function of the endothelial glyocalyx layer. Annu Rev Biomed Eng. 2007;9:121–67.

[96] Gouverneur M, Berg B, Nieuworp M, et al. Vascular-protective properties of the endothelial glyocalyx: effects of fluid shear stress. J Intern Med. 2006;259(4):393–400.

[97] Voyvodic PL, Min D, Liu R, et al. Loss of syndecan-1 induces a pro-inflammatory phenotype in endothelial cells with a dysregulated response to atheroprotective flow. J Biol Chem. 2014;289(14):9547–59.

[98] Huyhn J, Bordeleau F, Kraning-Rush CM, et al. Substrate stiffness regulates PDGF-Induced Circular Dorso Ruffle Formation Through MLCK. Cell Mol Bioeng. 2013;6(2).

[99] Saxena M, Liu S, Yang B, et al. EGER and HER2 activate rigidity sensing only on rigid matrices. Nat Mater. 2017;16(7):775–781.

[100] Yang B, Lieu ZZ, Wolfenson H, et al. Mechanosensing Controlled Directly by Tyrosine Kinases. Nano Lett. 2016;16(9):5951–61.

[101] Fu HL, Valiaithan RR, Arkwright R, et al. Discoidin domain receptors: unique receptor tyrosine kinases in collagen-mediated signaling [Research Support, N.I.H., Extramural Review]. J Biol Chem. 2013;288(11):7430–7.

[102] Vogel W, Gish GD, Alves F, et al. The discoidin domain receptor tyrosine kinases are activated by collagen [Research Support, Non-U.S. Gov’t]. Mol Cell. 1997;1(1):13–23.

[103] Vogel WF, Abdulhussein R, Ford CE. Sensing extracellular matrix: an update on discoidin domain receptor function [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov’t Review]. Cell Signaling. 2006;18(8):1108–16.

[104] Carafoli F, Bihan D, Stathopoulos S, et al. Crystallographic insight into collagen recognition by discoidin domain receptor 2 [Research Support, Non-U.S. Gov’t]. Structure. 2009;17(12):1573–81.

[105] Carafoli F, Mayer MC, Shiraishi K, et al. Structure of the discoidin domain receptor tyrosine kinases are activated by collagen [Research Support, Non-U.S. Gov’t]. J Biol Chem. 2001;276(20):15463–8.

[106] Tallquist MD, Molkentin JD. Redefining the identity of cardiac fibrobasts [Review Article]. Nat Rev Cardiol. 2017;14:484.

[107] Olase E, Arteta B, Benedicto A, et al. Loss of discoidin domain receptor 2 promotes hepatic fibrosis after chronic carbon tetrachloride through altered paracrine interactions between hepatic stellate cells and liver-associated macrophages. Am J Pathol. 2011;179(6):2894–904.

[108] Olase E, Ikeda K, Eng FJ, et al. DDR2 receptor promotes MMP-2-mediated proliferation and invasion by hepatic stellate cells. J Clin Invest. 2001;108(9):1369–78.

[109] Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell. 2010;141(7):1117–34.
[110] Mihai C, Chotani M, Elton TS, et al. Mapping of DDR1 Distribution and Oligomerization on the Cell Surface by FRET Microscopy. J Mol Biol. 2009;385(2):432–445.

[111] Noordeen NA, Carafoli F, Hohenester E, et al. A transmembrane leucine zipper is required for activation of the dimeric receptor tyrosine kinase DDR1. J Biol Chem. 2006;281(32):22744–22751.

[112] Abdulhussein R, Koo DH, Vogel WF. Identification of disulfide-linked dimers of the receptor tyrosine kinase DDR1 [Research Support, Non-U.S. Gov't]. J Biol Chem. 2008;283(18):12026–33.

[113] Leitinger B. Molecular analysis of collagen binding by the human discoidin domain receptors, DDR1 and DDR2: Identification of collagen binding sites in DDR2. J Biol Chem. 2003;278(19):16761–16769.

[114] Xu H, Abe T, Liu JKH, et al. Normal activation of dis- coidin domain receptor 1 mutants with disulfide cross-links, insertions, or deletions in the extracellular juxta- membrane region: Mechanistic implications. J Biol Chem. 2014;289(19):13565–13574.

[115] Juskaite V, Corcoran DS, Leitinger B. Collagen induces activation of DDR1 through lateral dimer association and phosphorylation between dimers. eLife. 2017;6:e25716.

[116] Mihai C, Chotani M, Elton TS, et al. Mapping of DDR1 distribution and oligomerization on the cell surface by FRET microscopy. J Mol Biol. 2009;385(2):432–45.

[117] Yeung D, Chmielewski D, Mihai C, et al. Oligomerization of DDR1 ECD affects receptor–ligand binding. J Struct Biol. 2013;183(3):495–500.

[118] Fu HL, Valiathan RR, Payne L, et al. Glycosylation at Asn211 regulates the activation state of the discoidin domain receptor 1 (DDR1). J Biol Chem. 2014;289(13):9275–87.

[119] Riento K, Ridley AJ. Rocks: multifunctional kinases in cell behaviour. Nat Rev Mol Cell Biol. 2003;4(6):446–56.

[120] Totsukawa G, Yamakita Y, Yamashiro S, et al. Distinct roles of ROCK (Rho-kinase) and MLCK in spatial regulation of MLc phosphorylation for assembly of stress fibers and focal adhesions in 3T3 fibroblasts. J Cell Biol. 2000;150(4):797–806.

[121] Staudinger LA, Spano SJ, Lee W, et al. Interactions between the discoidin domain receptor 1 and beta1 integrin regulate attachment to collagen. Biol Open. 2013;2(11):1148–59.

[122] Jiang Y, Xing X, Lu S. The effect of suppressing discoidin domain receptor expression on keloid formation and proliferation. Wounds. 2009;21(8):207–14.

[123] Staudinger LA, Spano SJ, Lee WS, et al. Role of discoidin domain receptor 1 in dysregulation of collagen remodeling by cyclosporin A. Int J Biochem Cell Biol. 2015;62:80–7.

[124] Licup AJ, Münzer S, Sharma A, et al. Stress controls the mechanics of collagen networks. Proc Natl Acad Sci. 2015;112(31):9573–9578.

[125] Hinz B, Phan SH, Thannickal VJ, et al. The myofibroblast: one function, multiple origins. Am J Pathol. 2007;170(6):1807–16.

[126] Piersma B, Wouters OY, de Rond S, et al. Ascorbic acid promotes a TGFβ1–induced myofibroblast phenotype switch. Physiol Rep. 2017;5(17).

[127] Krohn JB, Hutcheson JD, Martinez-Martinez E, et al. Discoidin domain receptor-1 regulates calcific extracel- lular vesicle release in vascular smooth muscle cell fibrocalcific response via transforming growth factor-beta signaling. Arterioscler Thromb Vasc Biol. 2016;36 (3):525–33.

[128] Franco C, Hou G, Ahmad PJ, et al. Discoidin domain receptor 1 (ddr1) deletion decreases atherosclerosis by accelerating matrix accumulation and reducing inflammation in low-density lipoprotein receptor-deficient mice. Circ Res. 2008;102(10):1202–11.

[129] Blissett AR, Garbellini D, Calomeni EP, et al. Regulation of collagen fibrillogenesis by cell-surface expression of kinase dead DDR2. J Mol Biol. 2009;385(3):902–11.

[130] Flynn LA, Blissett AR, Calomeni EP, et al. Inhibition of collagen fibrillogenesis by cells expressing soluble extra- cellular domains of DDR1 and DDR2. J Mol Biol. 2010;395(3):533–43.

[131] Flamant M, Placier S, Rodenas A, et al. Discoidin domain receptor 1 null mice are protected against hypertension-induced renal disease. J Am Soc Nephrol. 2006;17(12):3374–3381.

[132] Gross O, Girgenti R, Beirouss B, et al. Loss of collagen- receptor DDR1 delays renal fibrosis in hereditary type IV collagen disease [Research Support, Non-U.S. Gov't]. Matrix Biol. 2010;29(5):346–56.

[133] Kavvadas P, Dussaule JC, Chatziantoniou C. Searching novel diagnostic markers and targets for therapy of CKD. Kidney Int Suppl (2011). 2014;4(1):53–57.

[134] Kerroch M, Allier C, Dorison A, et al. Protective effects of genetic inhibition of Discoidin Domain Receptor 1 in experimental renal disease [Article]. Sci Rep. 2016;6:21262.

[135] Matsuyama W, Watanabe M, Shirahama Y, et al. Activation of discoidin domain receptor 1 on CD14-positive bronchoalveolar lavage fluid cells induces chemokine production in idiopathic pulmonary fibrosis. J Immunol. 2005;174(10):6490–8.

[136] Matsuyama W, Mitsuyama H, Watanabe M, et al. Role of collagen production in idiopathic pulmonary fibrosis. Chest. 2017;142(3):650–657.

[137] Muiznieks LD, Keeley FW. Molecular assembly and mechanical properties of the extracellular matrix: A fibrous protein perspective. Biochimica et Biophysica Acta (BBA)–Mol Basis Dis. 2013;1832(7):866–875.

[138] Hou G, Vogel WF, Bendeck MP. Tyrosine kinase activity of discoidin domain receptor 1 is necessary for smooth muscle cell migration and matrix metalloproteinase expression [Research Support, Non-U.S. Gov't]. Circ Res. 2002;90(11):1147–9.

[139] Mihai C, Chotani M, Elton TS, et al. Mapping of DDR1 Distribution and Oligomerization on the Cell Surface by FRET Microscopy. J Mol Biol. 2009;385(2):432–445.

[140] Roberts ME, Magowan L, Hall IP, et al. Discoidin domain receptor 1 regulates bronchial epithelial repair and matrix metalloproteinase production. Eur Respir J. 2011;37(6):1482–1493.
[141] Provenzano PP, Inman DR, Eliceiri KW, et al. Contact guidance mediated three-dimensional cell migration is regulated by Rho/ROCK-dependent matrix reorganization. Biophys J. 2008;95(11):5374–84.

[142] Meyer zum Gottesberge AM, Gross O, Becker-Lendzian U, et al. Inner ear defects and hearing loss in mice lacking the collagen receptor DDR1. Lab Invest. 2008;88(1):27–37.

[143] Meyer zum Gottesberge AM, Hansen S. The collagen receptor DDR1 co-localizes with the non-muscle myosin IIA in inner ear and contributes to the cytoarchitecture and stability of motile cells [journal article]. Cell Tissue Res. 2014;358(3):729–736.

[144] Stopak D, Harris AK. Connective tissue morphogenesis by fibroblast traction. Dev Biol. 1982;90(2):383–398.

[145] Hidalgo-Carcedo C, Hooper S, Chaudhry SI, et al. Collective cell migration requires suppression of actomyosin at cell-cell contacts mediated by DDR1 and the cell polarity regulators Par3 and Par6. Nat Cell Biol. 2011;13(1):49–58.

[146] Huang H, Svoboda RA, Lazenby AJ, et al. Up-regulation of N-cadherin by collagen I-activated discoidin domain receptor 1 in pancreatic cancer requires the adaptor molecule Shc1. J Biol Chem. 2016;291(44):23208–23223.

[147] Shintani Y, Fukumoto Y, Chaika N, et al. Collagen I-mediated up-regulation of N-cadherin requires cooperative signals from integrins and discoidin domain receptor 1. J Cell Biol. 2008;180(6):1277–1289.

[148] Nanthakumar CB, Hatley RJ, Lemma S, et al. Dissecting fibrosis: therapeutic insights from the small-molecule toolbox. Nat Rev Drug Discov. 2015;14(10):693–720.

[149] Marshall RP, Simpson JK, Lukey PT. Strategies for biomarker discovery in fibrotic disease. Biochimica et Biophysica Acta (BBA)–Mol Basis Dis. 2013;1832(7):1079–1087.

[150] Kerroch M, Guerrot D, Vandermeersch S, et al. Genetic inhibition of discoidin domain receptor 1 protects mice against crescentic glomerulonephritis. FASEB J. 2012;26(10):4079–91.

[151] Chen C, Deng J, Yu X, et al. Identification of novel inhibitors of DDR1 against idiopathic pulmonary fibrosis by integrative transcriptome meta-analysis, computational and experimental screening. Mol Biosyst. 2016;12(5):1540–1551.

[152] Dorison A, Dussaule JC, Chatziantoniou C. The role of discoidin domain receptor 1 in inflammation, fibrosis and renal disease. Nephron. 2017;137(3):212–220.

[153] Jeitany M, Leroy C, Tosti P, et al. Inhibition of DDR1–BCR signalling by nilotinib as a new therapeutic strategy for metastatic colorectal cancer. EMBO Mol Med. 2018 [In press].

[154] Ambrogio C, Gomez-Lopez G, Falcone M, et al. Combined inhibition of DDR1 and Notch signaling is a therapeutic strategy for KRAS-driven lung adenocarcinoma. Nat Med. 2016;22(3):270–7.