Cell-matrix and cell-cell contacts of myofibroblasts: role in connective tissue remodeling

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Introduction

Activation of fibroblasts into myofibroblasts, a crucial step in the process of wound healing and in the evolution of fibrocontractive diseases, is characterized by the development of intracytoplasmic stress fibers that confer to these cells the capacity of developing tension, and by the increased synthesis of extracellular matrix (ECM) components, such as collagen type I (1, 2). The most important marker of the fibroblast/myofibroblast phenotypic transition is the de novo expression of α-smooth muscle actin (α-SMA), the actin isoform typical of vascular smooth muscle cells (SMC) (3). It has been shown that α-SMA expression in stress fibers is essential for the acquisition of a high contractile activity by myofibroblasts, in the absence of the neo-expression of any other SMC-specific contractile protein, e.g. smooth muscle myosin (4–6). The work of several laboratories has demonstrated that the induction of the myofibroblastic phenotype requires the concerted action of growth factors, such as TGFβ1 (7, 8), specialized ECM molecules, such as the cellular fibronectin (FN) splice variant ED-A (9, 10) and a mechanically stressed environment (6, 11). When the continuity of the epithelial architecture has been reconstituted in normally healing wounds, myofibroblasts disappear massively through an apoptotic process (12). This process is lacking during the formation of hypertrophic scars or the onset of fibrocontractive diseases, where the persistence of cell contractile activity leads

Summary

It is presently accepted that fibroblast/myofibroblast modulation represents a crucial step in granulation tissue contraction and in the production of the connective tissue deformations typical of fibrocontractive diseases. In addition to synthesizing extracellular matrix (ECM) components, myofibroblasts can develop tensile force through the neoformation of α-smooth muscle actin (α-SMA) containing cytoplasmic stress fibers. Tension has been shown to be a crucial regulator of connective tissue remodeling. In order to coordinate tension distribution within connective tissue, cell-matrix and cell-cell contacts appear essential. This review addresses the formation, molecular structure and function of such structures that are characterized by their association with intracytoplasmic actin filaments. Actin associated cell-matrix adhesions appear to provide the interface between ECM components and intracellular stress fibers, thus contributing to the transmission of force to the substrate and to the detection of stress level in the matrix. Cell-cell adherens junctions appear to synchronize myofibroblast contractile activity. Further studies investigating the functions of these structures will be important for the understanding of the mechanisms of granulation tissue evolution and for the planification of strategies in view of influencing connective tissue deformations.

Keywords

α-smooth muscle actin, adherens junction, focal adhesion, wound healing, fibrosis

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to continuous matrix remodeling and retraction. Studies elucidating the mechanisms of apoptotic phenomena affecting granulation tissue may be important in order to develop preventive and/or therapeutic strategies for fibrocontractive changes.

Myofibroblasts communicate with each other electrochemically by forming gap junctions (13), which may play a role in achieving a coordinated production of tension within granulation tissue. In addition, myofibroblasts develop cell-matrix- as well as cell-cell-junctions (14-16) (Fig. 1). The cytoplasmic portion of the transmembrane linker proteins of both types of junctions is part of a complex that associates with actin filaments (17, 18), which participate in their organization and activity. Thus, the production and remodeling of these junctions are instrumental in the generation and maintenance of tensile force that represents a major activity of myofibroblastic cells in order to regulate granulation tissue evolution. Up to now not much attention has been devoted to the composition and function of these junctional complexes; the purpose of this review is to summarize the mechanisms involved in their development as well as those regulating force transmissions through these specialized contacts.

**Fibroblast matrix adhesions**

In contrast to normal dermal fibroblasts, myofibroblasts of both granulation tissue (15) and fibrocontractive diseases (19) develop complex adhesion structures with the ECM that have been originally called “fibronexus” (20) (Fig. 2). The fibronexus is thought to be important for the efficient transmission of myofibroblast contractile force to the ECM in order to promote wound contraction (2). However, little is known about the molecular composition and the development of the fibronexus during myofibroblast differentiation in vivo since most studies on cell-matrix junctions have been performed on cultured fibroblasts, mostly irrespective of their level of myofibroblast differentiation. Despite a large morphological and molecular heterogeneity, transmembrane integrins interact with proteins of the ECM in all cell-matrix junctions. The cytoplasmic tail of integrins is linked to actin filament bundles through a complex of structural and signaling molecules (18, 21, 22); for this reason we will use here the general term “actin-associated cell-matrix adhesions” (AACMAs). A discussion of the molecular interactions within the different types of AACMAs is beyond the scope of this report and we refer to excellent recent reviews (21, 23).

Various laboratories have used several denominations in order to define different types or different development degrees of AACMAs according to their size, localization within the cell, morphology and molecular composition. We use here a simplified classification of AACMAs formed in fibroblasts cultured on planar substrates including: fibrillar adhesions, immature focal adhesions (FAs), mature FAs and supermature FAs. In order to facilitate the reading, we indicate in Table 1 for each of these AACMA types the corresponding names used in the literature. It becomes more and more accepted that mechanical stress, exerted on the level of the ECM and/or produced by intracellular contractile force determines to a great extent which type of AACMA is formed (23, 24). Immature FAs or focal complexes (25) are small AACMAs that are generally described to form in the absence of tension, since they accumulate at the tips of lamellae when actin-myosin-based contraction is inhibited and they are independent of the activity of Rho-associated kinase (ROCK) (24); activation of ROCK by the small GTPase Rho is a major signaling step leading to the phosphorylation of

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**Figure 1:** Actin-associated junctions in myofibroblasts. Rat lung myofibroblasts were cultured on glass substrates, fixed after 5d and immunostained for α-SMA (red), paxillin (green) as a component of FAs and β-catenin (blue) as a marker for AJs. Myofibroblasts form large, supermature FAs with the ECM and AJs with adjacent cells; both structures are located at the terminal portion of α-SMA-positive stress fibers. Bar: 50 µm.

**Figure 2:** Transmission electron micrograph of the fibronexus. Myofibroblast from 9d-old rat wound granulation tissue form fibronexus contacts that appear as dense cytoplasmic plaques at which intracellular microfilaments (MF) form a continuum with extracellular FN fibrils (FN). The dotted line indicates the putative position of the cell border. Bar: 500 nm.
myosin light chain kinase and development of contractile force (26). However, application of mechanical force to FN receptors on the dorsal surface of fibroblasts was recently shown to induce the formation of immature FAs out of initial integrin contacts (27) and one study demonstrated their dissociation after inhibition of cell contractility (28).

Upon increase of intracellular and/or extracellular tension immature FAs transform into larger mature or “classical” FAs. Enhanced contractile activity by activation of Rho/ROCK increases the size of mature FAs (29, 30); conversely, they disassemble after loss of intracellular tension or modulate into immature FAs (31-34). Extracellular stress has been shown to substitute intracellular contractile activity in establishing mature FAs, since pulling the cell edge with the use of micro-needles enlarged FAs even in the presence of inhibitors of myosin and ROCK (35). Moreover, FAs have been stimulated to mature when pulled with the use of ECM protein-coated microbeads (36, 37) and stretched on silicone membranes (38). A number of studies demonstrated that the mechanical state of the ECM is a crucial factor determining the level of extracellularly applied tension: relaxing pre-stretched deformable silicone substrates was shown to reduce the size of fibroblast FAs (38) and growing cells on compliant polyacrylamide substrates inhibits FA maturation (39). Increasing the global matrix load of three-dimensional collagen gels leads to the rapid conversion of nascent adhesions into mature FAs (40, 41).

Mature FAs may further enlarge and evolve into so-called ‘supermature’ FAs (14), generally correlating with TGFβ-induced differentiation of myofibroblasts grown on stiff planar substrates (14) and in stressed collagen gels (42). Recently, FA supermaturation has been shown to depend on the high contractile activity mediated by α-SMA expression in stress fibers and is blocked by a fusion peptide containing the N-terminal sequence of α-SMA (43), a specific inhibitor of this α-SMA function (44). Supermature FAs differ from mature FAs by specifically expressing α-SMA and ED-A FN and by containing high levels of α5β1 integrin and tensin (14). Integrin α5β1 and tensin are often employed as markers for so-called fibrillar adhesions (45) that are, in contrast to mature (32) and supermature FAs (14, 46), αvβ3 integrin- and vinculin-negative. They associate with thin actin filament bundles in the cell center rather than with stress fibers (47) and remain almost unchanged after inhibition of actin-myosin contraction (45). During cell attachment, typical components of fibrillar adhesions (α5β1 integrin and tensin) are first co-localized with markers of mature FAs (αvβ3 integrin, vinculin) but separate with continuing cell spreading by moving towards the cell center in a process that requires actin-myosin contraction and a deformable ECM (48). This separation is prevented by growing fibroblasts on a non-deformable matrix of covalently immobilized FN molecules (47) or on chemically fixed sections of mouse embryo tissue (49).

Thus, it has been suggested that molecular markers of fibrillar adhesions and mature FAs co-occur in supermature FAs because they are subjected to high (intracellular) tension (14). This mutual expression is in conjunction with the dual function of supermature FAs in providing high cell adhesion (46), comparable to mature FAs, and in organizing (ED-A) FN (14), similar to fibrillar adhesions that are thought to open cryptic self-assembly sites by stretching the folded (relaxed) FN molecule (50, 51). At present, supermature FAs are most reliably identified by the specific expression of α-SMA and ED-A FN and the combined expression of vinculin and tensin.

Supermature FAs have been suggested to be a suitable model for the fibronexus in vivo, which is characterized by the firm co-alignment of intracellular actin filament bundles with

<table>
<thead>
<tr>
<th>Table 1: Molecular composition of different cell-matrix adhesions.</th>
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<td><strong>Length in vitro</strong></td>
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<tr>
<td>ED-A FN</td>
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<td>α-SMA</td>
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<tr>
<td>cytoplasmic actin</td>
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<td>α5 integrin</td>
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<td>β1 integrin</td>
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<td>αv integrin</td>
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<td>β3 integrin</td>
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<td>vinculin</td>
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<td>paxillin</td>
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<tr>
<td>tensin</td>
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<tr>
<td>focal adhesion kinase</td>
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<td>P-tyrosine</td>
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<td>talin</td>
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References for this table: (9, 14, 21, 23, 27, 43, 49)
extracellular FN fibrils (Fig. 2) that are connected to collagen in the wound matrix (16). The molecular composition of the fibronexus is largely unknown; however, similar to supermature FAs it contains α-SMA in stress fibers (own unpublished results) and α5β1 integrin (52) (Table 1). It is conceivable that the complexity of the fibronexus exceeds that of its in vitro counterparts, in particular by containing a variety of different integrins. It was recently shown that α4β1 integrin specifically binds to ED-A FN in fibroblasts (53) and is up-regulated in correlation with α-SMA expression in visceral SMCs during embryogenesis (54). Moreover, TGFβ significantly influences the expression pattern of fibroblast integrins (55); correlating with myofibroblast differentiation, TGFβ up-regulates α5β1 integrin in corneal (56) and human lung fibroblasts (57, 58), α4 integrin in human lung fibroblasts (58), α8β1 integrin in cardiac fibroblasts, integrin subunits α5, αv and α6 in human peritoneal fibroblasts (59) and α2 integrin in dermal fibroblasts (60). Furthermore, a number of integrins have been shown to be important in promoting the contraction of fibroblast-populated three-dimensional collagen gels, including α1β1 (61), α2β1 (61-65), α11β1 (66) and αvβ3 integrin (64), suggesting their implication in the contraction of the collagen-rich ECM of granulation tissue.

Transmission of high myofibroblast contractile activity to the ECM during wound contraction seems to be the major purpose of the fibronexus (2); however no force measurements have been performed in vivo. In vitro, the force exerted by mature FAs (average area of ~6 μm²) was shown to be linearly proportional to their size with an average force of ~5.5 nN μm² as shown by analyzing local deformations in patterned silicone substrates (67) and quantifying the deflection of micro-posts by FAs of SMC grown on a bed of microneedles (68). Thus, based on the size of supermature FAs (average area > 6 μm²) (69) one may assume considerable forces exerted at the level of these AACMAs. The fibronexus has also been suggested to immobilize fibroblasts in the wound bed by providing strong attachment. It is generally accepted that migration of fibroblasts in vitro occurs at an intermediate attachment strength (70, 71), which is provided by immature (72, 73) or mature FAs (74); migration capacity decreases with increasing size and number of FAs formed on planar substrates (75). Similar observations have been made for fibroblast migration in collagen gels, which decreases with decreasing ECM compliance and increasing formation of AACMAs and stress fibers (41). The high attachment strength that is provided by supermature FAs (43) leads to an almost complete immobilization of myofibroblasts in culture, a process which is promoted by the expression of α-SMA in stress fibers (43, 76).

A less studied but nevertheless fundamental role of the fibronexus could be the perception and transduction of signals from the ECM, a function that is generally accepted for AACMAs of cultured fibroblasts (22, 77, 78). AACMAs act as mechano-receptors and promote specific cell responses to changes in ECM rigidity (24, 79), such as the increase of fibroblast contractile activity in response to an increased matrix load (80, 81), which can be attributed to distinct integrin subsets (82). Moreover, myofibroblast differentiation depends on the perception of high extracellular stress (6, 11) and on the engagement of integrins with specific proteins of the ECM. Binding of integrin α4β1 to ED-A FN is inhibited by an epitope-mapped ED-A antibody (53), which was previously shown to block ED-A FN-mediated expression of α-SMA (9), suggesting the implication of α4β1 integrin in myofibroblast differentiation. In contrast, integrins αvβ5 and αvβ3 seem to down-regulate α-SMA expression via binding to vitronectin (83). In addition, a variety of collagen receptors are involved in matrix remodeling by regulating the expression of specific matrix metallo-proteinases (84). Finally, integrins seem to be directly involved in the local activation of TGFβ; the latent form of TGFβ (L-TGFβ) and the L-TGFβ-binding protein (LTBP) have been shown to bind to ECM proteins (85, 86) and to specifically associate with vitronectin (87) and with FN in the ECM of cultured fibroblasts (88) and myofibroblasts (89). Major progress in the understanding of the mechanisms of TGFβ activation was recently made by reporting the specific binding of L-TGFβ to αvβ1 integrin and its activation by αvβ6 integrin in lung fibrosis (90), suggesting the recruitment of L-TGFβ to AACMAs of distinct cells. It will be of interest to investigate whether the mechanical state of the ECM may provide a signal for specific TGFβ targeting to sites of local stress as present in the fibronexus.

**Cell-cell adherens junctions**

The fibronexus of myofibroblasts is instrumental in changing the molecular and mechanical properties of the ECM by applying intracellular force; in turn these changes serve as extracellular cues that are perceived by the fibronexi of cells that are embedded in the same matrix. In addition, myofibroblasts in wound and fibrotic tissues interact in a direct fashion by forming gap junctions (13) and actin-associated cell-cell adherens junctions (AJs). Although AJs have been described in granulation tissue at the level of electron microscopy as dense plaques underlying the plasma membranes of contacting myofibroblasts (15, 52) (Fig. 3), virtually nothing is known about the mechanisms of their formation, molecular nature and function. Most studies on AJs have been performed using other cell types; in epithelial and endothelial cells they principally fulfill a barrier function (91, 92) and act as tumor-suppressors (93). In embryogenesis they help to sort out different cell types during tissue morphogenetic processes (94, 95) and they mediate the formation of specific synaptic contacts during neuronal development (96, 97). On the cytoplasmic side of AJs, actin filaments bind directly to α-catenin that is part of a complex consisting of the cytosolic proteins γ-catenin (plakoglobin), p120CTN and β-catenin; the latter binds to the cytoplasmic tail of transmembrane...
The cadherins of contacting cells interact in a Ca²⁺-dependent fashion through a series of homologous extracellular cadherin repeats (EC1-EC5) (95, 99). Depending on the presence or absence of the conserved HAV cell recognition sequence in the EC1 domain (100), one can classify type I (classical) and type II (atypical) cadherins (101). EC1 is important for the formation of lateral cadherin dimers within the plasma membrane (102), which is a crucial step for their activation (103). Point-mutations in the EC1 domains of different cadherins have been shown to change their binding characteristics in cell aggregation assays, demonstrating its importance for homophilic trans-binding (104); however, other domains seem also to be involved in this recognition process (105). In some cases heterophilic interactions have been described for type I cadherins, such as N- and E-cadherin of liver and lens cells (106) and of fibroblast and epithelial cell lines (107); they seem to occur frequently among type II cadherins (108).

N-cadherin is the first identified and is the most common cadherin in fibroblasts (109, 110); moreover a number of studies demonstrated the expression of type I cadherins N-, P-, and R-cadherin, of type II cadherins VE-, OB-cadherin and Fat, of the protocadherin PC43 and yet uncharacterized FIB1, FIB2 and FIB3 in fibroblastic cells of different origin (Table 2). So far, no studies have been performed to systematically correlate the expression of certain cadherins with specific fibroblast functions; it will be of particular interest to investigate differences in cadherin expression between fibroblasts and myofibroblasts, which are conceivable for several reasons: 1) differentiated myofibroblasts exhibit a number of features similar to SMCs (2), which may include the expression of SMC-characteristic cadherins, such as N-, R-, T-cadherin and cadherin 6B (111). 2) The major myofibroblast inducer TGFβ changes the mor-

Table 2: Cadherins expressed in fibroblastic cells.

<table>
<thead>
<tr>
<th>Cadherin</th>
<th>Fibroblast</th>
<th>Detection</th>
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<tbody>
<tr>
<td><strong>N-Cadherin</strong></td>
<td>chicken embryo (109, 135)</td>
<td>WB, IF</td>
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<tr>
<td></td>
<td>rat BICR (136)</td>
<td>NB, WB</td>
</tr>
<tr>
<td></td>
<td>human gingival (137)</td>
<td>WB, IF</td>
</tr>
<tr>
<td></td>
<td>mouse C3H10T1/2 (138)</td>
<td>NB, WB</td>
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<tr>
<td></td>
<td>rat RAT-1, human M19 (107)</td>
<td>IF</td>
</tr>
<tr>
<td></td>
<td>mouse 3T3 (124)</td>
<td>IF</td>
</tr>
<tr>
<td></td>
<td>human normal and tumor colon, human corneal (139)</td>
<td>IF, WB, PCR</td>
</tr>
<tr>
<td></td>
<td>human corneal myofibroblasts (114)</td>
<td>IF, WB</td>
</tr>
<tr>
<td><strong>P-Cadherin</strong></td>
<td>human gingival, 3Y1 (137)</td>
<td>WB</td>
</tr>
<tr>
<td></td>
<td>rat NRK (123)</td>
<td>IF</td>
</tr>
<tr>
<td></td>
<td>rat 3Y1 (140)</td>
<td>IF</td>
</tr>
<tr>
<td></td>
<td>human corneal (139)</td>
<td>PCR</td>
</tr>
<tr>
<td><strong>R-Cadherin</strong></td>
<td>mouse C3H10T1/2 (138)</td>
<td>NB</td>
</tr>
<tr>
<td><strong>VE-Cadherin</strong></td>
<td>human gingival (137)</td>
<td>WB</td>
</tr>
<tr>
<td>(cad-5)</td>
<td>rat NRK and mouse 3T3 (123)</td>
<td>IF</td>
</tr>
<tr>
<td><strong>OB Cadherin</strong></td>
<td>mouse C3H10T1/2 (138)</td>
<td>NB, WB, PCR</td>
</tr>
<tr>
<td>(cad-11)</td>
<td>mouse embryonic mesenchymal (141-143)</td>
<td>ISH</td>
</tr>
<tr>
<td><strong>Fat</strong></td>
<td>mouse 3T3 (144)</td>
<td>WB, NB</td>
</tr>
<tr>
<td></td>
<td>human dermal (145)</td>
<td>PCR</td>
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<tr>
<td></td>
<td>mouse mesenchymal (146)</td>
<td>ISH</td>
</tr>
<tr>
<td><strong>PC43</strong></td>
<td>human dermal (145)</td>
<td>PCR</td>
</tr>
<tr>
<td><strong>FIB1-FIB3</strong></td>
<td>human dermal (145)</td>
<td>PCR</td>
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IF, immunofluorescence; ISH, in situ hybridization; NB, Northern blotting; PCR, polymerase chain reaction; WB, Western blotting
phology of AJs in endothelial cells and disrupts the integrity of endothelial cell monolayers (112); in epithelial cells, TGFβ induces a fibroblastic phenotype by switching E-cadherin expression to N-cadherin, a process called epithelio-mesenchymal transition (113). 3) Compared with their normal counterpart corneal myofibroblasts exhibit higher levels of cadherin expression and more extensive AJs (114).

In culture, AJs of fibroblasts are generally thought to be transient and mediate “contact-inhibition of movement” (115) and “contact-inhibition of cell division” (116) but little is known about their function in vivo. In fact, under physiological conditions tissue fibroblasts are usually surrounded and separated by high amounts of ECM proteins and rarely come into contact; during wound healing and in pathological situations such as fibrotic reactions fibroblasts proliferate and thus increase the cell/ECM ratio. The most compelling hypothesis for the function of fibroblast AJs in vivo is their implication in connective tissue retraction by coupling the stress fibers of contacting myofibroblasts. The existence of such multicellular contractile units is supported by the observation that dermal fibroblasts communicate through gap junctions (117), which are strongly up-regulated during differentiation of wound granulation tissue myofibroblasts (13) and of cultured corneal myofibroblasts (118).

The first evidence for a functional role of AJs in synchronizing contraction was provided by pulling one of two contacting fibroblasts with the use of electromagnets that act on internalized magnetic beads; this force application at AJs was shown to provoke the influx of extracellular Ca²⁺ across the cell membrane (119). It is well accepted that an increase in cytoplasmic Ca²⁺ increases actin-myosin contraction but in contrast to SMCs, elevated Ca²⁺ alone is not sufficient to induce myofibroblast contractile activity that depends on Rho/ROCK signaling as demonstrated in myofibroblasts of stressed collagen gels (120) and in granulation tissue strips (J.J. Tomasek, personal communication). AJs have been demonstrated to play a role in the closure of small epidermal wounds of embryos and in confluent cell monolayers by orchestrating the contractile activity of wound margin cells in a “purse-string” mechanism (121, 122). However, the mechanisms of wound contraction in approximately two-dimensional epidermal cell sheets differ from those of three-dimensional connective tissue contraction by embedded fibroblasts. Such distinct mechanisms are reflected by morphological differences; AJs of cultured epithelial (E-cadherin) and endothelial cells (VE-cadherin) are belt-like and associate with circumferential actin filaments (Fig. 4A), whereas AJs of fibroblasts are point- or strand-like and are located at the termini of stress fibers (109, 123) (Fig. 4B). Myofibroblast AJs are larger compared to those of fibroblasts and are distributed all along the terminal portion of α-SMA-positive stress fibers (Fig. 4C). In order to form multicellular units that are able to contract the ECM of wound granulation tissue, myofibroblast AJs should resist considerable intracellular and extracellular forces. It is conceivable that the high intracellular force developed by myofibroblasts itself leads to the evolution of AJs into large and stable contacts, analogous to supermature FAs (43). Indeed, mechanical stimulation of AJs by the spontaneous contractile activity of contacting fibroblasts provokes a viscoelastic response in their cytosol as resulting from cytoskeletal reorganization (124). Moreover, actin-myosin based contractile activity was shown to reinforce AJs of fibroblasts and of epithelial cells (121, 125, 126). In order to optimally transmit their contractile force to the ECM, multicellular units should be relatively small; large myofibroblast aggregates could exert higher forces but their efficiency would be determined by the amount of contacts with the ECM as limited by the available surface area; relative to an increasing unit volume its surface area is decreasing. Myofibroblasts could use AJs to determine the number of equivalent partners in the same contractile unit either by recognizing the amount of homophilically engaged cadherins or by computing the magnitude of force applied to their cadherins. Thus, AJs could function as sensors for the mechanical forces exerted by the contractile activity of contacting cells, analogous to the mechanoperception of forces at the ECM level by FAs (24). Hence, AJs of myofibroblasts could fulfill a dual function in connective tissue retraction by providing mechanical coupling and outside-in signaling. Cadherin-mediated signaling in the highly cellularized granulation tissue of late wounds may also be of importance in reducing the contractile activity of myofibroblasts and/or in triggering their disappearance. Interestingly, long-term homophilic engagement of E-cadherin was shown to reduce Rho activity in cultured epithelial cells (127, 128), suggesting a role of cadherins in down-regulating cell contractile activity. Corneal myofibroblasts in dense culture significantly decrease the expression of α-SMA and de-differentiate into...
α-SMA-negative fibroblasts (129); this has been attributed to contact-induced desensitization to TGFβ (130). These observations apparently contradict the above discussed implication of cadherins in improving myofibroblast contraction and are in conflict with the suggested role of Rho/ROCK-mediated contractility in reinforcing AJs (131). However, the paradox may be solved by taking into account that different cadherins could mediate different functions and that additional signals, such as the mechanical state of the matrix or the presence of growth factors could interact with cadherin signaling and thus promote distinct cellular responses.

In the late stages of wound healing, when the epidermal layer has been re-formed, myofibroblasts disappear by massive apoptosis (12) but the signals leading to this disappearance are still unknown. The high density of myofibroblasts in late granulation tissue suggests a possible role for AJs in this process; involvement of cadherin-mediated signaling in cell survival has been demonstrated in several cell-types (128, 132). In MDCK epithelial cells, cadherin engagement leads to the activation of phosphoinositide 3-kinase and downstream phosphorylation of Akt/PKB, a signaling cascade that is known to play a role in apoptosis (133). Moreover, conditional inactivation of the E-cadherin gene in differentiating alveolar epithelial cells of the mouse mammary gland leads to apoptosis and necrosis, rendering certain cadherins as potential cell survival factors (134). It will be of interest to study, whether specific cadherins are involved in promoting myofibroblast survival and apoptosis.

Discussion

The contractile activity of myofibroblasts is a crucial factor for connective tissue remodeling during wound healing and creates a stressed matrix, which in turn promotes myofibroblast differentiation in a mechanical feedback mechanism. When a normal wound is closed, ECM fibrils are maximally organized and carry the mechanical load completely (2); at this stage myofibroblasts disappear by apoptosis. However, in many pathological situations, such as hypertrophic scar formation and fibrosis myofibroblasts persist; this results in continuous connective tissue retraction. The physiological mechanisms leading to the interruption of the vicious cycle of up-regulated intracellular contractile activity and increased extracellular tension are not clear at present. In this review we have discussed the role of actin-associated cell-cell and cell-matrix junctions in myofibroblast function. AACMAs provide the interface between ECM fibrils and intracellular actin fibers; they function to transmit intracellular force to the substrate and to detect the level of stress in the matrix. AJs could synchronize the contractile activity of several myofibroblasts and inform a given cell about the exerted force and the differentiation state of its partners. The increased formation of homotypic myofibroblast AJs in dense tissue and the loss of mechanical tension in the closed wound matrix are potential signals in order to terminate myofibroblast action and survival. Thus, cell-cell and cell-matrix junctions, as specific key players in the perception and transduction of these signals represent possible targets for a therapeutic strategy in order to influence connective tissue deformations.

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Abbreviations

AACMA - actin-associated cell-matrix adhesion
AJ - adherens junction
ECM - extracellular matrix
FA - focal adhesion
FN - fibronectin
L-TGF - latent transforming growth factor
ROCK - Rho-associated kinase
SMC - smooth muscle cell
TGF - transforming growth factor
α-SMA - α-smooth muscle actin

References


