Chapter 5
Role of caveolin-1 in fibrotic diseases

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Abstract

Fibrosis underlies the pathogenesis of numerous diseases and leads to severe damage of vital body organs and, frequently, to death. Better understanding of the mechanisms resulting in fibrosis is essential for developing appropriate treatment solutions and is therefore of upmost importance. Recent evidence suggests a significant antifibrotic potential of an integral membrane protein, caveolin-1. While caveolin-1 has been widely studied for its role in the regulation of cell signaling and endocytosis, its possible implication in fibrosis remains largely unclear. In this review we survey involvement of caveolin-1 in various cellular processes and highlight different aspects of its antifibrotic activity. We hypothesize that caveolin-1 conveys a homeostatic function in the process of fibrosis by (a) regulating TGFβ1 and its downstream signaling; (b) regulating critical cellular processes involved in tissue repair, such as migration, adhesion and cellular response to mechanical stress; and (c) antagonizing profibrotic processes, such as proliferation. Finally, we consider this homeostatic function of caveolin-1 as a possible novel approach in treatment of fibroproliferative diseases.
Introduction

Fibrosis is commonly considered to be a result of deregulated tissue repair (Tomasek et al., 2002; Wynn, 2007). It is a pathological condition, where processes normally occurring during tissue repair, such as formation of connective tissue and wound contracture, persist and ultimately lead to disruption of normal tissue structure and organ dysfunction. The mechanisms underlying fibrosis are still largely unclear. It is commonly thought that the excess of transforming growth factor (TGFβ1) or/and the increased susceptibility of fibroblasts to it are possible causes of persistency of the tissue remodeling process (Serini and Gabbiani, 1996; Vaughan et al., 2000). As a consequence, fibroblasts express α-smooth muscle actin (αSMA) when differentiating into myofibroblasts. This results in a highly contractile cell type responsible for increased tissue contraction and production of extracellular matrix (ECM) components in tissue repair and fibrosis (Hinz, 2007; Hinz et al., 2012). However, another critical aspect in tissue repair is correct bidirectional signaling between fibroblasts and the ECM (Eckes et al., 2010; Tomasek et al., 2002; Wight and Potter-Perigo, 2011). Fibroblasts must be able to properly sense mechanical and compositional changes of their surrounding environment in order to remodel it accordingly and seize their activity when it is no longer required. Cellular functions vital in tissue remodeling, such as directional motility, ECM turnover and deposition, expression of αSMA and apoptosis, are carried out in accordance to the composition and mechanical properties of the surrounding ECM. There are a number of complex signaling pathways underlying these physiological events and it is important that these are well coordinated. Deregulation on any level may result in a loss of homeostasis and disproportionate deposition of ECM components and excessive tissue contraction, leading to fibrosis.

Caveolin-1 could play a role in monitoring this homeostasis by connecting various cell signaling events in tissue repair. Caveolin-1 is an integral membrane protein, which is increasingly acknowledged for its physiological importance in tissue repair and fibrosis. It is a part of the plasma membrane lipid rafts called caveolae, and plays a major role in signal transduction by organizing and coordinating various signaling events at restricted sites of the plasma membrane (Okamoto et al., 1998; Razani and Lisanti, 2001). Caveolin-1 is the main member of caveolin protein family and is essential for the formation of caveolae (Figure 1) (Parat, 2009; Williams and Lisanti, 2004). Other members include caveolin-2 and -3. Caveolin-2 is generally found associated with caveolin-1 and is thought to serve as an accessory protein for correct caveola formation. The two are ubiquitously found in different types of mammalian cells, whereas caveolin-3 appears to be restricted to muscular tissue. Caveolin-1 is implicated in numerous cellular processes, such as endocytosis, directional cell motility and cell cycle regulation (Parat, 2009; Patel et al., 2008). A number of reports have suggested its fibrosuppressive activity and shown its implication in several aspects of tissue remodeling (Del Galdo et al., 2008; Tourkina et al., 2008; Wang et al., 2006; Zhang et al.,
2011). Its therapeutic potential in fibrotic diseases is currently being considered. Knowledge of mechanisms by which caveolin-1 is regulating the tissue reparative processes is important for the understanding of fibrosis and the development of its treatment.

![Diagram of caveolin-1 in the plasma membrane]

**Figure 1: Location of caveolin-1 in the plasma membrane**

Caveolin-1 has an atypical topology for integral membrane protein: shaped as a hairpin, the C-terminus and N-terminus of caveolin-1 are both facing the cytoplasm and connected by a membrane-embedded hydrophobic domain. Caveolin-1 forms homo-oligomers through the oligomerization domain located at the N-terminal region. Caveolin scaffolding domain (CSD) is a part of the oligomerization domain. Via its CSD caveolin can interact with, and usually inhibit, a large number of signaling molecules, all of which possess the caveolin binding sequence motif. Examples of these signaling molecules are the TGFβ-receptor, Rho GTPases, Src, the epidermal growth factor receptor (EGFR) and β-integrins.
“Caveolin signaling hypothesis” and phospho-caveolin

The critical functional part of the caveolin-1 molecule is the caveolin scaffolding domain (CSD) located at its N-terminal region (Figure 1). Via this domain, caveolin-1 can interact with and inhibit a large number of signaling molecules that possess the caveolin-binding sequence motif. A few examples are the TGFβ-receptor, Rho GTPases, Src, the epidermal growth factor receptor (EGFR) and β-integrins (Okamoto et al., 1998; Razani and Lisanti, 2001).

Caveolin-1 can regulate signaling of associated molecules in three ways: (a) by direct interaction with the signaling molecule; (b) by compartmentalization of signaling platforms in restricted domains of the plasma membrane; or (c) by modulating signaling protein activation by caveolar endocytosis (Figure 2). Collectively, these phenomena are termed the “spatiotemporal regulation concept” or, alternatively “caveolin signaling hypothesis” (Okamoto et al., 1998; Parat, 2009).

Another noteworthy structural detail of caveolin-1 is the tyrosine-14 residue (Y14). Caveolin-1 gets phosphorylated on Y14 by small kinases, such as Src and Fyn (Grande-Garcia et al., 2007; Sanguinetti et al., 2003). This particular phosphorylated form, termed phospho-caveolin (pY14-Cav1), is implicated in cellular processes like cell motility and adhesion (see below) (Grande-Garcia and del Pozo, 2008; Grande-Garcia et al., 2007).

Figure 2. Spatiotemporal regulation of signaling molecules by caveolin-1 is achieved by A) Direct interaction with the signaling molecule, usually leading to inhibition of signaling; B) Compartmentalization of signaling platforms in caveolae; and C) Modulating signaling protein activation by caveolar endocytosis. Figure from Parat, 2009. Reprinted with permission from Elsevier.
**Role of caveolin-1 in fibrosis**

The relevance of caveolin-1 in fibrotic diseases has already been recognized in early studies with animal models. In radiation-induced lung fibrosis in rats and mini-pigs a downregulation of caveolin-1 was observed in lung epithelial cells prior to induction of fibrosis (Kasper et al., 1998). More recently, this downregulation of caveolin-1 was also seen in bleomycin-induced lung fibrosis (Tourkina et al., 2008; Wang et al., 2006). Consistently, caveolin-1 knock-out mice have revealed impaired wound healing and profound alterations in lung morphology, such as reduced alveolar spaces, increased wall thickening, fibrosis, and hypercellularity (Drab et al., 2001; Le Lay and Kurzchalia, 2005; Le Saux et al., 2008; Razani et al., 2001a; Wang et al., 2006; Zhang et al., 2011). More recently, a marked decrease of caveolin-1 has been reported in affected tissues in distinct human fibrotic diseases, such as idiopathic pulmonary fibrosis (IPF) (Wang et al., 2006), scleroderma (Tourkina et al., 2008), cardiac fibrosis (Miyasato et al., 2011), keloid scars (Zhang et al., 2011), and systemic sclerosis (Del Galdo et al., 2008). Moreover, reintroduction of caveolin-1 by various means has been shown to dramatically reduce fibrotic changes. Similarly, pretreatment with CSD peptide or caveolin-1 adenovirus was shown to prevent characteristic changes induced by fibrotic agents like bleomycin or TGFβ1 (Tourkina et al., 2008; Wang et al., 2010; Zhang et al., 2011).

In the literature, several mechanisms have been proposed by which caveolin-1 could exert its antifibrotic activity. However, a number of physiological properties of caveolin-1 have been described in different fields of research, which could also add to understanding of its fibrosuppressive character, but have not yet been reviewed in the light of fibrosis. This review will cover major relevant pathways in which caveolin-1 plays a role and outline its general mechanism of action in tissue repair and fibrosis.

1. **TGFβ1 signaling**

A number of studies have investigated the impact of caveolin-1 on TGFβ1 signaling in fibrosis. In distinct fibrotic diseases, such as scleroderma, IPF, keloids, cardiac fibrosis and systemic sclerosis, caveolin-1 was shown to be downregulated, while TGFβ1 signaling was enhanced (Del Galdo et al., 2008; Miyasato et al., 2011; Tourkina et al., 2008; Wang et al., 2006; Zhang et al., 2011). A similar negative correlation between TGFβ1 and caveolin-1 was observed in caveolin 1−/− mice and in the bleomycin induced fibrosis model in mice (Miyasato et al., 2011; Tourkina et al., 2008; Wang et al., 2006). In all cases, reintroduction of caveolin-1 attenuated the effects of TGFβ1 signaling. Caveolin-1 was reported to cause a decrease of type I collagen and fibronectin, and an increase of MMP mRNA expression. Furthermore, when introduced in the cells from healthy or affected donors, caveolin-1 has been reported to attenuate TGFβ1 signaling and reduce its subsequent effects. Conversely,
down-regulation of caveolin-1 increases TGFβ₁ signaling. The four pathways by which caveolin-1 is thought to interfere with TGFβ₁ are the following:

- Interaction with TGFβ type I receptor and prevention of Smad2 phosphorylation (1.1)
- Modulation of TGFβ type I/II receptor gene expression (1.2)
- Mediation of receptor complex turnover by internalization (1.3)
- Interference with latent TGFβ activation through regulation of MMPs and integrins (1.4).

1.1 Interaction with TGFβ type I receptor and prevention of Smad2 phosphorylation

Already a decade ago the first observations were made that caveolin-1 attenuates TGFβ₁ signaling by directly interacting with the TGFβ-receptor. In a study by Razani et al., the authors discovered that TGFβ type I receptor (TGFβRI) and its downstream effector SMAD-2 co-fractionated with caveolin-1 in caveolae enriched membrane domains of NIH-3T3 fibroblasts and that the receptor directly interacted with caveolin-1 via CSD (Razani et al., 2001b). This interaction led to sequestration of TGFβRI and prevented baseline SMAD phosphorylation. It was shown that full-length caveolin-1, but not the mutant containing a deletion in the CSD region, was able to inhibit constitutively active TGFβRI signaling, and that caveolin-1 interaction with TGFβRI was increased with the receptor stimulation. The authors stated that a negative regulation of TGFβ₁ by caveolin-1 was not cell-specific and they were also able to observe it in COS cell line. Consistently, other studies have also reported a negative regulation of TGFβ₁ signaling by caveolin-1 in different cell types, such as urethral smooth muscle and epithelial cells, leucocytes and hepatocytes (Chen et al., 2007; Mayoral et al., 2010; Stehr et al., 2004; Tourkina et al., 2010).

1.2 Modulation of TGFβ type I/II receptor gene expression

Besides direct inhibitory interaction, there is evidence that caveolin-1 regulates TGFβ-receptor gene expression. Lee et al. have shown that transfection of NIH3T3 fibroblasts with caveolin-1 led to a significant decrease of TGFβ type II receptor (TGFβRII) gene expression, while endogenous inhibition of caveolin-1 gene by siRNA had the opposite effect (Lee et al., 2007). By transfecting cells with serial deletion mutants of the TGFβRII promoter linked to a reporter gene, the authors identified two positive regulatory elements, PRE 1 and PRE 2. These elements were required for repression of TGFβRII by caveolin-1. Based on further investigations, it was suggested that caveolin-1 modulates binding activities of distinct unknown transcription factors bound to either PRE1 or PRE2. To our knowledge, no follow-up studies elucidating the nature of the transcription factors have been undertaken.

1.3 Mediation of receptor complex turnover by internalization

In a third mechanism, caveolin-1 can modulate signaling of the TGFβ-receptor activity by endocytic regulation. It is thought that TGFβ-receptors can be internalized via conventional
clathrin-coated endocytosis, as well as via caveolin-1-mediated lipid raft endocytosis (Chen, 2009; Di Guglielmo et al., 2003; Le Roy and Wrana, 2005). Clathrin-mediated endocytosis has been shown to enhance TGFβ-induced SMAD activation and subsequent transcriptional responses. Early endosomes with TGFβ-receptor-ligand complex function as a signaling organelle, recruiting downstream signaling elements such as SMAD2, and thus promote TGFβ signaling. Caveolin-1-mediated internalization on the other hand, promotes proteasomal degradation of the receptor through recruiting SMURF/SMAD7 TGFβ-inhibitory complex, resulting in a reduced TGFβ-receptor activity.

Interestingly, a number of signaling molecules take advantage of the alternative endocytic pathways by modulating TGFβ-receptor distribution in raft vs. non-raft membrane microdomains, and thus exerting pro- or anti-fibrotic effects. Examples are IL-6 and ADAM-12, which shift receptors to the non-raft fractions, thus enhancing TGFβ signaling (Seals and Courtneidge, 2003; Zhang et al., 2005). Heparin sulfate, hyaluronan-CD44, and CD109 have the opposite effect, since they promote TGFβ-receptor localization to the caveolae, thereby facilitating TGFβ-receptor endocytosis via the caveolar pathway and leading to proteasomal degradation of TGFβ-receptors (Bizet et al., 2011; Chen et al., 2006; Ito et al., 2004).

1.4 Latent TGFβ1 activation

Finally, caveolin-1 could also interfere with TGFβ1 signaling indirectly, through regulation of integrins and MMPs. Integrins and MMPs are involved in activation of latent TGFβ complex, which plays an important role in pathogenesis of fibrosis and myofibroblast differentiation (Annes et al., 2003; Margadant and Sonnenberg, 2010). Caveolin-1 has been described to regulate the membrane availability of MT1-MMP (Kim and Chung, 2008; Remacle et al., 2003), which has been implicated in latent TGFβ1 activation in several cell types (Karsdal et al., 2002; Mu et al., 2002; Tatti et al., 2008). Furthermore, caveolin-1 regulates the availability of integrins by endocytosis, and is in general implicated in integrin signaling, as described below in this review. Altogether, these findings suggest that caveolin-1 might have an important effect on TGFβ-integrin crosstalk and TGFβ1 activation. However, the link between caveolin-1- mediated regulation of MMPs and TGFβ1 remains to be experimentally addressed.

Thus, caveolin-1 can modulate TGFβ1 signaling by direct inhibition of the receptor, inhibition of the receptor gene expression, termination of TGFβ signaling by endocytosis, and possibly by affecting the activation of latent TGFβ. The relative importance of the described mechanisms for fibrosuppressive activity of caveolin-1, to our knowledge has not been addressed. Notably however, in most studies CSD peptide alone was sufficient to inhibit fibrotic effects of TGFβ signaling. This implies that the direct inhibitory interaction via CSD peptide with TGFβ-receptor or its downstream signaling pathways is probably the strongest contributing factor to anti-fibrotic properties of caveolin-1.
2. Caveolin-1 in cell matrix interaction

Besides exerting antifibrotic properties by regulating TGFβ1 and its downstream signaling, caveolin-1 is essential for normal tissue remodeling due to its critical role in cell adhesion and directional migration. Caveolin-1, specifically its Tyr 14 phosphorylated form (pY14-Cav1), is implicated in cell adhesion, polarity and directional migration mainly through its association with integrins and members of the Rho family of small GTPases, such as Rho, Rac, and Cdc42. These, in turn, play a major role in coordination of cytoskeletal rearrangement and formation and maturation of focal adhesions (Grande-Garcia and del Pozo, 2008; Grande-Garcia et al., 2007; Li et al., 2005; Nethe and Hordijk, 2011). Molecular mechanisms of caveolin-1-regulated adhesion and migration have been extensively reviewed elsewhere (Echarri et al., 2007; Grande-Garcia and del Pozo, 2008). Below we focus on the importance of caveolin-1 in fibrosis through its role in these processes.

2.1 Caveolin-1 in fibroblast adhesion

Proper fibroblast adhesion is essential for normal tissue reparative processes. The cells have to migrate to the site of damage, induce contraction of the tissue, and start to repair it. Focal adhesions play a principle role in force transmission during fibrosis and are a critical site of cell-ECM contact. They function as platforms for signal transduction in adherent cells. Their size, maturation and adhesive strength are thought to influence myofibroblast differentiation and levels of tissue contracture (Goffin et al., 2006; Hinz, 2006, 2007; Hinz and Gabbiani, 2003). Phospho-caveolin (pY14-Cav1) has been identified as a part of focal adhesions, co-localizing with β-integrins and focal adhesion kinase at adhesion sites (del Pozo et al., 2005; Gaus et al., 2006; Wary et al., 1998). The importance of caveolin-1 in focal adhesions has been demonstrated in caveolin-1−/− mouse embryonic fibroblasts. These cells, when plated on a fibronectin-coated surface, have an altered formation of focal adhesions. They were reported to be smaller, more abundant and immature, and distributed all over the ventral surface of the cells (Grande-Garcia et al., 2007).

The connection between caveolin-1 and integrins within focal adhesions has important physiological implications in anchorage-dependent cells. Loss of integrin-mediated adhesion stimulates caveolin-1-mediated internalization of rafts from the plasma membrane and their transport to the recycling endosomes, leading to dramatically decreased order of lipids in the plasma membrane, i.e. increased membrane fluidity (Echarri and Del Pozo, 2006; Echarri et al., 2007; Gaus et al., 2006; Norambuena and Schwartz, 2011). Changes in physical states of plasma membrane have implications for signaling. In a study using the fluorescent probe Laurdan and two-photon microscopy, Gaus et al. have shown that membrane order is highest in focal adhesions and exceeds even that in the lipid rafts. High membrane order in focal adhesions was dependent on membrane cholesterol, integrin engagement and pY14-Cav1 (Gaus et al., 2006). The authors hypothesized that the
ordered state within focal adhesions may have important consequences for signaling at these sites.

In addition, a number of studies suggest that pY14-Cav1 is necessary for regulation and stabilization of focal adhesion kinase (FAK) exchange in focal adhesions, enabling focal adhesion-mediated signaling and proper maturation of the adhesion sites (Goetz, 2009; Goetz et al., 2008; Nethe and Hordijk, 2011). Furthermore, in senescent fibroblasts, downregulation of caveolin-1 was reported to cause FAK inactivation, leading to disruption of focal adhesions and actin stress fibers (Cho et al., 2004).

Thus, caveolin-1 is critical for correct formation of cell-ECM adhesion complexes and normal signal transduction at the adhesion sites, via its role in membrane order and FAK regulation.

2.2 Caveolin-1 in fibroblast migration

Directional migration of fibroblasts to the site of damage is necessary for tissue remodeling. Caveolin-1 is known to be essential for directional cell migration in both planar surfaces and 3D structures (Grande-Garcia and del Pozo, 2008; Parat et al., 2003). pY14-Cav1 was shown to be indispensable for persistency of directional migration - in caveolin-1−/− MEFs directional and persistent migration seems to be completely abolished. This can be restored by re-expression of WT caveolin-1, but not with caveolin-1 containing a mutated phosphorylation site (Goetz et al., 2011; Grande-Garcia et al., 2007).

In addition to the above-mentioned mechanism of cell adhesion involving Rho and Rac GTPases, caveolin-1 is thought to regulate adhesion through integrin internalization (Bass et al., 2011; Vassilieva et al., 2008). The trans-membrane proteoglycan syndecan-4, which functions as a fibronectin co-receptor, was reported to attenuate adhesive strength in migrating fibroblasts by rapid triggering of caveolin-1-mediated α5β1-integrin endocytosis (Bass et al., 2011). The process was shown to be RhoG dependent. The authors underlined the importance of this mechanism for recognition of the change in ECM composition by fibroblasts following injury and subsequent increase in cell motility in response to local tissue damage. Thus, caveolin-1 regulates the strength of formation and disassembly of cell-ECM contact during migration.

2.3 Caveolin-1 in ECM remodeling and turnover

The importance of caveolin-1 in tissue remodeling has been demonstrated in caveolin-1−/− mice, which, among other changes, reveal impaired wound healing, altered lung mechanics with decreased lung compliance, as well as increased collagen deposition in the pulmonary tissue (Drab et al., 2001; Le Saux et al., 2008; Razani et al., 2001a). This characteristic type of tissue architecture in lungs of caveolin-1−/− mice is believed to be a general effect, attributed to an altered response of fibroblasts to TGFβ1, high vascularity, hypercellularity and other factors caused by caveolin-1 deletion.
Furthermore, caveolin-1 has also been shown to influence properties of the ECM specifically through its association with Rho GTPases and regulation of directional migration in fibroblasts. A recent study compared remodeling capabilities of caveolin-1^{-/-} versus wild type MEFs in vitro, using fibroblast-derived 3D matrices, which mimic the physiological environment (Goetz et al., 2011). The study concluded that through regulation of Rho GTPases (Rho and p190RhoGAP) caveolin-1 favored fibroblast elongation, directional migration and promoted force-dependent contraction of the matrices. The matrices remodeled by caveolin-1^{-/-} MEFs revealed disturbed collagen fiber organization and increased compliance as opposed to WT MEF-remodeled counterparts. The effect of caveolin-1 on the remodeling capability of fibroblasts required phosphorylation of the caveolin-1 Y14 residue. Re-expression of caveolin-1, but not its non-phosphorylatable mutant, was shown to rescue matrix-remodeling capability of caveolin-1^{-/-} MEFs. Thus, pY14-Cav1 was shown to be essential for proper remodeling of ECM due to its role in establishing adequate ECM structure and stiffness, and normal functioning of fibroblasts. It is interesting to note that the matrix stiffening capacities of caveolin-1 are in contrast with its antifibrotic properties.

In addition, caveolar endocytosis of α5β1-integrin has been proposed to contribute to ECM turnover, and hence influence ECM composition and further remodeling (Shi et al., 2010; Shi and Sottile, 2008; Sottile and Chandler, 2005). Shi et al. emphasized the importance of internalization and intracellular degradation of ECM proteins in lysosomes as an alternative pathway to extracellular degradation by MMPs, as previously shown by others (Everts et al., 1996). Shi et al. demonstrated that fibronectin matrix turnover to a large extent occurs through receptor-mediated endocytosis, followed by lysosomal degradation of fibronectin. Using fibronectin null myofibroblasts, Shi et al. identified β-integrins, including α5β1, as fibronectin matrix endocytic receptors and reported that caveolin-1 constitutively regulates α5β1-integrin endocytosis (Shi and Sottile, 2008). They showed in their model system, that siRNA against caveolin-1 inhibits α5β1-integrin endocytosis and fibronectin matrix turnover. In follow-up studies, Shi et al. have further emphasized the importance of caveolin-1-mediated fibronectin turnover in fibronectin-directed type I collagen deposition, i.e. deposition of type I collagen caused by fibronectin polymerization (Shi et al., 2010). Furthermore, the authors demonstrated that caveolin-1 inhibition caused decrease in type I collagen endocytosis and degradation.

In summary, caveolin-1 substantially influences ECM remodeling processes by regulation of adhesion and migration, and by regulation of integrin and fibronectin endocytosis. In addition, it has a general influence on overall tissue architecture due to its implication in numerous physiological processes, such as regulation of growth factor signaling, tissuevascularization, cell proliferation and others.
3. Caveolin-1 in mechanical tension and cell stretching

Cells are constantly exposed to mechanical tension. Induction of cellular signaling in response to mechanical stimuli (i.e. mechanotransduction) is of upmost importance in tissue repair processes, where mechanical properties of the surrounding ECM are constantly changing (Arora et al., 1999; Tomasek et al., 2002; Wight and Potter-Perigo, 2011). Cyclic stretch is known to play an important role in myofibroblast differentiation and responsiveness of the cells to profibrotic growth factors (Blaauboer et al., 2011).

Mechanotransductive properties of caveolin-1 are mainly attributed to its association with the actin cytoskeleton, integrins and small kinases (Parat, 2009; Parton and Simons, 2007; Radel and Rizzo, 2005). The role of caveolin-1 in cytoskeletal changes induced by shear stress in endothelial cells has been best studied, but falls beyond the scope of this review (Parat, 2009; Patel et al., 2008; Yu et al., 2006).

In addition, recent studies have demonstrated a purely mechanistic role of caveolae and caveolin-1 in cellular response to stretch (Gervasio et al., 2011; Sinha et al., 2011). Sinha et al. have challenged the homeostasis of the plasma membrane tension with different types of controlled mechanical stress (e.g. hypo-osmotic shock, tether pulling technique) and observed that cells respond to acute mechanical membrane stress by a rapid flattening of caveolae, in a passive process involving membrane mechanics and independent of the actin cytoskeleton or ATP (Sinha et al., 2011). Using the tether pulling technique, the authors came to the conclusion that caveolae are required for buffering the membrane tension in all cell types they examined (fibroblasts, HeLa, myoblasts) and proposed caveolae flattening as a physiological mechanism by which cells can respond immediately to sudden variations in membrane tension induced by acute mechanical stress.

As mentioned above, caveolae cannot be formed in absence of caveolin-1 and downregulation of caveolin-1 could lead to reduction in the number of caveolae. The absence or reduction of this “membrane tension buffering” mechanism provided by caveolae could contribute to fibrosis by increasing responsiveness of the cells to mechanical tension, which is known to play a crucial role in myofibroblast differentiation and modulation of their contractile activity (Hinz et al., 2001; Hinz et al., 2007). In addition, absence of this mechanism could alter mechanical properties of the cell itself, leading to modified perception of the surrounding matrix, i.e. mechanotransduction.

4. Caveolin-1 in cell proliferation

An increased cell proliferation is a common problem in various fibrotic diseases (Huang and Ogawa, 2012; Picardo and Khan, 2012; Xia et al., 2008). One of the reasons for uncontrolled tissue remodeling and excessive connective tissue formation is the persistence of fibroblasts and myofibroblasts in fibrotic tissue (Hardie et al., 2009; Tomasek et al., 2002;
Wight and Potter-Perigo, 2011). Caveolin-1 has been implicated in cell cycle regulation, cellular senescence and apoptotic signaling (Cho et al., 2004; Ohno-Iwashita et al., 2010; Wheaton, 2011). These regulatory abilities made it an extensive research topic in the cancer field: caveolin-1 is downregulated in cells evading apoptosis and undergoing uncontrolled proliferation. Furthermore, hypercellularity is a major phenotypic feature of caveolin-1−/− mice, and MEFs derived from knockout mice display excessive proliferation (Le Lay and Kurzchalia, 2005; Mercier et al., 2009; Razani et al., 2001a).

The overexpression of caveolin-1 is known to inhibit cell proliferation by blocking the progression through the G1 phase and to induce premature cellular senescence in primary cultures of murine fibroblasts (Volonte et al., 2002). In senescent fibroblasts caveolin-1 has been shown to be upregulated, determining the characteristic morphology of the cells, i.e. increased levels of focal adhesions, lamellipodia, and filopodia. Notably, knockdown of caveolin-1 in senescent fibroblasts was shown to reverse these morphological changes (Cho et al., 2004). The mechanism of caveolin-1-controlled cell senescence was proposed relatively recently and is thought to involve sequestration of Mdm2, the negative regulator of p53, into caveolar membranes (Bai et al., 2011; Volonte and Galbiati, 2011; Wheaton, 2011).

An additional mechanism of caveolin-1-controlled fibroblast proliferation was proposed by Xia et al. (Xia et al., 2010). These and other authors have previously found that polymerized type I collagen has a negative effect on proliferation of fibroblasts (Rhudy and McPherson, 1988; Schor, 1980; Xia et al., 2008). In their recent study Xia et al. (2010) demonstrated that downregulation of caveolin-1 in patients with IPF correlates with pathological activation of PI3K/Akt due to a decrease in phosphatase activity of PTEN, and this enables fibroblasts in IPF to escape the suppressive effect of polymerized collagen on proliferation. The authors showed that re-expression of caveolin-1 restored PTEN activity resulting in reduced AKT phosphorylation, and thus suppressed abnormal proliferation in fibrosis.

In addition, several indirect mechanisms of caveolin-1-controlled cell proliferation include regulation of anchorage dependent growth, inhibition of MAP kinase and negative regulation of epidermal growth factor signaling, which have been described in detail elsewhere (Cerezo et al., 2009; Engelman et al., 1998; Norambuena and Schwartz, 2011; Ohno-Iwashita et al., 2010; Park and Han, 2009; Wary et al., 1998).

Thus, the hyperproliferative phenotype, as well as the delayed apoptosis of fibroblasts seen in fibrotic conditions, correlate with and could, in part, be attributed to downregulation of caveolin-1.
5. Caveolin-1 in myofibroblast differentiation – a future research perspective

As mentioned earlier, persistency of the myofibroblast phenotype is a hallmark of fibroproliferative diseases. Elucidating the causes of excessive αSMA expression in fibrosis could offer a solution to treatment of fibroproliferative diseases and thus remains a top interest in fibrosis research. Unfortunately, studies on the connection between caveolin-1 and myofibroblast differentiation are still scarce. Tourkina et al. have assessed the influence of the CSD peptide on αSMA expression by fibroblasts from healthy donors and scleroderma patients, which were shown to have decreased levels of caveolin (Tourkina et al., 2008). Introduction of the peptide or overexpression of caveolin-1 adenovirus resulted in inhibition of αSMA expression in affected, but not in the healthy cells. Conversely, knockdown of caveolin-1 by siRNA increased αSMA expression in healthy fibroblasts, but not in scleroderma-derived counterparts. This was attributed to involvement of distinct signaling pathways regulating αSMA expression in healthy versus affected fibroblasts, however the exact mechanisms still remain to be elucidated. Similarly, Wang et al. reported inhibition by caveolin-1 of TGFβ1-induced αSMA expression in human pulmonary fibroblasts (Wang et al., 2006).

Overall, it is likely that caveolin-1 can antagonize αSMA expression by inhibiting TGFβ1 signaling. However, it is interesting that downregulation of caveolin-1 also compromises cell adhesion, mechanotransduction, and cell contractility, all of which are pivotal for myofibroblast differentiation. In fibrosis, where caveolin-1 is downregulated, fibroblasts will exhibit impaired contractility, adhesion and mechanotransduction, while their surrounding ECM will have reduced stiffness. These conditions are far from favorable for myofibroblast differentiation (Tomasek et al., 2002), however fibroblasts in fibrosis still excessively express αSMA. This suggests that in fibroproliferative diseases fibroblasts are different from the healthy fibroblasts and employ additional mechanisms for αSMA expression. In addition, findings of Tourkina et al. that caveolin-1 differentially regulates αSMA expression in normal and affected fibroblasts also suggest involvement of distinct mechanisms of myofibroblast differentiation in healthy vs. fibrotic conditions, as proposed by the authors (Tourkina et al., 2008). Elucidating these distinct mechanisms might be beneficial for development of fibrosis treatment. Studies on the role of caveolin-1 in myofibroblast differentiation could contribute to distinguishing pathological myofibroblast differentiation from the normal physiological process.
Conclusion

Caveolin-1 is essential for a proper execution of critical processes in tissue repair, such as adhesion, migration and buffering of mechanical tension. In addition, it inhibits major profibrotic signaling pathways and antagonizes potentially profibrotic physiological events, such as cell proliferation. Figure 3 depicts an overview of physiological events and signaling pathways relevant in fibrosis and tissue repair, which involve caveolin-1.

Figure 3: Overview of different roles of caveolin-1 in fibrotic diseases.
Caveolin-1 is essential for execution of critical processes in tissue repair, such as migration, cell adhesion and ECM turnover due to its association with Rho GTPases and integrins. In addition, caveolin-1 has antagonizing activity on processes which favor development of fibrosis. Namely, caveolin-1 regulates membrane tension through rapid disassembly of caveolae, and antagonizes cell proliferation through its involvement in cell cycle regulation and by facilitating PTEN phosphatase activity. Finally, caveolin-1 inhibits TGFβ₁ on four different levels, as depicted in the scheme. Ellipse on the graph denotes positive regulation, while rectangular frame stands for inhibitory activity.

It is important to emphasize that the antifibrotic property of caveolin-1 should be regarded as a collective, homeostatic function, rather than as a result of inhibition of a particular profibrotic pathway. This underlines the therapeutic potential of caveolin-1 in fibrotic diseases: given its physiological function, caveolin-1 could serve as a neutralizing agent of
fibrotic signaling, instead of selectively blocking a particular profibrotic signaling pathway, which has proven to be difficult and ineffective (Gauglitz et al., 2011; Lafyatis, 2006).

A principal objective for the cells in tissue remodeling is to adequately react to a rapidly changing environment; hence the main challenge in fibrosis is the exact timing, coordination and regulation of intensity of various signaling processes. The plasma membrane can be considered as a forefront for reception and transduction of these signaling processes and it is crucial that the dynamics at the membrane are not lagging behind the changes in ECM composition or cytokine concentration. This puts caveolin-1, as an integral membrane protein, at the ideal location to perform its function of coordinating and neutralizing cellular signaling processes at the plasma membrane.

The CSD peptide could be a promising means for treatment of fibrosis, since it does not require adenoviral transfection. However, so far this has not been tested sufficiently for clinical application. Research as reviewed in this paper will result in more insight in the mechanisms of caveolin-1 involvement in fibrotic processes and bring us closer to the possible use of CSD peptide as an anti-fibrosis therapy.

Thus, caveolin-1 has a pivotal role in regulating several processes involved in tissue remodeling, allowing it to safeguard tissue homeostasis and prevent fibrosis.
References


