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SCLERODERMA (J VARGA, SECTION EDITOR)

The Primary Cilium: Emerging Role as a Key Player in Fibrosis

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Abstract

Purpose of Review The myofibroblast is the culprit in the pathogenesis of fibrosis in systemic sclerosis (SSc). Activation of morphogen signaling pathways has been shown to be critically involved in organ fibrosis. Remarkably, the cellular receptors and key molecules from these signaling pathways are localized in the primary cilium. The primary cilium is a unique cellular organelle present in virtually all cells. This article summarizes recent studies evaluating the association between primary cilia and morphogen signaling driving myofibroblast transition and subsequent fibrosis.

Recent Findings Emerging observations implicate dysfunctional primary cilia in fibrosis in many different tissues and organs. Primary cilia seem to be necessary for the initiation of the transition and sustained activation of myofibroblasts.

Summary We summarize recent progress in this field and propose the primary cilium as a potential mediator of fibrosis pathogenesis in SSc. Understanding the contributions of primary cilia in fibrosis may ultimately inform the development of entirely new approaches for fibrosis prevention and treatment.

Keywords Primary cilia · Ciliopathies · Myofibroblasts · Systemic sclerosis

Introduction

Systemic sclerosis (SSc) is a complex and clinically heterogeneous disease associated with fibrosis in multiple organs [1]. Genetics makes only a modest contribution to disease susceptibility (4.7%). GWAS studies have shown that the majority of the susceptibility loci for SSc are genes associated with the immune system, apoptosis-related genes, and fibrotic-related genes [2]. Environmental and occupational factors also make a major contribution to the disease and can lead to modifications at the epigenetic level. Thus, a significant effort has been made in determining DNA methylation and histone modifications underlying pathogenic alterations of gene expression in SSc [3].

The myofibroblast, a highly contractile mesenchymal cell, plays a central role in the pathogenesis of fibrosis in SSc and other fibrotic conditions [4]. Several cells can differentiate into myofibroblasts depending on the tissue where they are present. Indeed, mesenchymal progenitor cells, fibroblasts, and epithelial and endothelial cells, as well as preadipocytes, have been proposed as sources of myofibroblasts driving fibrosis [5–9].

Activation of morphogen signaling pathways results in myofibroblast transition. Although transient activation may be necessary to initiate tissue repair, sustained morphogen signaling pathways lead to aberrant signaling and pathologic fibrosis. Hedgehog (HH), Wnt, TGF-β, and other signaling pathways have been shown to be critically involved in organ fibrosis and SSc, and excellent recent reviews have summarized relevant findings [2, 10, 11]. Remarkably, the receptors and key molecules involved in morphogen signaling pathways are localized in a specialized cellular organelle called the primary cilium. Primary cilia are antenna-like organelles present in almost all of vertebrate cells. Because they concentrate several morphogen signaling proteins in their structure, primary cilia play critical roles in development and cellular proliferation and

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differentiation [12]. Surprisingly, to date, there are only a few studies evaluating the association between primary cilia and morphogen signaling driving myofibroblast transition and subsequent fibrosis. Here, we summarize recent progress in characterizing this association and propose the primary cilium as a potential novel target for prevention and treatment of fibrosis.

Primary Cilia Structure and Signaling

The primary cilium is a microtubule-based structure that protrudes from the cell surface. It can be divided in three compartments: (1) the basal body, derived from the mother centriole; (2) the transition zone, located above the basal body; and (3) the axoneme, consisting of nine doublet microtubules that extend from the basal body [13, 14] (Fig. 1a). The primary cilium is dynamically regulated during the cell cycle. During ciliogenesis, the primary cilium assembles during the G1 phase of the cell cycle and continues to grow as cells move to G0, reaching its maximum length (1–10 μm, depending on the cell type). Disassembly of the primary cilium starts as cells transition from the G phase into S phase, and the primary cilium is completely absent before entry into mitosis [15, 16] (Fig. 1b).

The ciliary proteins are synthesized in the cytosol and transported in the cilium by intraflagellar transport (IFT), a bidirectional transport system operated by IFT proteins and motors. IFT proteins form two network complexes named IFTA, which includes IFT43, IFT121, IFT122, IFT139, IFT140, and IFT144, in a complex that binds to dynein-2 motors to transport proteins from the cilium tip to the cell body; and IFTB complex, which contains IFT20, IFT22, IFT25, IFT27, IFT38, IFT46, IFT52, IFT54, IFT56, IFT57, IFT70, IFT74, IFT80, IFT81, IFT88, and IFT172, forming a complex that interacts with kinesin-II motors to transport proteins from the basal body to the cilium tip [17•]. IFT transport mechanisms not only participate in the assembly/disassembly of primary cilium structures but also play key roles in the transport of receptors and signaling molecules in and out of the primary cilia [18•].

Primary cilia can be viewed as cellular antennae that serve as the central processing units for a multiplicity of signaling pathways. Their unique structure is enriched in receptors and signal transducers that coordinate the output of cellular signaling pathways. Some of these events are regulated within sub-compartments of the primary cilium and are influenced by the differentiation state and the microenvironment of the cells in which they are present [19••]. Mutations that lead to dysfunctional primary cilia are increasingly recognized to be associated with a pleiotropic group of heterogeneous disorders called ciliopathies [20••].

Cilia as Signaling Loci for Morphogen Signaling

A growing body of research over the last 15 years has uncovered important signaling pathways that are localized in primary cilia [21]. These include transforming growth factor β (TGF-ß), Wnt, and HH signaling. TGF-β signaling includes a group of ligands that bind to a heterotetrameric receptor conformed by type I (RI) and type II (RII) serine/threonine kinases. TGF-β receptors localize in the primary cilium, and their internalization from the surface of the primary cilium membrane is via clathrin-mediated endocytosis, inducing phosphorylation and activation of transcription factors SMAD2/3 [22•]. Activated SMADs form a trimeric complex with SMAD4, which translocates into the nucleus for targeted activation of gene expression. This signaling pathway is modulated by the inhibitory effect of SMAD7, localized at the base of the primary cilium. TGF-β signaling can also crosstalk with other signaling pathways, including MAP kinase signaling by activation of ERK1/2 through a mechanism independent of clathrin-mediated endocytosis. Activation of ERK1/2, p38, and JNK integrates TGF-β signaling into a larger signaling network that controls diverse cellular responses. Moreover, primary cilia TGF-β receptors can activate HH signaling by interacting with smoothened (SMO), leading to activation of GLI transcription factors that translocate to the nucleus to regulate gene expression (Fig. 2a) [19••]. In this regard, it is important to recognize that TGF-β signaling plays critical roles in cell-cycle control, migration, and differentiation, and, remarkably, is one of the main signaling pathways driving myofibroblast transformation and fibrosis [2].

Wnts are a large family of pleiotropic secreted lipoproteins implicated in wound healing, development, and fibrosis. The multiple Wnt ligands can transduce their signals through non-canonical or canonical pathways involving β-catenin [23]. In the canonical pathway, Wnt ligands bind to frizzled receptors that are located in the cell membrane covering the primary cilium [24]. Activation of these receptors triggers the formation of complexes with co-receptors LRP5/6 and disheveled (DVL), promoting stabilization of cytoplasmic β-catenin, which then leads to nuclear translocation of this protein to regulate gene expression by binding to Lef/Tcf transcription factors [25] (Fig. 2b). Activation of Wnt signaling is associated with transcription of genes important for cell proliferation, differentiation, and survival [26]. Importantly, this signaling pathway has also been implicated in SSc [2]. Aberrantly elevated expression of nuclear β-catenin and its target gene, *AXIN2*, occur in the skin and lungs of patients with SSc [27, 28]. A growing body of evidence shows that Wnt can also elicit fibrotic responses through TGF-β signaling [22•, 29], indicating that both signaling pathways are interdependent.

HH signaling pathways regulate several cellular processes during development and tissue homeostasis. Transduction of

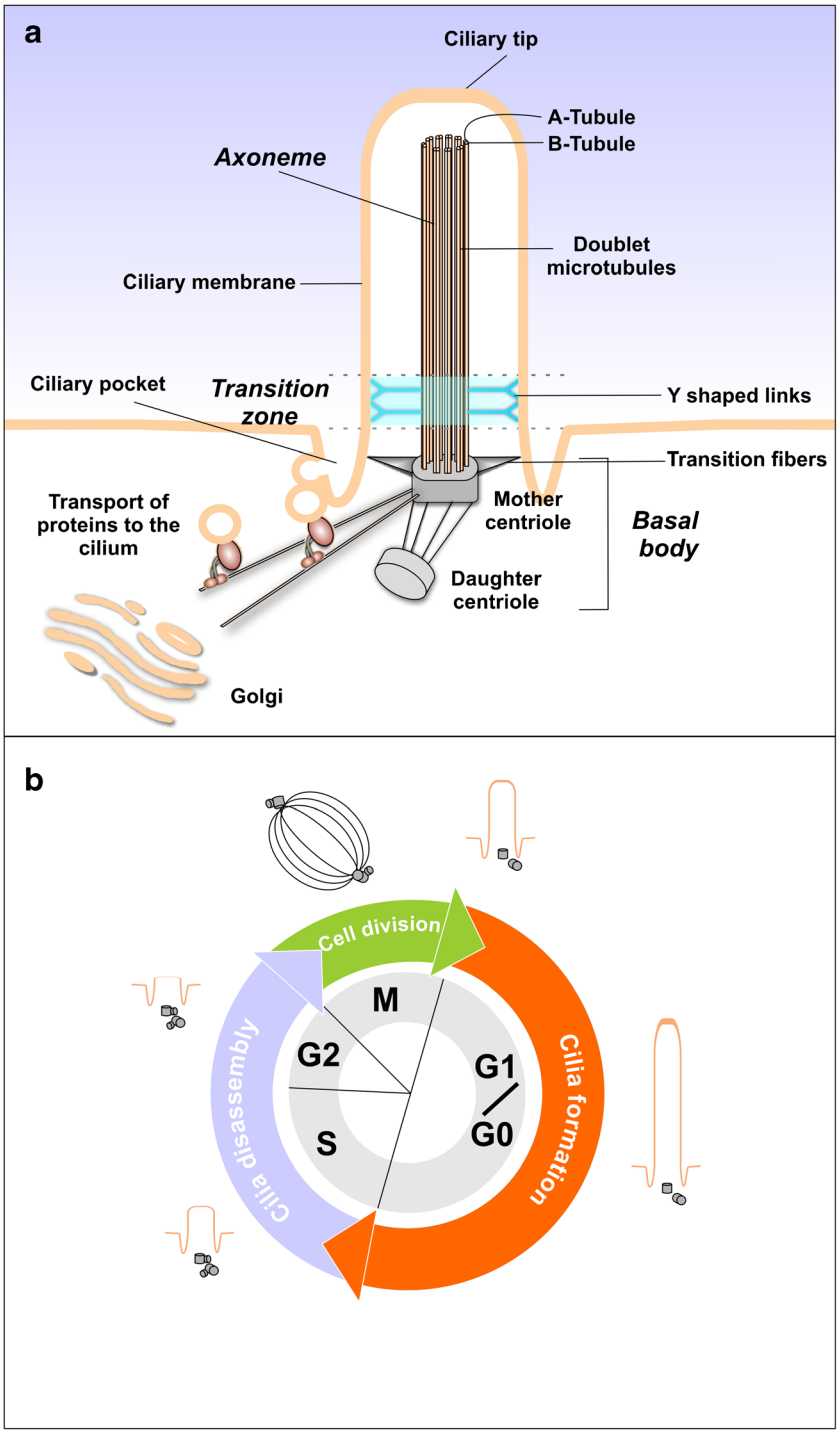

Fig. 1 The primary cilium structure and cell cycle. **a** Schematic representation of primary cilia structure. The axoneme is represented by nine doublets of microtubules (A and B tubules). The axoneme is covered by ciliary membrane. The transition zone is characterized by Y-shaped links (light blue) that mediate interaction with the ciliary membrane. Transition fibers extend from the distal appendages of the mother centriole. The basal body is made up by the mother and daughter centrioles in non-motile cilia. **b** Ciliogenesis is synchronized with the cell cycle. During the course of G1, the primary cilium develops from the mother centriole and reaches the highest length at G0. During S phase, both centrioles duplicate to form new daughter centrioles and disassembly of the cilium starts. By entry into mitosis, the cilium is completely disassembled

the signal is mediated through two transmembrane proteins Patched1 (Ptch1) and SMO, both present along the primary cilium membrane [30]. In the absence of sonic hedgehog ligand (SHH), Ptch1 keeps the pathway off by inhibiting the activity of SMO. When SMO is inactive, the GLI transcription factors are proteolytically processed to make a repressor that binds to HH target genes and blocks their transcription. Conversely, binding of HH ligands to Ptch1 inhibits its activity and triggers endocytic internalization of Ptch1 from the ciliary membrane. Inactivation of Ptch1 is associated with activation and enrichment of SMO, which then promotes conversion of full-length GLI into a transcriptional activator (GLIA), followed by induction of targeted gene expression [31] (Fig. 2c). The intermediate steps between SMO activation and GLIA formation and nuclear translocation are not well understood [19••]. However, it has become clear that HH

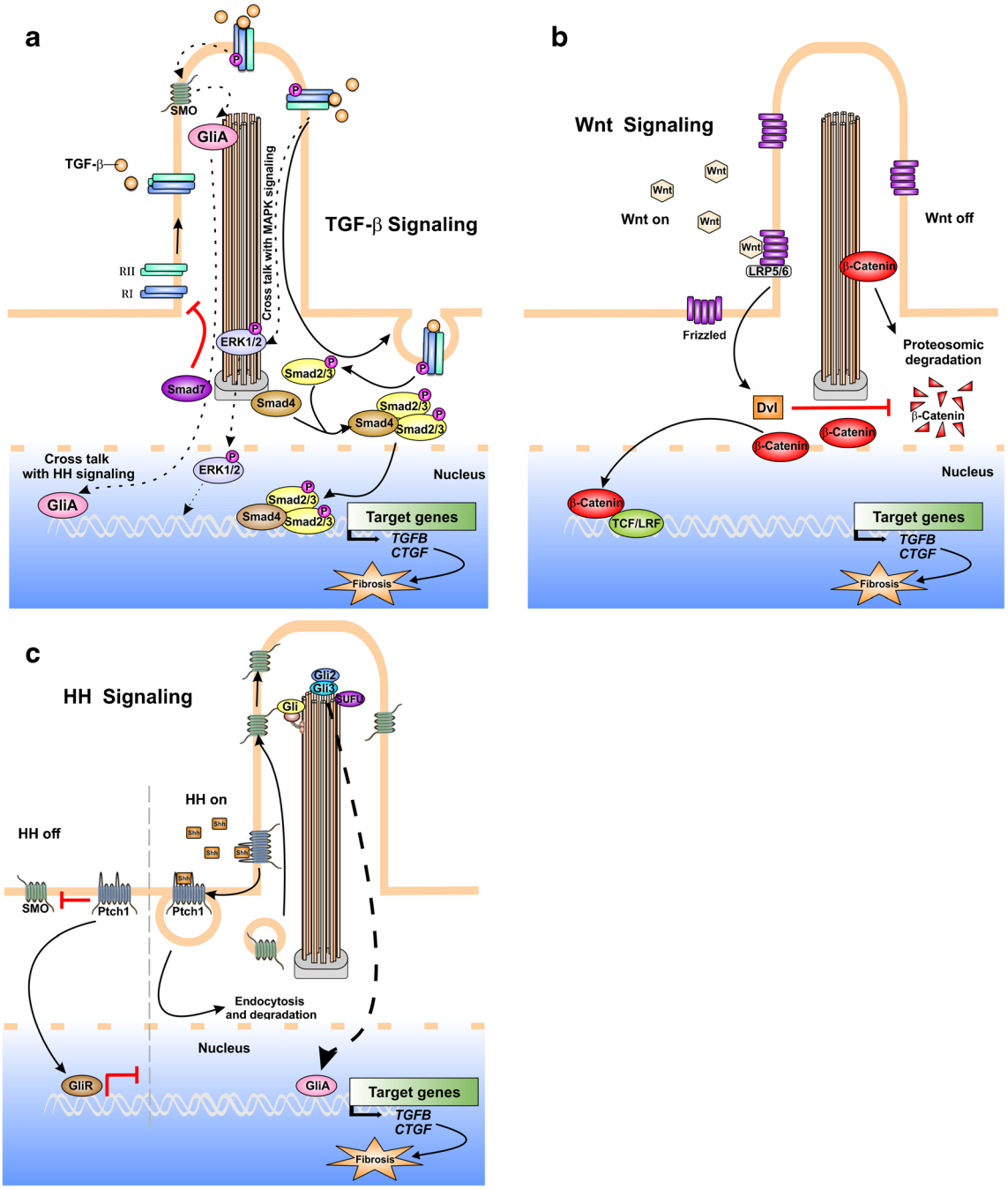

Fig. 2 Schematic representation for morphogen signaling transduction regulated by the primary cilium. **a** TGF-β signaling. Receptors type I (RI) and type II (RII) for TGF-β ligand localize in the ciliary membrane and form a heterotetrameric complex that after binding with TGF-β is internalized from the surface of the primary cilium membrane via clathrin-mediated endocytosis. After endocytosis, they induce phosphorylation and activation of transcription factors SMAD2/3. Phosphorylated SMAD2/3 forms a trimeric complex with SMAD4, which translocates into the nucleus for targeted activation of profibrotic genes expression. This signaling pathway is modulated by the inhibitory effect of SMAD7, localized at the base of the primary cilium. TGF-β signaling can also crosstalk with other signaling pathways, including MAP kinase signaling by activation of ERK1/2 through a mechanism independent of clathrin-mediated endocytosis. TGF-β receptors can also activate HH signaling by interacting with SMO, leading to activation of GLI transcription factors that translocate to the nucleus to regulate gene expression. **b** Wnt signaling. Wnt ligands bind to frizzled receptors that are located in the cell membrane covering the primary cilium. Activation of these receptors triggers the formation of complexes with co-receptors LRP5/6 and DVL, promoting stabilization of cytoplasmic β-catenin, which then translocates to the nucleus to regulate profibrotic gene expression by binding to Lef/Tcf transcription factors. In case of absence of Wnt ligands, β-catenin is degraded by the proteasome complex. **c** HH signaling. In the absence of HH ligands, receptor Ptch1 keeps the pathway off by inhibiting the activity of SMO. When SMO is inactive, the GLI transcription factors are proteolytically processed to make a repressor that binds to HH target genes and blocks their transcription. Conversely, binding of HH ligands to Ptch1 inhibits its activity, and triggers endocytic internalization of Ptch1 from the ciliary membrane. Then SMO becomes activated and promotes conversion of full-length GLI into GLIA, followed by induction of targeted profibrotic gene expression

signaling is not only important during embryonic development. HH signaling also controls myofibroblast activation leading to fibrosis in the skin [32, 33] and several tissues and organs [34–37].

Ciliopathies and Fibrosis

Ciliopathies are a heterogeneous group of disorders characterized by abnormal formation and function of cilia [38, 39]. The ciliopathies are strongly associated with genetic mutations. Indeed, to date, more than 200 genes have been implicated in ciliopathies, and at least another 241 genes that have been associated with ciliary structures and/or functions that could potentially result in known or novel ciliopathies if disrupted in humans [20••, 40]. The phenotypes of complete loss or dysfunction of cilia are extremely heterogeneous and complex. In the case of complete or partial loss of mature cilia, serious multi-organ disorders may develop. In the case of defects of morphologically normal cilia, late-onset polycystic kidney disease and retinal degeneration represent the most common clinical manifestations. In addition, other manifestations include anosmia, dystonia, cardiac anomalies, infertility, obesity, CNS abnormalities, and skeletal dysplasia [19••]. Remarkably, ciliopathies also manifest fibrosis in tissues and solid organs. For example, polycystic kidney disease (PKD), a ciliopathy associated with disrupted primary cilia, shows several features including tubulointerstitial fibrosis in the kidneys as well as liver fibrosis [41]. Interestingly, mice lacking Cdc42, a small GTPase present in primary cilia, have impaired ciliogenesis and fibrosis in the kidney [42]. Moreover, the congenital model of cilia dysfunction $IFT88^{Orpk}$ mouse shows periportal fibrosis in the liver [43]. Recent evidence suggests that skin fibrosis in SSc is associated with a marked reduction in the expression of SPAG17, a poorly characterized cellular protein that is involved in ciliogenesis [44]. The mechanisms linking ciliopathies with fibrosis have not been elucidated. However, recent compelling evidences suggest the involvement of primary cilia in myofibroblast transition.

Myofibroblast Transition and Primary Cilia

A unifying fibrosis paradigm is myofibroblast plasticity: a process by which quiescent tissue-resident cells transform into activated myofibroblasts responsible for extracellular matrix accumulation and fibrosis [45]. Myofibroblasts can originate from various cell types, including fibroblasts, preadipocytes, monocytes, pericytes, endothelial cells, and epithelial cells [29]. In this process, cell-type-specific molecules for each transition are required. Although some of these molecules and networks are known, the disease- and tissue-specific mechanisms underlying progenitor-to-myofibroblast transition and sustained activation remain poorly understood [45, 46]. Key morphogen signaling pathways, including TGF-β, HH, and Wnt, have been implicated in driving or sustaining pathological myofibroblast transition [2, 10, 11].

As mentioned above, these pathways are regulated and spatiotemporally coordinated by the primary cilium. Recently, a group of laboratories have shown compelling results indicating that primary cilia are essential for the regulation of myofibroblast transition. Studies from Egorova and collaborators [47] used endothelial cells from mice lacking IFT88 protein (IFT88Tg737RPW mice). Interestingly, they observed that cultured endothelial cells from these mutant animals showed the absence of primary cilia, gain of expression of myofibroblast markers including α-SMA and N-cadherin, and loss of endothelial cell markers like CD31 when they were exposed to increased shear stress. This group also observed increased activation of TGF-β/SMAD2 signaling in both in vitro and in vivo studies. Results from their research suggest that lack of primary cilia primes shear-induced endothelial-to-mesenchymal transition. However, mouse embryonic fibroblasts (MEFs) with the same deletion in IFT88 show stunted primary cilia and reduced activation of TGF-β/SMAD2 signaling after stimulation with TGF-β [22•], suggesting that a well-developed and functional primary cilia may be important for the activation of TGF-β signaling in fibroblasts. It should be noted that IFT88 belongs to a group of proteins required for ciliogenesis and the transport of proteins along the primary cilia [48]. In this context, it is possible that mutations in IFT88 not only affect primary cilia structure but may also alter the transport of TGF-β receptors into the primary cilium [22], resulting in reduced TGF-β signaling.

Rozycki and collaborators [49••] performed elegant studies to examine primary cilia and their impact in cultured epithelial cells. Their results showed that primary cilia undergo a dynamic biphasic change during epithelial-myofibroblast transition (EMyT) as well as fibroblast-to-myofibroblast transition induced by TGF-β. Under these experimental conditions, the mechanism for myofibroblast transformation appears to require the initial presence of primary cilia to sense TGF-β ligand. Subsequently, during the transition process, cells lose these organelles and become myofibroblasts. In this context, ciliary loss may be mediated by a mechanism involving increased levels of myosin phosphorylation accompanied by activation of Rac1, SMAD3, and reactive oxygen species (ROS). Moreover, disrupted expression of the ciliary protein, KIF3A, revealed that alterations in primary cilia before TGF-β stimulation prevent the transition of precursors into myofibroblasts, but once the critical signaling has been initiated, loss of primary cilia may, on the contrary, facilitate the transition [49••].

Different mechanisms seem to be regulating the transition of adipose progenitors into myofibroblasts. Arrighi and collaborators [50] showed that TGF-β stimulation promotes transition of

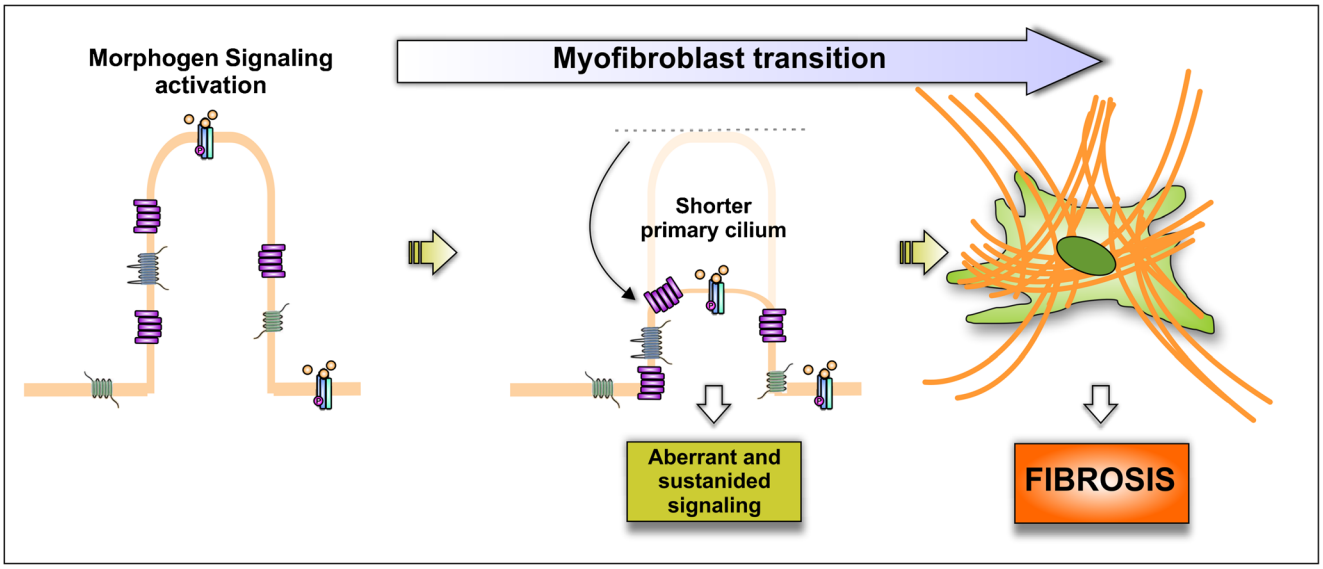

Fig. 3 The primary cilium modulates progenitor-myofibroblast transition. Compelling evidence suggests that the primary cilium may be important for the mechanism driving myofibroblast transition. The role of the primary cilium may be associated with a biphasic mechanism where the first phase requires the coordination and transduction of fibrotic morphogen signaling pathways by the primary cilium. The second phase seems to be cell-type-specific and may involve loss or reduction in the length of the primary cilium, resulting in sustained myofibroblast activation and subsequent fibrosis

adipose progenitors to myofibroblasts accompanied by reduction in primary cilia length, but not in loss of this organelle as seems to be the case for myofibroblast transition from endothelial cells and fibroblasts [49••]. Interestingly, myofibroblasts originating from adipose progenitors increase GLI-1 expression after induction with SHH ligand, indicating that although the primary cilium is shorter in this case, it may still be able to respond to HH signaling. Remarkably, deciliation of adipose progenitors induced by HPI-4 (inhibitor of dynein motor) treatment or by knockdown of KIF3A, before TGF-β stimulation, decreased the ability of TGF-β to induce myofibroblast transformation, in agreement with Rozycki and collaborators [49••]. It would be interesting to determine whether similar time course experiments as carried out by Rozycki et al. would also show enhanced myofibroblast transformation in adipose progenitors if the ciliary disruption is performed a few hours before TGF-β stimulation. The primary cilium seems to be also important for the transition of human lung fibroblasts to myofibroblasts induced by TGF-β [51]. Similarly, work from Villalobos and collaborators [52] showed that primary cilia present in fibroblasts are associated with regulation of fibrosis and tissue remodeling in the heart.

We have summarized recent data indicating that the primary cilium is necessary for the acquisition and the maintenance of a myofibroblast phenotype. The precise mechanisms underlying these phenomena remain poorly understood. The role of the primary cilium may be associated with coordination and transduction of fibrotic morphogen signaling pathways. This process seems to be cell-type-specific. However, the primary cilium role in myofibroblast differentiation and fibrosis may be biphasic. A well-developed primary cilium seems to be required for morphogen ligands to bind to their receptors, and when the signal has been initiated, loss or shortening of primary cilium length may be responsible for sustained myofibroblasts activation. Perhaps the well-recognized association of ciliopathies with fibrosis may be linked to defects in primary cilia length, but with morphogen receptors still present in the cilium membrane. Therefore, the signal can be initiated, but may activate aberrant downstream events leading to sustained activation or crosstalk with other regulatory signals resulting in pathogenic fibrosis (Fig. 3). Indeed, the growing list of signaling pathways that are connected to primary cilia is indicative of the importance of these organelles in orchestrating the integration and crosstalk between pathways in a spatiotemporal manner [19••].

Conclusions

While the role of the morphogens TGF-β, Wnt, and HH in the pathological myofibroblast differentiation in SSc and other fibrotic diseases is becoming well established, the precise mechanisms underlying progenitor-to-myofibroblast transition and sustained activation remain poorly understood [45, 46]. There is now compelling evidence that the primary cilium is a master regulator of morphogen signaling pathways, and as such may represent a key piece in the puzzle for the pathogenic mechanism of fibrosis.

Over the past 15 years, a growing body of research has uncovered important cellular signaling pathways that function via primary cilia. In addition, proteomic and genomic studies have determined that the ciliary composition of receptors and regulatory proteins is cell- and tissue-specific. Moreover, in individual cells, the receptors and regulatory proteins present in the primary cilium change over time, enabling the cell to carry out specific spatiotemporal regulation of signaling according to the environment in which the cell is present [19••]. Such specialized and perfectly synchronized transduction of signaling machinery makes the primary cilium a unique organelle. Any event that destabilizes this perfect orchestra may result in aberrant primary cilia signaling. Therefore, ciliopathies are characterized by a high degree of heterogeneity. To date, the role of ciliogenesis and altered ciliary function in SSc and other fibrotic diseases has not been established. For instance, it would be interesting to know whether proteins important for cilia assembly as well as proteins associated with ciliary dysfunction are differentially regulated in SSc. Are the number of the primary cilia or the cilia length different in biopsies from SSc patients? Understanding the contributions of primary cilia to fibrosis may ultimately inform the development of entirely new approaches for fibrosis therapy.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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