DOI: 10.1111/exd.12255
www.wileyonlinelibrary.com/journal/EXD

Canonical Wnt signalling as a key regulator of fibrogenesis – implications for targeted therapies?

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Abstract: Canonical Wnt signalling belongs to the so-called morphogen pathways and plays essential roles in development and tissue homeostasis. Being such a crucial regulatory pathway, Wnt signalling is tightly controlled at different levels. However, uncontrolled activation of canonical Wnt signalling has been implicated into the pathogenesis of various human disorders. In the last years, aberrant Wnt signalling has been demonstrated in fibrotic diseases including systemic sclerosis (SSc). In this review, we will discuss the current state of research on canonical Wnt signalling in SSc. Activation of canonical Wnt signalling induces fibroblast activation with subsequent myofibroblast differentiation and excessive collagen release resulting in tissue fibrosis. Genetic or pharmacological blockade of Wnt activation ameliorates experimental fibrosis in different preclinical models. These findings have direct translational implications because several small molecule inhibitors of Wnt signalling are currently evaluated in clinical trials and some already showed first promising results.

Key words: collagen – fibroblast – fibrosis – morphogen – scleroderma – stem cell

Accepted for publication 27 September 2013

Introduction

Fibrosing disorders are characterized by an excessive accumulation of extracellular matrix (ECM) components which disrupts the physiological tissue architecture leading to the dysfunction of the affected organ (1). The loss of organ function is the major cause for the high morbidity and mortality of these diseases. Systemic sclerosis (SSc) is a systemic fibrosing disease that affects multiple organ systems such as the skin, the lungs, the heart and the gastrointestinal tract. SSc is the connective tissue disease with the worst outcome. The overall 10-year survival rate of diffuse cutaneous SSc, the more severe subtype, ranges from 60% to 70% (2,3). Fibrosing disorders in general have been suggested to account for approximately 45% of deaths in industrialized countries, thereby highlighting the great medical need for effective antifibrotic therapies.

The first histopathological hallmarks in early stages of SSc are apoptosis of endothelial cells and perivascular inflammatory infiltrates, whereas later stages of SSc are dominated by excessive deposition of ECM in affected tissues (1,4). The ECM components, mainly consisting of collagen types I, III, VI and VII, fibronectins and glycosaminoglycans, are released by persistently activated fibroblasts (4). The underlying mechanisms and pathways leading to the persistent activation of fibroblasts are only partially understood. Current concepts suggest that vascular alterations and profibrotic mediators released from infiltrating leucocytes trigger fibroblast activation in early stages of SSc (1). Nevertheless, during the course of the disease, fibroblast activation becomes independent of external stimuli, which seems at least in part mediated by epigenetic alterations in fibroblasts (1,4). While research has long concentrated on profibrotic cytokines like TGFβ, PDGF, IL4, IL13 and MCP1, several studies of the last years highlight the key role of morphogen or stem cell pathways in the pathogenesis of fibrotic diseases (5–11). Although the Notch and the Hedgehog pathways also act as potent profibrotic mediators, this review will focus on the effects of canonical Wnt signalling on fibroblast activation and potential implications for targeted therapies.

Canonical Wnt signalling

Wnt signalling can be subclassified into canonical and non-canonical cascades. Non-canonical Wnt signalling includes the planar cell polarity pathway and Wnt/Calcium pathways (12–14). The planar cell polarity pathway is induced by activation of Rho and Rac leading to the induction of the kinases JNK and ROCK. Wnt may also activate Src and JNK kinases via activation of Ryk and Ror receptors, although this mechanism is less well understood. Finally, non-canonical Wnt signalling can also lead to calcium release from intra-cellular compartments via activation of phospholipase C (PLC) and the inositol trisphosphate receptor (IP_3R) (12). The canonical Wnt cascade is a key pathway during embryonic development, but is also crucial for stem cell renewal and tissue homeostasis in the adult (15,16). While canonical Wnt signalling has been extensively studied and implicated into the pathogenesis of multiple diseases including SSc, the roles of non-canonical Wnt signalling remain largely enigmatic.

The central integrator of canonical Wnt signalling is β-catenin whose levels are regulated by the so-called β-catenin destruction complex consisting of glycogen synthase kinase 3β (GSK3β), casein kinase 1 (CK1), the adenomatous polyposis coli (APC) tumor suppressor protein and the scaffolding protein axin. In the absence of Wnt proteins or with high concentrations of endogenous Wnt antagonists, β-catenin is phosphorylated by GSK3β and CK1 which triggers its subsequent ubiquitination and proteasomal degradation (12). Wnt proteins compose a family of secreted glycoproteins, which is defined by the property of 22 cysteine

residues forming disulphide bridges and resulting in a globular structure (17). When the local concentration of Wnt proteins outweighs the endogenous inhibitors, Wnt proteins bind to Frizzled (Fz) membrane receptors and lipoprotein related protein (LRP) coreceptors, which recruits dishevelled (DVL), axin and GSK3β to the plasma membrane, thereby destabilizing the β-catenin destruction complex. β-catenin is no longer degraded, but accumulates and translocates to the nucleus where it interacts with T-cell factor/lymphoid enhancer-binding factor-1 (TCF/Lef-1) transcription factors and several cofactors like p300 and CREB-binding protein (CBP) to stimulate the transcription of target genes (Fig. 1) (12).

This core pathway can be modified and fine-tuned at several levels. In humans, ten different Fz receptors and 19 different Wnt ligands enable a plethora of different ligand–receptor interactions, which may affect the signalling outcome (18). Moreover, several families of endogenous inhibitors like secreted frizzled-related proteins (SFRPs), Dickkopf (DKK) proteins or Wnt inhibitory factor (WIF) can antagonize Wnt signalling. Both SFRPs and WIF bind Wnt proteins, thereby preventing the interaction of Wnts and the Fz receptor. In contrast, DKK proteins bind to LRP coreceptors and are thought to therefore disturb complex formation of the Fz receptor with its LRP coreceptor (18–20). Furthermore, tankyrases (TNKS1 and 2), which belong to the poly(ADP-ribose) polymerase (PARP) protein family, modulate the stability of the β-catenin destruction complex by parylation and subsequent ubiquitination of axin (21,22). Finally, several transcriptional cofactors like p300 and CBP interact with β-catenin/TCF complexes, thereby regulating the transcriptional outcome of canonical Wnt signalling (Fig. 1) (12,23).

The Wnt pathway in fibrotic diseases

Numerous studies within the last years highlight a central role of canonical Wnt signalling in various fibrotic conditions. Apart from SSc, canonical Wnt signalling has also been implicated in pulmonary, renal and liver fibrosis as well as in keloid formation, suggesting that aberrant activation of Wnt signalling may be a common denominator of fibrotic diseases (5,24–31). For SSc, increased levels of nuclear β-catenin and of target genes such as axin2 have been demonstrated in fibrotic skin and lungs of SSc patients, and those findings were also mimicked in murine models of SSc such as bleomycin-induced fibrosis or Tight-skin-1 (Tsk1) mice (5,11,32). The aberrant activation of canonical Wnt signalling is caused by elevated expression of Wnt proteins, mainly Wnt1 and Wnt10b, but also by decreased expression of endogenous inhibitors such as DKK1, DKK2, SFRP1 and WIF (5,11,31,33,34). The mechanisms leading to the downregulation of Wnt inhibitors in SSc are complex and involve epigenetic mechanisms such as DNA methylation-induced gene silencing as well as direct inhibitory effects of profibrotic cytokines such as TGFβ (5,34,35).

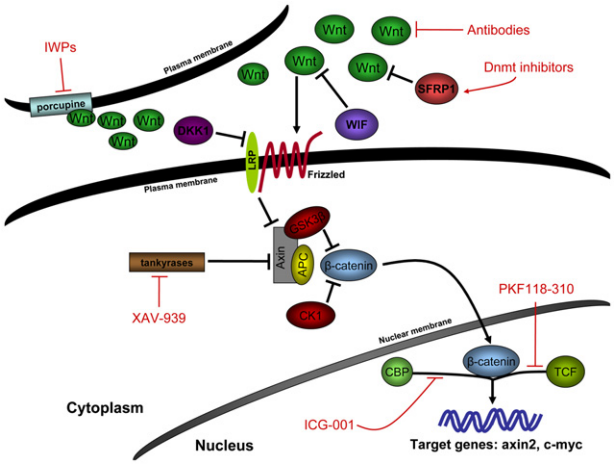

Figure 1. Canonical Wnt signalling and potential therapeutic interventions in fibrosis. The acyltransferase porcupine is essential for the release of Wnt ligands, which can be blocked by several small molecule inhibitors called IWPs. Activation of the Wnt pathway can be antagonized either by selective antibodies against Wnt proteins or by inhibition of Dnmts, which reactivates the expression of endogenous antagonists like DKK1 or SFRP1. Tankyrases can be targeted by small molecules such as XAV-939, thereby stabilizing the β-catenin destruction complex and inhibiting canonical Wnt signalling. Once β-catenin has translocated into the nucleus, it forms complexes with TCF/Lef transcription factors and several cofactors, including CBP, to induce target gene transcription. Small molecules inhibit the interaction of β-catenin with TCF (e.g. PKF118-310) or with CBP (ICG-001) and therefore disrupt β-catenin-mediated gene transcription.

Canonical Wnt signalling has profound effects on fibroblast activation and fibrogenesis. Wnt proteins stimulate the differentiation of resting fibroblasts into myofibroblasts and increase the release of ECM *in vitro* (5,31). *In vivo*, activation of the Wnt pathway by different approaches such as overexpression of Wnt10b, stabilization of β-catenin or inhibition of GSK3β results in rapid and progressive skin fibrosis (5,11,33,36,37). Fibroblast-specific stabilization of β-catenin by deletion of exon 3, which contains the phosphorylation sites for the degradation of β-catenin, culminates in increased dermal thickness, elevated myofibroblast counts and increased hydroxyproline content already within 2 weeks after induction of Cre-mediated recombination (33,37). Rapid onset of fibrosis was also observed upon pharmacological inhibition of GSK3 in mice (36). Moreover, overexpression of Wnt10b under an adipocyte-specific promoter leads to massive skin fibrosis with tenfold increases in collagen content in 12-week-old mice. As in human SSc, Wnt10b transgenic mice also display a prominent subcutaneous atrophy (5,11).

Of interest, canonical Wnt signalling closely interacts with TGFβ signalling, another major profibrotic pathway, which drives fibroblast activation with subsequent myofibroblast differentiation and aberrant collagen release. TGFβ activated canonical Wnt signalling with increased nuclear accumulation of β-catenin, induction of TCF/Lef reporter activity and transcription of the target gene axin2 in cultured fibroblasts and in murine skin (5). Furthermore, inhibition of TGFβ signalling reduced Wnt signalling in different experimental models, highlighting that TGFβ indeed contributes to the aberrant activation of canonical Wnt signalling in fibrosis. Moreover, inhibition of Wnt signalling in turn abrogated the pro-fibrotic effects of TGFβ in several preclinical models in cell culture experiments *in vitro* and in animal models *in vivo*, demonstrating that Wnt activation is required for TGFβ-mediated fibrosis (5,33,38,39). TGFβ regulates Wnt signalling in part on the level of its endogenous inhibitors, thereby increasing the responsiveness of SSc fibroblasts to Wnt proteins. TGFβ inhibits the expression of DKK1 in a p38-dependent manner to activate canonical Wnt signalling (5). Further studies revealed that both DKK1 and SFRP1 are downregulated in SSc and experimental fibrosis via promoter hypermethylation-induced transcriptional silencing which is triggered by TGFβ (34,35) (unpublished data). However, the interaction between canonical Wnt signalling and

the TGFβ cascade may not be unidirectional. A recent study demonstrated that Wnt3a stimulates the expression of TGFβ1 and induces phosphorylation of Smad2 and Smad3, indicating that canonical Wnt signalling may also activate the TGFβ cascade (31). Taken together, these findings highlight a close interaction of both pathways and suggest a vicious cycle of aberrant Wnt and TGFβ signalling in SSc.

Translational implications

The aberrant activation in different fibrotic disorders and its potent profibrotic effects in various model systems nominate canonical Wnt signalling as interesting candidate for antifibrotic therapies. This is further supported by the antifibrotic effects of targeting canonical Wnt signalling in experimental fibrosis (5,33,39). Targeting of the transcriptional coactivator β-catenin by fibroblast-specific knockout effectively ameliorated bleomycin-induced fibrosis as a model for early inflammatory stages of SSc (33). Furthermore, overexpression of the endogenous inhibitor DKK1 strongly ameliorated fibrosis in models resembling both early and later stages of the human disease such as bleomycin-induced fibrosis, Tsk-1 mice and fibrosis induced by overexpression of a constitutively active TGFβ-receptor I (TGFBR1^{CA}) (5). The third approach, knockdown of Wntless (Wls; also known as EVI/Sprinter/Mig14/Gpr177), reduces the secretion of all Wnt ligands and thus simultaneously targets canonical and non-canonical Wnt signalling. Knockdown of Wls also exerted potent antifibrotic effects in preventive as well as in therapeutic approaches (39).

Thus, blockade on different levels of the Wnt cascade – either on the level of ligand secretion or on the level of endogenous inhibitors or on the transcriptional level – is effective in antifibrotic treatment. Nevertheless, canonical Wnt signalling has long been considered 'undruggable', as the Wnt core pathway lacks typical pharmacological targets that can be easily inhibited. In addition, concerns about adverse effects caused to Wnt signalling in stem cell maintenance dampened the enthusiasm for canonical Wnt signalling as a therapeutic target. In the last couple of years, however, several small molecule inhibitors with novel mechanisms of action have been developed (40). These inhibitors target tankyrases, β-catenin/TCF interactions, binding of transcriptional coactivators to β-catenin and the acyl transferase porcupine which is essential for Wnt ligand modification and release (Fig. 1). Of note, first results from early clinical trials indicate that those inhibitors are effective and that their use is not limited by toxic side effects (40).

Some of those Wnt inhibitors have recently been assessed in experimental models of SSc with promising antifibrotic effects. The tankyrase inhibitor XAV-939 efficiently inhibited canonical Wnt signalling in experimental fibrosis with reduced nuclear accumulation of β-catenin and decreased expression of the Wnt target gene c-myc. XAV-939 was well-tolerated and exerted potent antifibrotic effects in bleomycin-induced fibrosis and in an *in vivo* model with overexpression of TGFBR1^{CA}, thereby again highlighting the interaction of TGFβ and Wnt signalling (38). PKF118-310, which inhibits the binding of β-catenin to TCF transcription factors, and ICG-001, which blocks the binding of transcriptional coactivators to β-catenin, also exerted potent antifibrotic effects in bleomycin- and TGFBR1^{CA}-induced skin fibrosis in well-tolerated doses (41).

Given that DNA hypermethylation-induced silencing of endogenous Wnt inhibitors also contributes to aberrant Wnt activation, inhibition of DNA methyltransferases (Dnmts) may also be an interesting strategy to inhibit Wnt signalling in SSc. One Dnmt inhibitor, 5-aza-2′-deoxycytidine (5-aza, Decitabine), was recently evaluated in experimental skin fibrosis. Treatment with 5-aza reactivated the expression of the Wnt inhibitors DKK1 and SFRP1 and effectively blocked Wnt signalling with reduced nuclear accumulation of β-catenin and decreased expression of axin2. The inhibition of canonical Wnt signalling translated into potent antifibrotic effects with decreased dermal thickening, reduced accumulation of collagen and impaired myofibroblast differentiation upon bleomycin-challenge (34). Although Dnmt inhibitors do not inhibit canonical Wnt signalling selectively, Dnmt inhibitors currently offer a major advantage, as their clinical programmes are more advanced than those of Wnt inhibitors. Whereas Wnt inhibitors have just completed phase I trials, 5-aza or 5-azacytidine (5-azaC, Vidaza) have already been approved for clinical routine in myelodysplastic syndromes and leukaemia (42–44). Thus, Dnmt inhibitors would be available for clinical trials in patients with SSc and other fibrotic diseases without delay.

Conclusion

During the last years, canonical Wnt signalling emerged as a core pathway in the pathogenesis of SSc and other fibrotic diseases. Aberrant activation of this signalling cascade potently stimulates fibroblast activation and tissue fibrosis in different tissues. Pharmacologic and genetic inactivation of canonical Wnt signalling effectively inhibits fibrosis in different preclinical models including cell culture experiments and several mouse models. These data suggest that targeting canonical Wnt signalling might be a potential novel target for the treatment of SSc and other fibrotic diseases. These findings have direct translational implications because several small molecule inhibitors have already entered early clinical trials, demonstrated good tolerability and would also be available for evaluation in patients with SSc or other fibrotic diseases.

However, considering the regulatory role of the canonical Wnt pathway in stem cell self-renewal and the lack of experience with long-term inhibition of Wnt signalling, several points need to be addressed before initiating clinical trials with Wnt inhibitors in patients with fibrotic diseases. First, the effects of Wnt inhibition should be evaluated in additional organ systems as the outcomes of Wnt signalling differ in a tissue-, cell-type- and context-specific manner. Second, the safety of chronic inhibition of the canonical Wnt pathway should be carefully assessed, with a particular focus on intestinal stem cells whose depletion would further complicate gastrointestinal involvement in SSc. Finally, the Wnt cascade tightly interacts not only with TGFβ signalling but also with the other morphogen pathways Hedgehog and Notch, which are also aberrantly activated in fibrotic diseases and contribute to disease progression (6–10). These interactions should be analysed in more detail to exclude potential counter-regulatory effects upon Wnt inhibition. A better knowledge of the interplay of morphogen pathways may also offer options for combined inhibition of morphogen pathways with dose-adjusted regimens.

Author Contribution

Both authors contributed to the drafting and writing of the manuscript.

Conflict of interest

The authors have declared no conflicting interests.

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