EXTENDED REPORT

Vitamin D receptor regulates TGF- β signalling in systemic sclerosis

Pawel Zerr,¹ Stefan Vollath,¹ Katrin Palumbo-Zerr,¹ Michal Tomcik,^{1,2}
Jingang Huang,¹ Alfiya Distler,¹ Christian Beyer,¹ Clara Dees,¹ Kolja Gela,³
Oliver Distler,⁴ Georg Schett,¹ Jörg H W Distler¹

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¹Department of Internal Medicine III and Institute for Clinical Immunology, University of Erlangen-Nuremberg, Erlangen, Germany

²Department of Rheumatology of the First Faculty of Medicine, Institute of Rheumatology, Charles University, Prague, Czech Republic

³Department of Orthopedic Trauma Surgery, University of Erlangen-Nuremberg, Erlangen, Germany

⁴Center of Experimental Rheumatology and Zurich Center of Integrative Human Physiology, University Hospital Zurich, Zurich, Switzerland

Correspondence to

Dr Jörg Distler, Department of Medicine 3 and Institute for Clinical Immunology, University of Erlangen-Nuremberg, Ulmenweg 18, Erlangen D-91054, Germany; Joerg.distler@uk-erlangen.de

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ABSTRACT

Background Vitamin D receptor (VDR) is a member of the nuclear receptor superfamily. Its ligand, 1,25-(OH)₂D₃, is a metabolically active hormone derived from vitamin D₃. The levels of vitamin D₃ are decreased in patients with systemic sclerosis (SSc). Here, we aimed to analyse the role of VDR signalling in fibrosis.

Methods VDR expression was analysed in SSc skin, experimental fibrosis and human fibroblasts. VDR signalling was modulated by siRNA and with the selective agonist paricalcitol. The effects of VDR on Smad signalling were analysed by reporter assays, target gene analyses and coimmunoprecipitation. The effects of paricalcitol were evaluated in the models of bleomycin-induced fibrosis and fibrosis induced by overexpression of a constitutively active transforming growth factor- β (TGF- β) receptor I (TBRI^{CA}).

Results VDR expression was decreased in fibroblasts of SSc patients and murine models of SSc in a TGF- β -dependent manner. Knockdown of VDR enhanced the sensitivity of fibroblasts towards TGF- β . In contrast, activation of VDR by paricalcitol reduced the stimulatory effects of TGF- β on fibroblasts and inhibited collagen release and myofibroblast differentiation. Paricalcitol stimulated the formation of complexes between VDR and phosphorylated Smad3 in fibroblasts to inhibit Smad-dependent transcription. Preventive and therapeutic treatment with paricalcitol exerted potent antifibrotic effects and ameliorated bleomycin- as well as TBRI^{CA}-induced fibrosis.

Conclusions We characterise VDR as a negative regulator of TGF- β /Smad signalling. Impaired VDR signalling with reduced expression of VDR and decreased levels of its ligand may thus contribute to hyperactive TGF- β signalling and aberrant fibroblast activation in SSc.

D (1,25-(OH)₂D₃). 1,25-(OH)₂D₃ activates vitamin D receptor (VDR), a member of the superfamily of nuclear receptors.⁷ The transcriptional activity of VDR is regulated by its ligand and recruitment of transcriptional coactivators or by dimerisation with other transcription factors such as retinoid X receptors or Smad3.⁷ VDR signalling is best known for its essential role in calcium and phosphorus homeostasis.^{5–7} However, VDR signalling has multiple roles beyond the regulation of calcium homeostasis and is increasingly recognised as a key regulator of cell proliferation, differentiation and immunomodulation.^{5–7–8} Moreover, accumulating evidence suggests that vitamin D deficiency is a common feature in various autoimmune diseases, but may also increase the risk of cancer and infectious disease.⁶ Severe vitamin D deficiency as in genetically predisposed individuals impairs self-tolerance and immune responses by comprising the functions of dendritic cells, regulatory T cells, Th1 cells and B cells.⁶ Decreased levels of vitamin D have been observed in various autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes and multiple sclerosis.⁶ For SSc, several independent studies from different countries reported significantly reduced levels of vitamin D.^{9–16} One study indicated an inverse correlation between the levels of vitamin D and the modified Rodnan Skin Score,⁹ suggesting that deficiency in vitamin D and impaired VDR signalling may directly contribute to the pathogenesis of SSc.

Several compounds that modulate VDR signalling are currently in clinical use. Besides the precursor vitamin D, several active VDR ligands are approved. The endogenous VDR agonist 1,25-(OH)₂D₃ has been used for the treatment of secondary hyperparathyroidism.¹⁷ However, the therapeutic use of 1,25-(OH)₂D₃ is often complicated by hypercalcemia and hyperphosphatemia. To overcome this limitation, synthetic VDR agonists were designed that bind to VDR with very high affinity and have a decreased risk of hypercalcemia and hyperphosphatemia.¹⁸ One of these non-hypercalcemic VDR agonists in clinical use is paricalcitol. Paricalcitol is well tolerated and has been shown to suppress the levels of parathyroid hormone more effectively than 1,25-(OH)₂D₃ without inducing hypercalcemia and hyperphosphatemia in clinical studies.¹⁹

Inspired by the clinical observations on vitamin D deficiency in SSc, we aimed to characterise the

The major hallmark of systemic sclerosis (SSc) is an excessive accumulation of extracellular matrix, which is caused by a pathological activation of fibroblasts.¹ Transforming growth factor- β (TGF- β) has been identified as a central mediator of fibroblast activation in SSc.² However, the intracellular signalling cascades, by which it stimulates the production of extracellular matrix, are only partially understood, and the current knowledge has not yet been translated into antifibrotic therapies.^{3–4}

Vitamin D is primarily synthesised in the skin, but dietary intake of vitamin D contributes approximately 20% to the total requirement.^{5–6} Vitamin D is hydroxylated in two steps in the liver and kidneys to generate its active form, 1,25 dihydroxyvitamin

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role of VDR in fibroblast activation in SSc. We demonstrate that SSc patients are not only deficient in vitamin D, VDR is also strongly downregulated in a TGF- β -dependent manner in SSc fibroblasts. We also characterise VDR as a negative regulator of TGF- β /Smad signalling that prevents aberrant activation of fibroblasts. Furthermore, we demonstrate that VDR agonist paricalcitol inhibits TGF- β signalling and effectively prevents experimental fibrosis in two different mouse models.

MATERIALS AND METHODS

Patients and fibroblast cultures

All SSc patients fulfilled the American College of Rheumatology (ACR) criteria for SSc. The median age of SSc patients was 52 years (range 19–78 years) and their median disease duration was 4 years (range 0.5–10 years). The study was approved by the ethical committee of the University of Erlangen-Nuremberg. Informed consent was obtained from all participants. Fibroblasts were obtained from SSc patients, healthy volunteers, mice deficient for VDR (VDR^{-/-}) and wild-type littermates (VDR^{+/+}).^{20–21} In selected experiments, fibroblasts were stimulated with combinations of TGF- β at a concentration of 10 ng/mL (R&D Systems, Wiesbaden, Germany) and/or paricalcitol at a concentration of 40 ng/mL (Abbott, Wiesbaden, Germany).

Immunofluorescence staining

Immunofluorescence staining for VDR was performed according to previously established protocols^{22–23} using polyclonal rabbit-anti VDR (Abcam, Cambridge, UK). Counterstaining was performed in the use of polyclonal mouse-anti-Vimentin or pSmad3 (both Abcam). Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI).

Western blot analysis

Protein samples were transferred to polyvinylidene difluoride (PVDF) membranes and incubated with polyclonal rabbit-anti-VDR antibodies (Abcam) at a dilution of 1:100. β -Actin served as a loading control. Signals were detected with species-specific immunoglobulin antibodies labelled with horseradish peroxidase (Dako, Hamburg, Germany) and enhanced luminol-based chemiluminescent (ECL) Plus reagent.

Quantitative real time-PCR

Gene expression was quantified by SYBR Green real time-PCR using the MxPro-3500P System (Agilent, Böblingen, Germany).^{24–25} Samples without enzyme in the reverse transcription reaction (non-RT controls) were used as negative controls. β -Actin was used to normalise for the amounts of cDNA.

VDR overexpression and knockdown

Human VDR was amplified from whole blood and cloned into the pEF1/myc-His B plasmid (Invitrogen, Karlsruhe, Germany), resulting in the expression of human VDR tagged with myc. Empty pEF1/myc-His B plasmid without VDR served as control.

For VDR knockdown experiments, VDR siRNA (Eurogentec, Cologne, Germany) with the following sequences human VDR 5'-GGAGUUCAUUCUGACAGAU55-3' (forward), 5'-AUCUGU CAGAAUGAACUCC55-3' (reverse) was used. Non-targeting siRNAs (Ambion) served as controls. Human dermal fibroblasts were transfected using the Amaxa 4D-Nucleofector (Amaxa, Cologne, Germany).^{26–27}

Quantification of collagen protein

The amount of soluble collagen in cell culture supernatants was quantified using the SirCol collagen assay (Biocolor, Belfast, Northern Ireland).^{28–29}

Stress fibre staining

Stress fibres were visualised with phalloidin as describe previously.^{30–31} Quantification was performed using the ImageJ software (V1.42q, National Institutes of Health, USA).

Coimmunoprecipitation

Human fibroblasts were transfected with the pEF1/myc-His B plasmid containing VDR (pEF1_VDR) or empty pEF1/myc-His B plasmid (pEF1). Cytoplasmatic and nuclear extracts were incubated with Protein G Sepharose, mouse anti-Myc-Tag (Cell Signaling, Germany) or mouse IgGs (Santa Cruz Biotechnology, Heidelberg, Germany). Finally, western blotting against c-Myc (Cell Signaling), Smad3 (Cell Signaling) and pSmad3 (Abcam) was performed. β -Actin and lamin A/C (Cell Signaling) served as references.

SMAD reporter assays

Human dermal fibroblasts were transfected with replication-deficient adeno-associated virus AdCAGA-Luc encoding the luciferase enzyme under a CAGA promoter. Luciferase activity was determined with a microplate luminometer (Berthold Technologies, Germany).³²

Bleomycin-induced dermal fibrosis

VDR^{-/-} mice on a C57/Bl6 background have been described before.³³ Wild-type C57/Bl6 (VDR^{+/+}) littermates were used as controls. Skin fibrosis was induced by local injections of bleomycin every other day for 4 weeks.^{34–35} Subcutaneous injections of sodium chloride served as controls. For pre-established fibrosis, mice were prechallenged with bleomycin for 3 weeks before treatment was initiated. Treatment and injections of bleomycin were continued for additional 3 weeks as described.^{24–30–36}

TBRI-induced dermal fibrosis

Replication-deficient adeno-associated virus encoding constitutively active TGF- β receptor type I (AdTBR) was injected into the skin.^{36–37}

Treatment with paricalcitol

Paricalcitol (Zemlar; 5 μ g/mL; Abbott, Wiesbaden, Germany) was injected at a dose of 0.3 μ g/kg/d subcutaneously.

Analysis of murine skin

Dermal thickness,³⁸ hydroxyproline content^{39–40} and α -smooth muscle actin positive myofibroblasts^{41–42} were analysed as described previously.

Statistics

Data were expressed as mean $\pm$ SE of the mean. Student t test was used for statistical analyses. p Values of less than 0.05 were considered statistically significant.

RESULTS

The expression of VDR is decreased in SSc

The mRNA levels of VDR were reduced in lesional skin of SSc patients compared with matched healthy individuals (figure 1A). The decreased expression of VDR in SSc skin was confirmed on the protein level by immunofluorescence. Double staining with

Figure 1 Vitamin D receptor (VDR) expression is decreased in systemic sclerosis (SSc) and in experimental fibrosis. (A) The mRNA levels of VDR mRNA are significantly reduced in SSc skin as compared with healthy skin (n=5 each). (B) Immunofluorescence staining for VDR with co-staining for vimentin and DAPI demonstrated a reduced staining for VDR in SSc fibroblasts. Representative images are shown at 200- and 1000-fold magnification. (C) Semiquantitative analyses confirmed the downregulation of VDR protein in fibroblasts in SSc skin (n=10). (D) The downregulation of VDR persisted in cultured SSc fibroblasts (n=5). (E and F) mRNA (n=6) (E) and protein (n=5) (F) levels of VDR are decreased in the skin of bleomycin-challenged mice as compared with NaCl injected controls.

the fibroblast marker vimentin demonstrated that SSc fibroblasts express particularly low levels of VDR (figure 1B). Semiquantitative analyses confirmed the pronounced downregulation of VDR in SSc fibroblasts as compared with fibroblasts in healthy skin (figure 1C). Decreased expression of VDR in cultured SSc fibroblasts was maintained even after several passages in vitro (figure 1D). We also observed decreased expression of VDR in experimental models of SSc. Subcutaneous injections with bleomycin reduced the mRNA and protein levels of VDR in murine skin (figure 1E,F). Immunofluorescence with co-staining with vimentin highlighted a similar expression pattern in bleomycin-challenged mice as in human SSc with strongly decreased VDR levels in fibroblasts (see online supplementary figure S1). No correlation was observed between the serum levels of 1,25-(OH)₂D₃ and VDR expression in SSc skin.

TGF-β downregulates the expression of VDR

We next investigated the molecular mechanisms underlying the downregulation of VDR in SSc. TGF-β is a key mediator of fibroblast activation in fibrotic diseases and SSc fibroblasts are characterised by endogenous and persistent activation of TGF-β signalling. We thus speculated that TGF-β may contribute to the decreased expression of VDR. Indeed, persistent exposure of healthy dermal fibroblasts to TGF-β significantly reduced the expression levels of VDR (figure 2A,B). Stimulation with TGF-β induced an initial peak of VDR mRNA and protein. However, exposure to chronically increased levels of TGF-β as in fibrotic disorders significantly decreased the mRNA and protein levels VDR with VDR protein becoming almost undetectable after 96 h (figure 2B). To confirm the inhibitory effects of TGF-β in vivo, we

analysed the levels of VDR in the skin of mice overexpressing TGF-β receptor I (TBRI^{CA}). Consistent with the in vitro results, the mRNA and protein levels of VDR were decreased in TBRI^{CA} mice compared with LacZ control mice (figure 2C,D). Immunofluorescence confirmed the downregulation of VDR in dermal fibroblasts of TBRI^{CA} mice (see online supplementary figure S2). Moreover, the expression levels of VDR correlated inversely with the levels of pSmad3 as an indicator of active canonical TGF-β in bleomycin- and TBRI^{CA}-induced fibrosis (see online supplementary figures S3 and S4). To further demonstrate that the decrease of VDR in SSc is mediated by TGF-β, we analysed the expression of VDR in bleomycin-challenged mice treated with the selective TGF-β receptor type I inhibitor SD-208. Treatment with SD-208 prevented the bleomycin-induced downregulation of VDR (figure 2E), thereby highlighting that TGF-β signalling is essentially required to downregulate VDR in fibrosis.

VDR negatively regulates TGF-β-induced fibroblast activation

To investigate the functional effects of the decreased expression of VDR, we inactivated VDR in healthy dermal fibroblasts by siRNA. siRNA against VDR effectively decreased the expression of VDR. Knockdown of VDR enhanced the sensitivity of fibroblasts to the stimulatory effects of TGF-β. The stimulatory effects of TGF-β on col1a1 mRNA (figure 3A) on the release of collagen protein (figure 3B) and on the formation of stress fibres (figure 3C) were more pronounced in fibroblasts deficient of VDR than in fibroblasts transfected with non-targeting siRNA.

In contrast, activation of VDR signalling by paricalcitol, a highly selective VDR agonist, inhibited TGF-β signalling in

Figure 2 Persistent TGF- β stimulation decreases the expression of vitamin D receptor (VDR). (A and B) Chronic stimulation with transforming growth factor- β (TGF- β) results in persistent downregulation of VDR mRNA (n=4) (A) and protein (B) after an initial peak. (C and D) Overexpression of TGF- β receptor I (TBRI^{CA}) significantly reduces mRNA (n=5) (C) and protein (n=5) (D) levels of VDR in murine skin. (E) Treatment with the selective TBRI inhibitor SD-208 prevents downregulation of VDR mRNA in bleomycin-induced skin fibrosis (n=5).

fibroblasts. Treatment of TGF- β -stimulated fibroblasts with paricalcitol completely prevented the upregulation of col 1a1 mRNA (figure 3D), release of collagen protein (figure 3E) and formation of stress fibres (figure 3F) by TGF- β . However, paricalcitol did not alter the basal expression of collagen in resting fibroblasts, indicating that paricalcitol selectively interferes with TGF- β signalling.

To analyse whether VDR and paricalcitol interfere with TGF- β signalling by regulation of Smad-dependent transcription, we first performed Smad-reporter assays using human fibroblasts transfected with a luciferase construct under the control of four Smad-binding elements sites. Treatment with paricalcitol significantly reduced TGF- β -induced luciferase activity, but did not reduce the basal activity of the Smad-reporter (figure 4A). Consistent with the results of the Smad-reporter, paricalcitol also inhibited the stimulatory effects of TGF- β on the expression of Smad target genes such as PAI-1 (figure 4B), Smad7 (figure 4C) and connective tissue growth factor (CTGF) (figure 4D). To analyse whether activated VDR directly interacts with Smad proteins to repress Smad-dependent transcription, we perform Co-IPs in fibroblasts overexpressing c-myc-tagged VDR. As Smad3 is the central R-Smad for the pro-fibrotic effects of TGF- β in fibroblasts, we first analysed a potential interaction of VDR with Smad3. In the absence of paricalcitol, VDR interacted with Smad3, but not with pSmad3. However, activation of VDR by paricalcitol induced binding of VDR to pSmad3 and also increased the total levels of Smad3 that were coimmunoprecipitated with VDR. Thus, paricalcitol may inhibit TGF- β signalling by inducing binding of VDR to pSmad3 (figure 4E).

Treatment with paricalcitol ameliorates experimental fibrosis

We next evaluated the antifibrotic effects of paricalcitol in bleomycin-induced skin fibrosis. Challenge with bleomycin induced leukocyte infiltration and accumulation of collagen with

prominent dermal thickening (figure 5A). Preventive treatment with paricalcitol effectively reduced bleomycin-induced fibrosis. Dermal thickening (figure 5B), differentiation of resting fibroblasts into metabolically active myofibroblasts (figure 5C), hydroxyproline content (figure 5D) and the mRNA levels of col1a1 (figure 5E) were all reduced in bleomycin-challenged mice treated with paricalcitol as compared with vehicle-treated, bleomycin-challenged mice.

However, in contrast to its effects in wild-type mice expressing normal levels of VDR, paricalcitol did not reduce fibrosis in VDR-deficient mice (figure 5A–E). This finding confirms that the antifibrotic effects of paricalcitol are mediated by activation of VDR and not by off-target mechanisms.

To evaluate paricalcitol in another model with different underlying pathomechanisms, we used the mouse model of TBRI^{CA}-induced fibrosis. Preventive treatment with paricalcitol exerted potent antifibrotic effects (figure 6A) and significantly ameliorated TBRI^{CA}-induced dermal thickening (figure 6B), decreased myofibroblast differentiation (figure 6C), reduced the hydroxyproline content (figure 6D) and the mRNA levels of col1a1 (figure 6E).

To analyse whether activation of VDR signalling may also induce be effective in therapeutic dosing regimes, we evaluated the effects of paricalcitol in a modified model of pre-established, bleomycin-induced fibrosis. Indeed, when treatment with paricalcitol was initiated after 3 weeks of prechallenge with bleomycin, it did prevent further progression of fibrosis, and induced regression of fibrosis and reduced the dermal thickness, myofibroblast counts and hydroxyproline content to below pretreatment levels represented by placebo-treated mice injected with bleomycin for 3 weeks followed by injections of NaCl for another 3 weeks (see online supplementary figure S5).

Of note, paricalcitol was well tolerated in antifibrotic doses. No evidence of toxicity was observed on regular clinical examinations or during necropsy.

Figure 3 Vitamin D receptor (VDR) negatively regulates transforming growth factor-β (TGF-β)-induced fibroblast activation. (A–C) siRNA-mediated knockdown of VDR enhances the sensitivity of fibroblasts towards the pro-fibrotic effects of TGF-β with increased levels of col1a1 mRNA (n=7) (A), collagen protein (n=6) (B) and enhanced stress fibre formation (C) upon stimulation with TGF-β compared with fibroblasts transfected with non-targeting siRNA. (D–F) Treatment with the VDR agonist paricalcitol prevents TGF-β-induced induction of col1a1 mRNA (n=6) (D), collagen protein (n=6) (E) and stress fibre formation (n=12) (F).

DISCUSSION

We demonstrate in the present study that VDR signalling is impaired in SSc with strongly reduced expression levels of VDR in SSc fibroblasts and murine models of SSc. We further demonstrate that the reduction of VDR is mediated by TGF-β.

Persistent activation of TGF-β signalling decreased the expression of VDR, whereas selective inhibition of TGF-β signalling prevented the downregulation of VDR in experimental fibrosis. Consistent with endogenous activation of TGF-β signalling in cultured SSc fibroblasts⁴³ and the inhibitory effects of TGF-β on

Figure 4 Vitamin D receptor (VDR) inhibits Smad3-dependent transcription. (A) Incubation with paricalcitol inhibits the stimulatory effects of transforming growth factor-β (TGF-β) on luciferase activity under the control of Smad-binding elements (SBE) (n=5). (B–D) Treatment with paricalcitol reduces the expression of the prototypical Smad target genes PAI-1 (n=6) (B), Smad7 (n=6) (C) and connective tissue growth factor (CTGF) (n=6) (D). (E) Coimmunoprecipitation demonstrates that paricalcitol stimulates binding of VDR to phosphorylated Smad3 in TGF-β-stimulated fibroblasts (n=4).

Figure 5 Paricalcitol prevents bleomycin-induced skin fibrosis. Treatment with paricalcitol ameliorated the histological features of fibrosis (A), reduced dermal thickening (B), prevented the differentiation of resting fibroblasts into myofibroblasts (C), decreased the hydroxyproline content (D) and normalised the mRNA levels of col 1a1 (E) in wild-type mice, but not in vitamin D receptor (VDR)-deficient mice. Representative images of H&E- and trichrome-stained sections are shown at 200-fold magnification in (A) (n=6–8 per group).

Figure 6 Paricalcitol ameliorates transforming growth factor- β (TGF- β) receptor I (TBR1^{CA})-induced skin fibrosis. Paricalcitol reduces TBR1^{CA}-induced skin fibrosis (A) and reduces dermal thickening (B), myofibroblast differentiation (C), hydroxyproline content (D) and col 1a1 mRNA (E). Representative images of H&E- and trichrome-stained sections are shown at 200-fold magnification in (A) (n=6 per group).

VDR, the levels of VDR were also reduced in cultured SSc fibroblasts even after several passages in culture. However, VDR signalling in SSc is disturbed at the level of the receptor and vitamin D₃; the precursor of its endogenous ligand 1,25-(OH)₂D₃, is decreased in SSc. Several independent studies from different countries with more than 1000 SSc patients in total reported significantly reduced levels of vitamin D₃ in patients with SSc as compared with age- and sex-matched healthy individuals.^{9 11–15} Moreover, reduced VDR signalling is indirectly supported by the high prevalence of osteopenia and osteoporosis in SSc patients.^{15 44 45} Together, these data highlight severely impaired VDR signalling in SSc.

Our studies identify VDR as a negative regulator of fibroblast activation that interferes with the pro-fibrotic effects of TGF-β. Activated VDR binds to phosphorylated Smad3 and blocks Smad-dependent transcription (see supplementary figure S6). The reduced expression of VDR in SSc together with the decreased levels of its ligand vitamin D₃ thus impairs the regulatory effects of VDR signalling and enhances the sensitivity of SSc fibroblasts to the pro-fibrotic effects of TGF-β. Indeed, healthy fibroblasts become more sensitive to TGF-β and release more collagen upon knockdown of VDR.

Our results are supported by clinical observations. A recent study demonstrated an inverse correlation of the serum levels of vitamin D₃ and the modified Rodnan Skin Score,⁹ thereby further highlighting the causal relationship of impaired VDR signalling and fibrosis in SSc.

The simultaneous deficiency in VDR and its ligand in fibroblasts suggest that supplementation with standard doses of vitamin D₃ will not be sufficient to normalise VDR signalling in SSc. Indeed, supplementation with standard doses of vitamin D failed to correct the decreased vitamin D levels and restore VDR signalling in SSc patients.¹⁶ Very high doses of vitamin D₃ or 1,25-(OH)₂D₃, on the other hand, may cause hypercalcemia and hyperphosphatemia as dose-limiting toxicity. However, these concerns can be overcome by the use of novel synthetic VDR agonists such as paricalcitol. Paricalcitol activates VDR more potently than 1,25-(OH)₂D₃ and has a significantly decreased risk of hypercalcemia and hyperphosphatemia.

Indeed, antifibrotic doses of paricalcitol were well tolerated in our study without evidence of toxicity on clinical monitoring or on necropsy. Treatment with paricalcitol restored VDR signalling in SSc fibroblasts, inhibited Smad-dependent transcription and reduced the pro-fibrotic effects of TGF-β on fibroblasts in vitro. Preventive as well as therapeutic treatment with paricalcitol also effectively ameliorated fibrosis in two murine models with different underlying pathologies representing different stages of SSc patients.⁴⁶ Moreover, VDR agonists may also be effective in other fibrotic diseases. Indeed, VDR agonists have recently been shown to prevent activation of hepatic stellate cells in liver cirrhosis and epithelial-to-mesenchymal transition in kidney fibrosis.^{47–50} VDR agonists may also modulate the outcome of fibrotic diseases by regulating T cell differentiation and B cell activation.⁶ Although further studies in additional model systems are required, our findings have direct translational implications because paricalcitol is approved for clinical use. Moreover, several other VDR agonists that likely resemble the antifibrotic effects of paricalcitol such as 20S-hydroxyvitamin D₃ are under clinical investigation and showed promising effects in murine models.⁵¹

In summary, we demonstrate that VDR is a negative regulator of TGF-β signalling. Impaired VDR signalling with reduced levels of vitamin D₃ and decreased expression of VDR results in hypersensitivity of SSc fibroblasts to TGF-β signalling and may

contribute to the uncontrolled activation of fibroblasts in SSc. In contrast, activation of VDR with paricalcitol inhibits TGF-β signalling and ameliorates experimental fibrosis (see supplementary figure 65). These findings may have clinical implications, considering the availability of well-tolerated, non-hypercalcemic VDR agonists for clinical trials.

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Pawel Zerr, Stefan Vollath, Katrin Palumbo-Zerr, Michal Tomcik, Jingang Huang, Alfiya Distler, Christian Beyer, Clara Dees, Kolja Gela, Oliver Distler, Georg Schett and Jörg H W Distler

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