

β -Catenin Mediates Mechanically Regulated, Transforming Growth Factor- β 1-Induced Myofibroblast Differentiation of Aortic Valve Interstitial Cells

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Objective—In calcific aortic valve disease, myofibroblasts and activation of the transforming growth factor- β 1 (TGF- β 1) and Wnt/ β -catenin pathways are observed in the fibrosa, the stiffer layer of the leaflet, but their association is unknown. We elucidated the roles of β -catenin and extracellular matrix stiffness in TGF- β 1-induced myofibroblast differentiation of valve interstitial cells (VICs).

Methods and Results—TGF- β 1 induced rapid β -catenin nuclear translocation in primary porcine aortic VICs in vitro through TGF- β receptor I kinase. Degrading β -catenin pharmacologically or silencing it with small interfering RNA inhibited TGF- β 1-induced myofibroblast differentiation without altering Smad2/3 activity. Conversely, increasing β -catenin availability with Wnt3A alone did not induce differentiation. However, combining TGF- β 1 and Wnt3A caused greater myofibroblast differentiation than TGF- β 1 treatment alone. Notably, in VICs grown on collagen-coated PA gels with physiological stiffnesses, TGF- β 1-induced β -catenin nuclear translocation and myofibroblast differentiation occurred only on matrices with fibrosa-like stiffness, but not ventricularis-like stiffness. In diseased aortic valves from pigs fed an atherogenic diet, myofibroblasts colocalized with increased protein expression of Wnt3A, β -catenin, TGF- β 1, and phosphorylated Smad2/3 in the fibrosa.

Conclusion—Myofibroblast differentiation of VICs involves matrix stiffness-dependent crosstalk between TGF- β 1 and Wnt signaling pathways and may explain in part why the stiffer fibrosa is more susceptible to disease. (*Arterioscler Thromb Vasc Biol.* 2011;31:00-00.)

Key Words: heart valves ■ Wnt ■ β -catenin ■ extracellular matrix mechanics ■ myofibroblast ■ transforming growth factor- β

Calcific aortic valve disease (CAVD) is the most common valve disease in North America and Europe and is among the most frequent of all cardiovascular diseases.¹ A hallmark of CAVD is increased myofibroblast differentiation of valve interstitial cells (VICs) in lesions,² marked by increased α -smooth muscle actin (α SMA) expression and its incorporation into actin stress fibers.³ Increased myofibroblast presence is associated with increased valve tissue remodeling, fibrosis,⁴ and calcification.⁵ Notably, sclerotic lesions form preferentially in the fibrosa,^{6,7} the stiffer layer of the aortic valve leaflet.^{8,9} Aortic VICs contain a large subpopulation of multipotent mesenchymal progenitors,¹⁰ and their pathological differentiation is regulated by not only biochemical cues but also extracellular matrix stiffness.¹¹ However, the molecular mechanisms involved in VIC myofibroblast differentiation and the cause of the preferential occurrence of myofibroblasts in the fibrosa are not clear.

Transforming growth factor- β 1 (TGF- β 1)/Smad signaling is activated in CAVD.⁵ TGF- β 1 has been shown to induce VIC myofibroblast differentiation in a matrix stiffness-dependent

manner.¹² However, the mechanisms by which TGF- β 1 induces VIC myofibroblast differentiation and the role of matrix stiffness are not fully established. TGF- β 1 binds to TGF- β receptor I and II (TGF- β RI and II). On binding, TGF- β RI kinase is activated to phosphorylate Smad2/3 (pSmad2/3), which subsequently translocates to cell nucleus to regulate transcriptional activity. Although Smad2/3 phosphorylation and nuclear translocation are critical to TGF- β 1-induced myofibroblast differentiation, Smad proteins alone are not sufficient to activate transcription. TGF- β signaling relies on its crosstalk with other signaling pathways and recruitment of other transcriptional factors to regulate transcriptional responses.^{13–16}

A signaling pathway that crosstalks with TGF- β /Smad signaling is the canonical Wnt pathway. For example, TGF- β 1 induces rapid nuclear translocation of β -catenin, a central component of Wnt signaling, to modulate proliferation and osteogenic differentiation of mesenchymal progenitor cells.¹⁷ Notably, TGF- β 1-induced β -catenin nuclear translocation is cell type dependent.¹⁷ Wnt3A and β -catenin expression are upregulated

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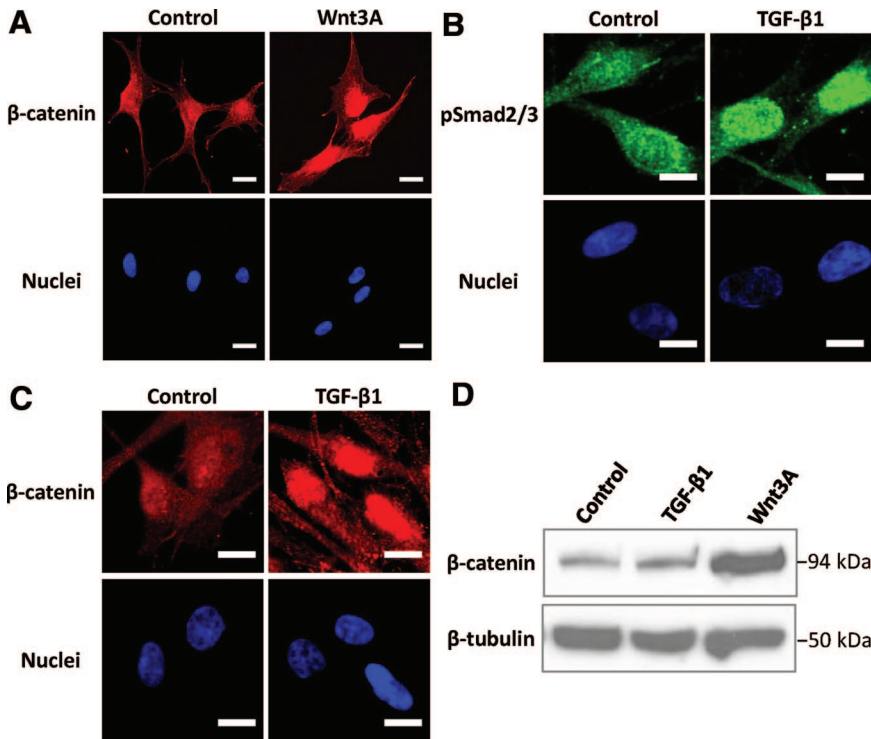


Figure 1. TGF- β 1 induces β -catenin nuclear translocation in VICs. A, Wnt3A-conditioned medium induced β -catenin nuclear translocation in VICs after 2 hours of incubation. B and C, After stimulation of VICs with 5 ng/mL TGF- β 1 for 2 hours, not only did pSmad2/3 translocate into the nuclei, indicating the activation of TGF- β 1 signaling, but β -catenin also translocated into the nuclei. D, Unlike Wnt3A-conditioned medium, TGF- β 1 only mildly increased β -catenin levels in VICs after 2 hours of treatment. Scale bars, 20 μ m.

in CAVD, where they have been hypothesized to mediate VIC osteogenic differentiation.¹⁸ However, the role of β -catenin in regulating cell differentiation may not be limited to osteogenic processes and appears to be context-dependent. For example, in the presence of TGF- β 1, Wnt3A induction of the osteogenic transcription factor Runx2 is inhibited in C3H10T1/2 cells, and instead myofibroblast genes, SM22 α and α SMA, are upregulated through a β -catenin dependent mechanism.¹⁹ β -Catenin has also been shown to regulate myofibroblast differentiation in endothelial cells,²⁰ epithelial cells,²¹ renal fibroblasts,²² and lung fibroblasts.^{23,24} Moreover, we²⁵ and others^{26,27} have shown that β -catenin activity can be modulated by mechanical forces, suggesting that β -catenin may mediate mechanically regulated signal transduction. Taking this evidence together, we hypothesized that β -catenin mediates TGF- β -induced VIC myofibroblast differentiation in a matrix stiffness-dependent manner.

In this report, we demonstrate that (1) β -catenin is required for TGF- β 1-induced VIC myofibroblast differentiation, and (2) TGF- β 1-induced β -catenin nuclear translocation and myofibroblast differentiation are dependent on matrix stiffness, occurring only on matrices with stiffness that mimics that of the fibrosa. These results provide new insights into the molecular mechanisms involved in VIC myofibroblast differentiation and suggest a novel mechanics-based mechanism that may explain in part why the stiffer fibrosa is more susceptible to disease.

Methods

An expanded Methods section is available online at <http://atvb.ahajournals.org>.

Porcine hearts were obtained from 8-month-old pigs from a local abattoir (Quality Meat Packers, Toronto, Ontario, Canada). For each experiment, VICs were isolated from a minimum of 10 hearts. Experiments were repeated at least 3 times for each study. VICs were routinely maintained in complete medium (Dulbecco's modified Eagle's medium with 10% fetal bovine serum [Hyclone, Logan,

Utah] and 1% penicillin and streptomycin) at 37°C in a humidified incubator with 5% CO₂.

To study β -catenin and pSmad2/3 activity, freshly isolated VICs were plated at 10⁴ cells/cm² in complete medium for 2 days before experiments. For TGF- β 1 treatment, 5 ng/mL TGF- β 1 (100-21C, PeproTech, Embury, Ontario, Canada) in complete medium was applied to VICs for 2 hours. For SD208 treatment, VICs were pretreated with 2 μ mol/L SD208 in complete medium for 12 hours before applying TGF- β 1 with SD208 in complete medium for another 2 hours. For endostatin treatment, VICs were pretreated with 15 μ g/mL endostatin (CLPRO248-3, Cedarlane, Burlington, Ontario, Canada) in complete medium for 12 hours before application of TGF- β 1 with endostatin in complete medium for another 2 hours. As a complementary approach, VICs were transfected with small interfering RNAs (siRNAs) targeting β -catenin using N-TER nanoparticle siRNA transfection system (N2913, Sigma-Aldrich, Oakville, Ontario, Canada). For experiments using Wnt3A-conditioned medium, L-cell-conditioned medium was used as control and TGF- β 1 was diluted in Wnt3A-conditioned medium or L-cell-conditioned medium to 5 ng/mL. VICs were treated with conditioned medium with or without TGF- β 1 for 2 hours. In complementary experiments, VICs were treated with 15 ng/mL recombinant Wnt3A protein. To study VIC myofibroblast differentiation, VICs were plated at 10⁴ cells/cm² in complete medium for 12 hours before experiments. TGF- β 1, SD208, endostatin, and Wnt3A treatments were the same as above, except VICs were treated for 48 hours instead of 2 hours.

Polyacrylamide (PA) gels coated with type I collagen were used for matrix stiffness-dependent studies. Micropipette aspiration was used to measure PA gel and aortic valve tissue layer-specific stiffness.

Results

TGF- β 1 Induces β -Catenin Nuclear Translocation in VICs

In control culture conditions, β -catenin was kept mostly in the cytoplasm at low levels in VICs (Figure 1A). As expected, Wnt3A-conditioned medium induced significant nu-

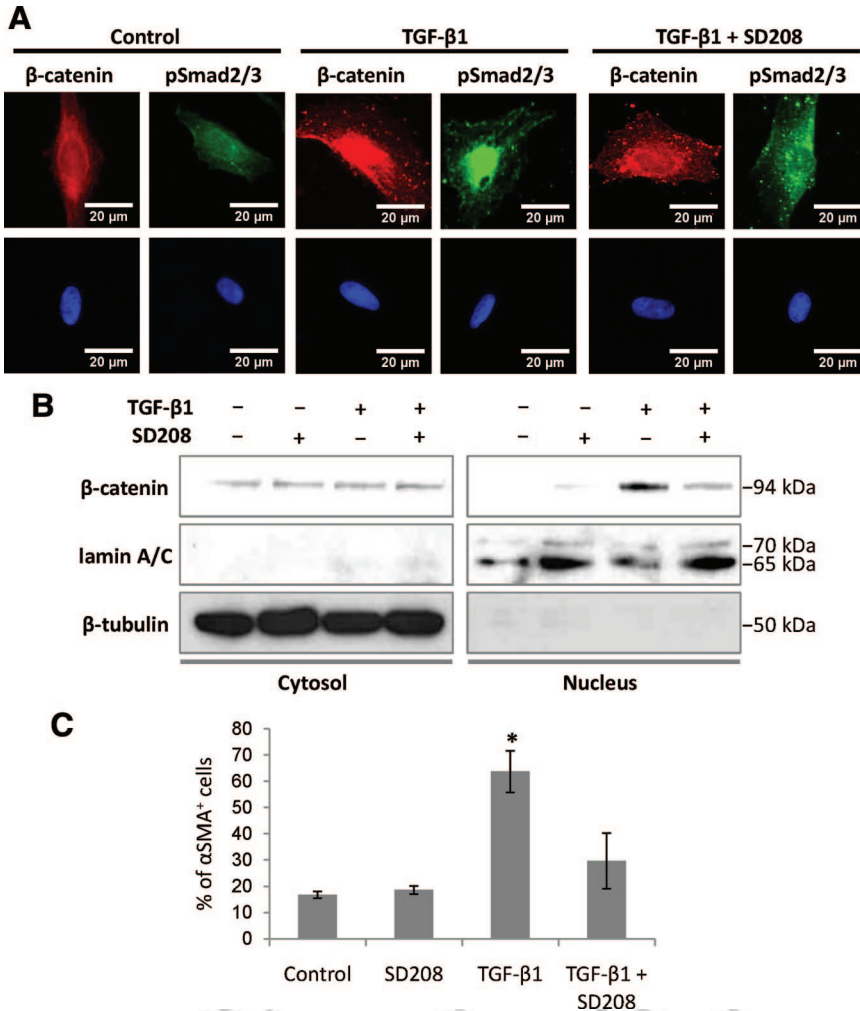


Figure 2. TGF- β 1-induced β -catenin nuclear translocation and myofibroblast differentiation occurs through TGF- β RI. A and B, Inhibiting TGF- β RI using 2 μ mol/L SD208 inhibited not only pSmad2/3 nuclear translocation but also β -catenin nuclear translocation. C, SD208 treatment inhibited TGF- β 1-induced myofibroblast differentiation in VICs. * P <0.05, n =3 per group. Scale bars, 20 μ m.

clear translocation of β -catenin in VICs after 2 hours of treatment (Figure 1A). Interestingly, TGF- β 1 not only induced phosphorylated Smad2/3 (pSmad2/3) nuclear translocation (Figure 1B), indicating that TGF- β 1 signaling was activated, but also induced significant nuclear translocation of β -catenin after 2 hours of treatment (Figure 1C). In contrast to treatment with Wnt3A-conditioned medium, however, TGF- β 1 did not significantly increase the total β -catenin level in VICs (Figure 1D).

TGF- β 1 Induces β -Catenin Nuclear Translocation and Myofibroblast Differentiation Through TGF- β Type I Receptor

To investigate the mechanism by which TGF- β 1 induces β -catenin nuclear translocation, VICs were pretreated for 12 hours with SD208, a highly specific inhibitor for TGF- β RI kinase,²⁸ before application of 5 ng/mL TGF- β 1 for another 2 hours. Not only did SD208 treatment block nuclear translocation of pSmad2/3, demonstrating the effective inhibition of TGF- β 1 signaling by SD208, but it also inhibited the nuclear translocation of β -catenin (Figure 2A and 2B). These results indicate that TGF- β 1-induced β -catenin nuclear translocation was mediated by TGF- β RI kinase activity.

We also found that TGF- β RI kinase activity was required for TGF- β 1-induced myofibroblast differentiation in VICs.

Without SD208, TGF- β 1 significantly increased the percentage of α SMA-positive myofibroblasts (P <0.05) after 48 hours, as expected. However, TGF- β 1-induced myofibroblast differentiation was inhibited by SD208 (P <0.05) (Figure 2C and Supplemental Figure I).

TGF- β 1-Induced Myofibroblast Differentiation Depends on β -Catenin Availability

To investigate the role of β -catenin in TGF- β 1-induced myofibroblast differentiation, β -catenin availability was manipulated using endostatin or Wnt3A-conditioned medium. Endostatin treatment significantly decreased the β -catenin level in the cytoplasm and therefore reduced TGF- β 1-induced β -catenin nuclear translocation (Figure 3A). However, Smad2/3 phosphorylation and nuclear translocation were not affected by the endostatin treatment (Figure 3A). Strikingly, although pSmad2/3 was not altered, endostatin treatment significantly inhibited TGF- β 1-induced myofibroblast differentiation (Figure 3B and Supplemental Figure II) and α SMA transcription (Figure 3C). To confirm the role of β -catenin in mediating TGF- β 1-induced myofibroblast differentiation, siRNA was used to knock down β -catenin expression. After transfecting VICs with siRNAs for 24 hours, the expression of β -catenin was significantly reduced (Figure 3D). Immediately after siRNA treatment, TGF- β 1

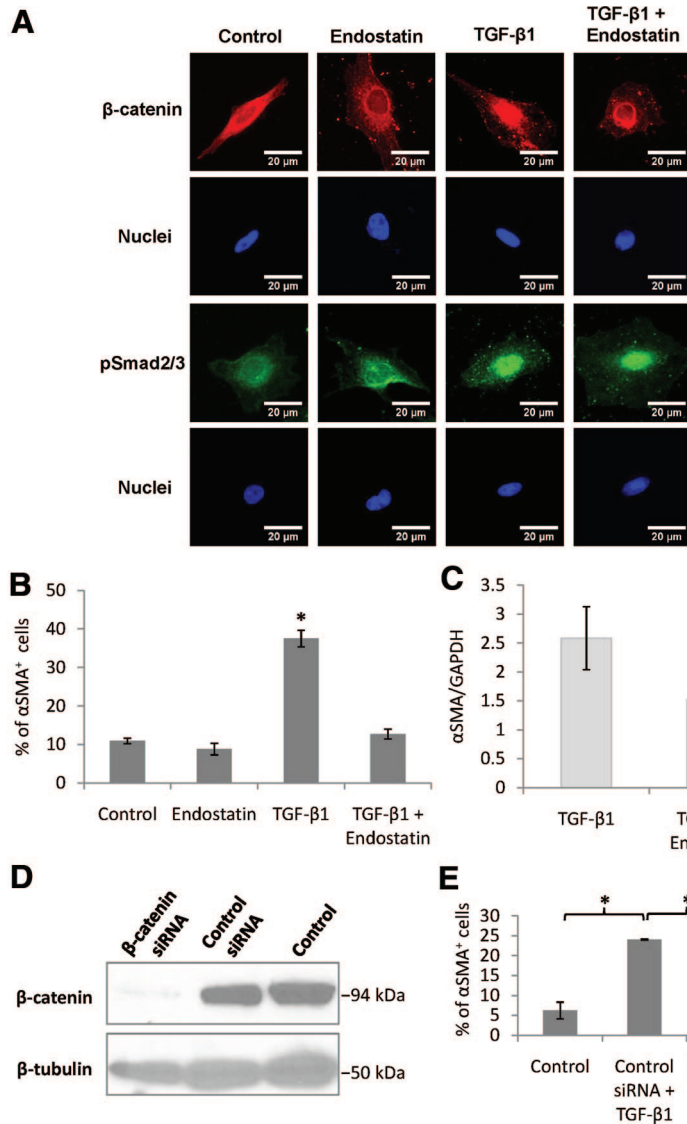


Figure 3. Reducing β -catenin availability inhibits TGF- β 1-induced myofibroblast differentiation. A, Degrading cytoplasmic β -catenin using 15 μ g/mL endostatin reduced TGF- β 1-induced β -catenin nuclear translocation without altering pSmad2/3 nuclear translocation. B and C, Endostatin treatment significantly inhibited TGF- β 1-induced myofibroblast differentiation (B) and α SMA transcription (C). D, Transfecting VICs with siRNAs targeting β -catenin significantly reduced β -catenin protein level after 24 hours. E, Silencing β -catenin significantly inhibited TGF- β 1-induced myofibroblast differentiation. * P <0.05, n=3 per group. Scale bars, 20 μ m.

was applied for 48 hours to induce myofibroblast differentiation. As expected, β -catenin siRNA treatment significantly inhibited TGF- β 1-induced myofibroblast differentiation (Figure 3E). These results indicate that β -catenin is required for TGF- β 1-induced myofibroblast differentiation of VICs.

To further investigate the role of β -catenin in TGF- β 1-induced myofibroblast differentiation, Wnt3A-conditioned medium was used to increase the availability of β -catenin. Wnt3A significantly increased β -catenin levels in the cytoplasm and nucleus but had no effect on Smad3 phosphorylation and nuclear translocation (Figure 4A). When TGF- β 1 and Wnt3A were both present, they synergistically induced greater β -catenin nuclear translocation (Figure 4A). Interestingly, whereas Wnt3A treatment alone failed to induce α SMA transcription ($P=1.0$) and myofibroblast differentiation ($P=0.4$) (Figure 4B and 4C), combining Wnt3A with TGF- β 1 induced greater myofibroblast differentiation (Figure 4B, Supplemental Figure III) and α SMA transcription (Figure 4C) than TGF- β 1 treatment alone (P <0.05). A similar result was obtained when VICs were treated with 15 ng/mL recombinant Wnt3A instead of conditioned medium

(Supplemental Figure IV). These results suggest that the crosstalk between TGF- β 1 and Wnt3A signaling pathways regulates VIC myofibroblast differentiation and that the extent of TGF- β 1-induced α SMA transcription and myofibroblast differentiation is dependent on the availability of β -catenin.

Myofibroblasts Colocalize With Wnt and TGF- β Signaling Components in Sclerotic Aortic Valves

We examined normal and diseased porcine aortic valve leaflets for the spatial distributions of myofibroblasts and TGF- β and Wnt signaling components in situ. In normal aortic valves, α SMA expression was not detectable, and TGF- β and Wnt signaling components were absent. However, in serially sectioned diseased aortic valve leaflets from hypercholesterolemic pigs, there were abundant α SMA-positive cells colocalized with TGF- β 1, pSmad2/3, Wnt3A, and β -catenin proteins primarily in lesions in the fibrosa (Figure 5). These results confirm that myofibroblast differentiation occurs preferentially in the fibrosa and suggest the association between TGF- β and Wnt signaling pathways and

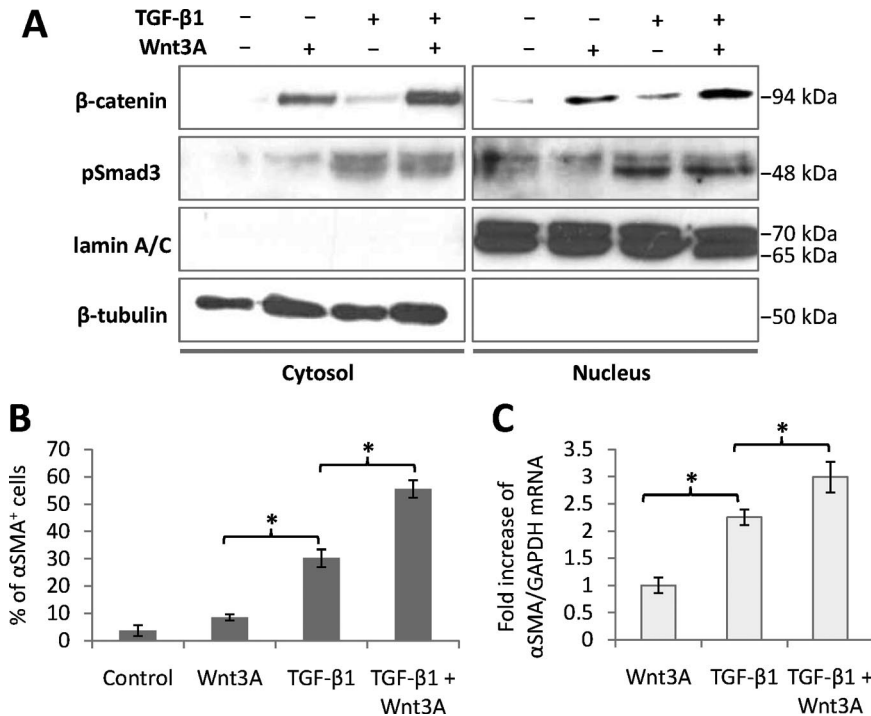


Figure 4. TGF- β 1 and Wnt3A synergistically induce myofibroblast differentiation and α SMA transcription. A, Wnt3A-conditioned medium increased both cytosolic and nuclear β -catenin levels but had no effect on Smad3 phosphorylation and nuclear translocation. TGF- β 1 and Wnt3A synergistically induced greater β -catenin nuclear translocation. B and C, Wnt3A-conditioned medium alone did not induce myofibroblast differentiation or α SMA transcription. However, combining Wnt3A with 5 ng/mL TGF- β 1 caused greater myofibroblast differentiation (B) and α SMA transcription (C) than TGF- β 1 treatment alone. * $P < 0.05$, $n = 3$ per group.

their involvement in VIC myofibroblast differentiation early in disease development.

TGF- β 1-Induced β -Catenin Nuclear Translocation and Myofibroblast Differentiation Depend on Matrix Stiffness

To investigate the effect of the local mechanical environment on TGF- β 1-induced β -catenin nuclear translocation and myofibroblast differentiation, VICs were seeded on type I collagen-coated PA gels with a range of stiffness (compressive moduli of 3, 11, 22, 50, and 144 kPa) and glass coverslips. We found that there was a stepwise increase of β -catenin nuclear translocation with increasing stiffness (Figure 6A) after 2 hours of TGF- β 1 treatment. VIC response to TGF- β 1 was similar on 3- and 11-kPa gels ($P = 0.58$), with β -catenin nuclear translocation occurring in less than 10% of VICs. TGF- β 1-induced β -catenin nuclear translocation increased significantly ($P < 0.01$) to $\approx 40\%$ of VICs on 22-kPa gels, which was not different than the percentage on 50-kPa gels ($P = 0.21$). The percentage of VICs that responded to TGF- β 1 increased again to $\approx 70\%$ on the 144-kPa gels ($P < 0.01$) but did not increase on the glass coverslips ($P = 0.11$).

VIC myofibroblast differentiation, indicated by cells with α SMA⁺ stress fibers, in response to TGF- β 1 over this range of stiffness followed the same trend as the percentage of cells with β -catenin nuclear translocation in the presence of TGF- β 1 (Figure 6B). On the soft matrices (3 and 11 kPa), there was no significant increase in the proportion of myofibroblasts relative to the control (no TGF- β 1) condition; on the medium-stiffness matrices (22 and 50 kPa), the fraction of myofibroblasts increased by $\approx 16\%$ ($P < 0.05$); and on the stiff matrices (144 kPa and glass), the percentages increased by $\approx 40\%$ ($P < 0.01$). This stepwise increase of myofibroblast

differentiation with increasing matrix stiffness was not observed when β -catenin expression was silenced using siRNA (Figure 6B). These data indicate that TGF- β 1-induced β -catenin nuclear translocation and myofibroblast differentiation depend on matrix stiffness.

To determine the in vivo relevancy of the PA gel stiffness range tested in vitro, we used micropipette aspiration to measure and directly relate PA gel stiffness to the local stiffness of aortic valve leaflet tissue. Local valve extracellular matrix stiffness is heterogeneous both within a layer and between the layers of porcine aortic valve leaflets.⁹ Although there is significant overlap between the fibrosa and ventricularis stiffness ranges, the fibrosa is significantly stiffer than the ventricularis on average ($P < 0.05$) and contains several focal regions that are stiffer than any in the ventricularis (Figure 6C). Moreover, when we compared these fibrosa and ventricularis stiffness ranges to the PA gel stiffness range, we found that there were regions in the fibrosa that had stiffness comparable to the 22-kPa PA gels, whereas the ventricularis stiffness range was entirely below 22 kPa. Notably, 22 kPa was the stiffness required for significant β -catenin nuclear translocation and myofibroblast differentiation in VICs in the presence of TGF- β 1 (Figure 6A and 6B). These results suggest that stiff focal regions in the fibrosa provide a local mechanical environment that is permissive, and therefore more susceptible, to VIC myofibroblast differentiation.

Discussion

β -Catenin is a central component of the canonical Wnt signaling pathway. Although the Wnt/ β -catenin signaling pathway is distinct from the TGF- β /Smad pathway, their interaction has been reported to be important in regulating cell function during development^{29–31} and postnatally.^{32–35} In the present study, we demonstrated a novel role of β -catenin

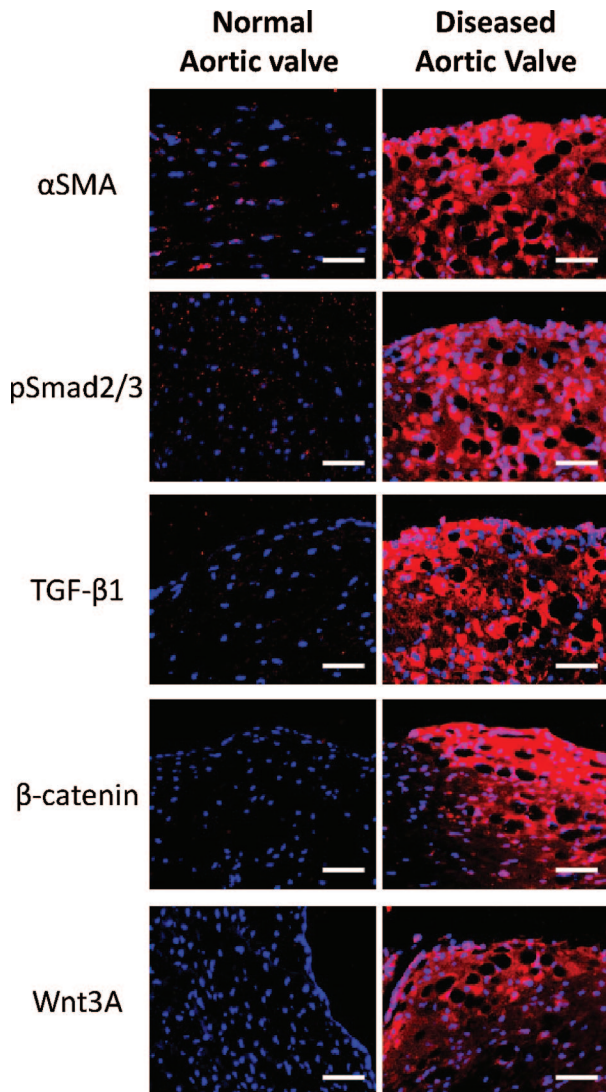


Figure 5. Myofibroblasts colocalize with TGF- β 1 and Wnt3A signaling components in the fibrosa of diseased aortic valves. In normal aortic valves (left column), α SMA expression was not detectable, and TGF- β and Wnt signaling components were absent. However, in diseased aortic valves from hypercholesterolemic pigs (right column, serial sections of a diseased aortic valve leaflet), abundant α SMA-positive cells were colocalized with TGF- β 1, pSmad2/3, Wnt3A, and β -catenin proteins primarily in lesions in the fibrosa. Scale bars, 50 μ m.

in mediating TGF- β 1-induced myofibroblast differentiation in VICs. We showed that TGF- β 1-induced α SMA transcription and myofibroblast differentiation were dependent on the availability of β -catenin. Although Wnt3A alone failed to induce α SMA transcription or myofibroblast differentiation, increasing the β -catenin level using Wnt3A-conditioned medium potentiated the ability of TGF- β 1 to induce greater α SMA transcription and myofibroblast differentiation than TGF- β 1 treatment alone. Consistent with our findings in primary valve fibroblasts, Shafer and Towler recently reported that Wnt3A converges with TGF- β 1 signaling to upregulate SM22 α and α SMA transcription in the C3H10T1/2 mesenchymal progenitor cell line.¹⁹ We also found that in diseased aortic valves, α SMA was upregulated in the fibrosa layer in regions where TGF- β /Smad and

Wnt/ β -catenin signaling components were also upregulated, suggesting that the crosstalk between TGF- β and Wnt signaling pathways may be involved in inducing VIC myofibroblast differentiation in CAVD.

The mechanism by which TGF- β 1 induces β -catenin nuclear translocation in VICs is not clear. Wnt3A induces β -catenin nuclear translocation by inhibiting β -catenin degradation through Dishevelled proteins.³⁶ Unlike Wnt3A, TGF- β 1 only mildly increased the β -catenin level in VICs (Figures 1C and 4A), which is consistent with findings in human mesenchymal progenitor cells.¹⁷ It is possible that TGF- β 1 induces β -catenin nuclear translocation independently of Dishevelled proteins in VICs. Smad3 has been reported to interact with β -catenin to form a protein complex.^{17,37} The formation of a Smad3/ β -catenin complex not only protects β -catenin from proteasome degradation but also facilitates β -catenin nuclear translocation and transcriptional activity.³⁷ Within the nucleus, Smad binding element and lymphoid enhancer binding factor/T-cell factor (the binding sites for Smad3 and β -catenin, respectively) can physically interact on binding to Smad3 and β -catenin to elicit different transcriptional activity from when there is Smad3 or β -catenin binding alone.^{19,29,38} We found that pSmad2/3 and β -catenin nuclear translocation were both required to induce α SMA transcription and myofibroblast differentiation and that Smad2/3 phosphorylation by TGF- β RI kinase was required for TGF- β 1-induced β -catenin nuclear translocation. It is likely that Smad3-dependent β -catenin nuclear translocation mediates TGF- β 1-induced α SMA transcription and myofibroblast differentiation in VICs. However, the mechanism by which β -catenin regulates α SMA promoter activity remains to be determined.

The dependence of TGF- β 1-induced myofibroblast differentiation on matrix stiffness was first demonstrated on floating (soft, \approx 8 kPa³⁹) and anchored (stiff, \approx 20 kPa³⁹) collagen gels in gingival fibroblasts⁴⁰ and VICs.¹² TGF- β 1 induces myofibroblast differentiation only on the stiffer anchored gels. Later, using silicone-based matrices, it was shown that on soft matrices (\leq 11 kPa), cells were not able to form sufficient focal adhesions to support myofibroblast maturation.⁴¹ In this study, we used PA gels to allow fine-tuning and a wider range of stiffness than can be achieved with collagen gels to better study the effect of matrix stiffness. Consistent with previous findings, we found that VICs were not responsive to TGF- β 1 on the soft matrices (3 and 11 kPa). Interestingly, the dependence of TGF- β 1 effects on matrix stiffness were stepwise and nonlinear, suggesting threshold control of TGF- β 1 signaling by matrix stiffness. We found that the TGF- β 1-induced β -catenin nuclear translocation showed a stepwise dependence on matrix stiffness similar to that of the TGF- β 1-induced myofibroblast differentiation. This suggests that matrix stiffness may not only affect the downstream α SMA organization and myofibroblast maturation but also target upstream components of the TGF- β 1 signaling pathway. The molecular mechanisms by which matrix stiffness modulates the effects of TGF- β 1 are not clear, although we previously showed that TGF- β RI expression in VICs is higher on stiffer matrices,¹¹ suggesting a

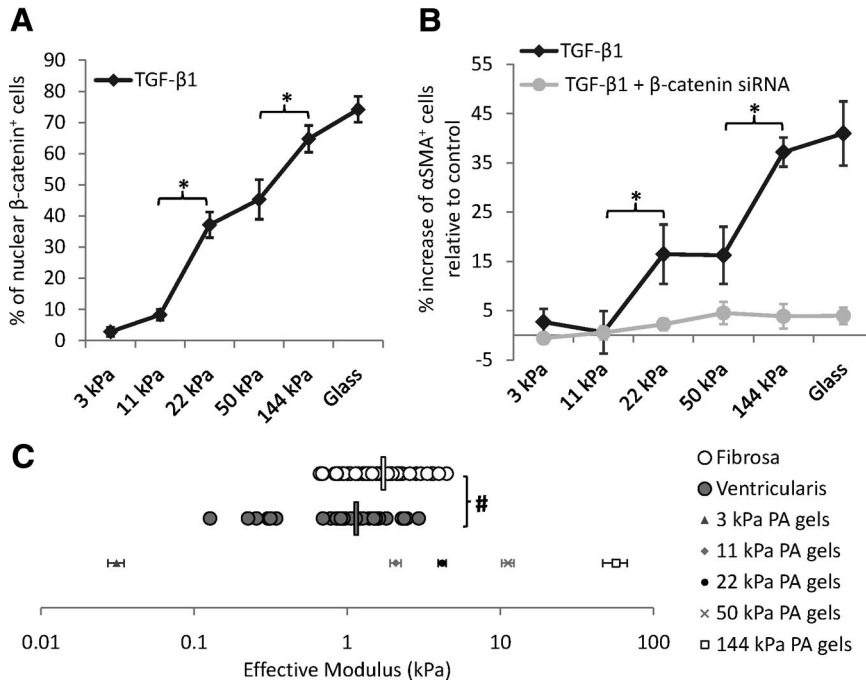


Figure 6. TGF- β 1-induced β -catenin nuclear translocation and myofibroblast differentiation occurs only on matrices of fibrosa-like stiffness. A, TGF- β 1-induced β -catenin nuclear translocation showed a stepwise dependence on matrix stiffness, with the first and second thresholds at 22 and 144 kPa, respectively. B, TGF- β 1-induced myofibroblast differentiation showed a similar stepwise dependence on matrix stiffness, which was not observed when β -catenin expression was silenced. * $P < 0.05$, $n = 3$ per group. C, Using micropipette aspiration, we found that the fibrosa layer is significantly stiffer than the ventricularis layer (from⁹). Moreover, there were regions in the fibrosa, but not in the ventricularis, whose stiffness was similar to the 22-kPa PA gels, the stiffness threshold for TGF- β 1-induced β -catenin nuclear translocation and myofibroblast differentiation. The vertical bars mark the mean stiffness measurements in the fibrosa and the ventricularis groups. PA gel moduli are presented as mean \pm standard error. # $P < 0.01$.

mechanism by which VIC sensitivity to TGF- β 1 can be modulated by matrix stiffness.

Tissue stiffness plays an important role in regulating myofibroblast differentiation in vivo. During wound healing, tissue stiffening by mechanical tension is required for myofibroblast differentiation in granulation tissue.⁴² In liver fibrosis, increases in liver stiffness precede myofibroblast differentiation of hepatic stellate cells.⁴³ The fibrosa layer of normal aortic valves is significantly stiffer than that of the ventricularis,^{8,9} although there is significant spatial heterogeneity within each layer. Importantly, we showed here that the fibrosa layer, but not the ventricularis layer, contained regions with stiffness that was comparable to 22-kPa PA gels, which was the first threshold stiffness for TGF- β 1-induced β -catenin nuclear translocation and myofibroblast differentiation in VICs. Thus, the preferential occurrence of myofibroblasts in the fibrosa layer in CAVD may partially result from intrinsically stiff regions in the fibrosa that provide a local mechanical environment that permits VICs to be more responsive to TGF- β 1. The stiffening of valve leaflets as CAVD progresses may further increase the response of VICs to TGF- β 1.

In conclusion, we demonstrated complex interplay between TGF- β and Wnt signaling pathways and the local mechanical environment in regulating VIC myofibroblast differentiation. Our results showed that β -catenin plays a critical role in mediating TGF- β 1-induced myofibroblast differentiation. Moreover, TGF- β 1-induced β -catenin nuclear translocation and myofibroblast differentiation occur only on the fibrosa-like stiffness. These results may explain in part why focal regions in the stiffer fibrosa are more susceptible to disease.

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Disclosures

None.

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