Hydrogel cultures reveal TRPV4 regulation of myofibroblast activation and proliferation in Valvular Interstitial Cells

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Statement of Purpose: Aortic Valve Stenosis (AVS) is a progressive disease characterized by stiffening and thickening of valve leaflets, leading to disrupted blood flow and eventual left ventricular pressure-overload.[1,2] There are no known effective pharmacological therapeutic options for AVS patients, and surgical or transcatheter valve replacement remains the standard treatment for patients with severe disease. Valvular interstitial cells (VICs) are fibroblast-like cells that make up the main cell population in valve leaflet tissue and are responsible for promoting homeostasis, tissue maintenance and repair.[3–5] Routine mechanical perturbations to valve tissue can cause VICs to activate to myofibroblasts, which are characterized by the assembly of alpha-smooth muscle actin (αSMA) into stress fibers and increased migration, proliferation, and secretion of ECM components.[6] During AVS, myofibroblasts activation persists, causing aberrant accumulation of extracellular matrix (ECM) proteins and a stiffening of the tissue.[6] Calcium signaling through the mechanosensitive Transient Receptor Potential (TRP) channels play regulatory roles in myofibroblast activation through mechanotranduction. Transient Receptor Potential Vanilloid type 4 (TRPV4) is a stretch-activated nonspecific cation channel that has been shown to be a regulator in fibrosis development and is a promising potential therapeutic target for fibrosis. We posit that TRPV4 activity may regulate αSMA production and myofibroblast activation processes in VICs and valve fibrosis.

Methods: Hydrogels were polymerized by mixing 5% 8-arm 20 kDa PEG-DBCO with 2mM PEG4-GRGDSG cell adhesive peptide and 4-arm 10 kDA PEG-Azide at a ratio of 4:1 DBCO to azide groups. To produce soft hydrogels, gel solution was immediately sandwiched between an azide-functionalized coverslip and a Sigmacote (Sigma) treated glass slide. To produce stiff hydrogels, preformed soft hydrogels formed were soaked in a 2 mM photo-initiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) solution in phosphate buffered saline (PBS) for 20 minutes at 37˚C. Hydrogels were treated with 365 nm light at 10 mW/cm2 for 120 seconds. For experimental use, cells isolated from porcine valve tissue were seeded at a density of 15,000 cells/cm2 in VIC growth media consisting of M199 media supplemented with 1% FBS, penicillin-streptomycin, and fungizone. 12mm hydrogels were used for immunostaining experiments, 25mm hydrogels were used for real-time quantitative polymerase chain reaction (RT-qPCR) and western blot (WB) experiments. For TRPV4 inhibitor studies, VIC growth medium was supplemented with GSK2193874 (Tocris) in DMSO (Sigma-Aldrich) in concentrations indicated by the experiments.

Results: We verified TRPV4 functionality in VICs using live calcium imaging during application of small molecule modulators of TRPV4 activity. We designed hydrogel biomaterials that mimic mechanical features of healthy or diseased valve tissue microenvironments, respectively, to investigate the role of TRPV4 in proliferation (Fig 1A) and myofibroblast activation (Fig 1B). Our results show that TRPV4 regulates VIC proliferation in a microenvironment stiffness-independent manner. While there was a trend toward inhibiting myofibroblast activation on soft microenvironments during TRPV4 inhibition, we observed near complete deactivation of myofibroblasts on stiff microenvironments. We further identified Yes-activated protein (YAP) as a downstream target for TRPV4 activity on stiff microenvironments as TRPV4 inhibition significantly decreases YAP nuclear localization (Fig 1C).

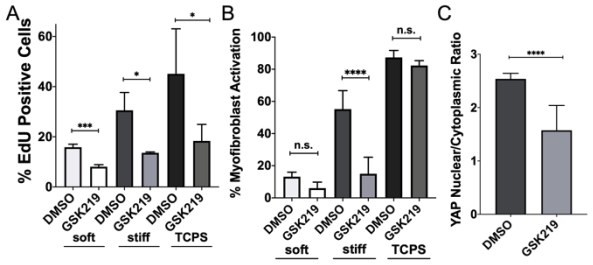

Figure 1. Cell response to TRPV4 inhibition on different microenvironments measuring A) proliferation by percent EdU positive cells B) percent myofibroblast activation by presence of αSMA fibers. C) VIC YAP nuclear localization on stiff hydrogels.

Conclusions: Mechanosensitive TRPV4 channels regulate VIC myofibroblast activation, whereas proliferation regulation is independent of the microenvironmental stiffness. Collectively, the data suggests differential regulation of stiffness-induced proliferation and myofibroblast activation. Our data further suggests a regulatory role for TRPV4 regarding YAP nuclear localization. TRPV4 is an important regulator for VIC myofibroblast activation, which is linked to the initiation of valve fibrosis. Although more validation studies are necessary, we suggest TRPV4 as a promising pharmaceutical target to slow aortic valve stenosis progression.

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