Statin therapy transforms cardiac fibroblast function in human failing heart

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PROBLEM
The effect of statin therapy, a commonly used lipid lowering strategy in patients at risk for cardiovascular disorders, on cardiac fibroblast function is not known.

BACKGROUND
Cardiac fibrosis underlies the progression of atrial fibrillation and heart failure. Fibroblast proliferation and differentiation precedes fibrosis. Statins (HMG-CoA 3-hydroxy-3-methylglutaryl-CoenzymeA reductase inhibitor) therapy is recommended (by ACC/AHA) for patients having cardiovascular disease. Apart from the established lipid lowering effect, statins have other effects reported in animal models but its effect on human ventricular fibroblasts (HVF) responsible for extracellular matrix secretion and fibrosis, is unknown. As excessive fibrosis is associated with heart failure (HF) and fibroblast-myofibroblast trans-differentiation precedes fibrosis, we tested the hypothesis that statin therapy interferes with the normal proliferation and differentiation function of HVFs from HF patients.

OBJECTIVE
To determine the effect of statin therapy on cardiac fibroblasts, isolated from failing heart patients either under statin therapy or not, and to determine the signaling mechanisms involved in this effect.

METHODS
Cell culture: Primary cultures of HVf from HF patients undergoing (VAD) implantation under statin therapy (HF+Statin) for at least 1 year (n = 5) or not (n = 4), non-diseased HVF from trauma victims (n = 3), were compared. Proliferation assay: The fibroblast proliferation was assessed by ethynyl 3-deoxyuridine (EdU), a thymidine analogue, incorporation assay, and cell counts using hemocytometer.
Immunoblotting: Expression of a smooth muscle actin (a-SMA) and GAPDH was assessed in fibroblast culture lysates. Immunohistochemistry: Myofibroblasts were identified with immunolocalization of a-SMA using appropriate primary/secondary antibodies, visualized under confocal microscopy and quantified using Fluoview software.
PCA Array: Transcriptomic changes were studied from total RNA using RT2 Profiler™ PCR array. Antibody Array: Exploratory antibody array was performed by Full Moon Biosystems Inc. Statistical Analysis: unpaired Student’s t-test or one-way ANOVA

RESULTS
STATIN THERAPY MITIGATES FIBROBLAST DIFFERENTIATION:
Fig. 1: Representative western blotting images(top) of HVFs lysates probed for the expression of a-SMA. Failing heart HVFs that were NOT under statin therapy showed significantly higher expression of a-SMA compared to the failing heart HVFs that were under statin therapy for at least one year. Bar graph(bottom) displays the pooled average image densities of a-SMA bands normalized to the corresponding GAPDH bands. Bands were quantified using Image J software. *P<0.03 vs Control; **P<0.01 vs HF+Statins

STATIN THERAPY ALTERS THE TRANSCRIPTOME OF CARDIAC FIBROBLASTS:
Fig. 5: Transcriptomic changes were studied from total RNA using RT2 Profiler™ PCR array. Data were analyzed by Student’s t-test. Among the 84 transcription factors (TFs) profiled, statin therapy upregulated significantly the following 13: CREB1, SMOG1, TCF7L2, MEF2A, ATF3, and SP3 that are mainly involved in signaling pathways of GPCR, bone morphogenetic proteins, Wnt, MAPK/ERK, and miscellaneous pathways, respectively. TTPA2A tends to be downregulated by two fold, but not statistically significant.

Fig. 6: Exploratory antibody array shows upregulation of CA125, Kupf70(p80), SREC1, Apolipoprotein D, ER Ca pump, clck8, il-3, Laminin B1, neurofilament, ERMCC, INK, superoxide dismutase, Collagen IV, ESK-1B, and Fascin, in failing hearts which are downregulated by statin therapy.

CONCLUSIONS
Statin interferes in the human ventricular fibroblasts differentiation function by associated changes in the transcriptome and signaling molecules involved in fibrosis. This anti-fibrotic effect of statins may be harnessed in therapeutics to mitigate the progression of cardiac fibrosis and heart failure, apart from its lipid-lowering effect.