Simvastatin Prevents TGFβ1 Induced SMAD2/3 Phosphorylation in Human Ventricular Fibroblasts: Involvement of Protein Phosphatase

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PROBLEM
Cardiac fibroblasts play key role in abnormal arrhythmias and heart failure (HF) because they mediate the process of unwanted excessive cardiac fibrosis as a consequence of maladaptive cardiac remodeling affecting contractility and conductivity of the heart. Transforming growth factor (TGF)-β, a critical mediator of fibrogenic activities that contribute to the development of cardiac hypertrophy and HF.

BACKGROUND
Ventricular fibrosis characterized by activation of ventricular fibroblasts (VFs) and excessive matrix deposition associated with cardiac injury results in progressive cardiac dysfunction, ultimately leading to heart failure. A major role in this process has been attributed to transforming growth factor beta (TGFβ1) signaling mediated through phosphorylation of SMAD2/3 proteins. Statins are cholesterol-lowering drugs reported to reduce cardiac fibrosis through cholesterol independent pathway, but the underlying mechanisms mediating their anti-fibrotic effects are not fully defined.

OBJECTIVE
We hypothesize simvastatin, a commonly used statin, reduces TGFβ1 induced ventricular fibrosis through activation of SMAD phosphatase PPA2A and PPM1A.

METHODS
Cultures of ventricular fibroblasts (passage 3) plated at density of 4,000 cells/cm² in the absence and presence of TGFβ1 (5ng) with or without simvastatin (1μM) were studied. Proliferation assays: Fibroblast proliferation (population doubling), cell counts from triplicate wells were evaluated using a hemocytometer and the number of cells at each time-point were counted and expressed as mean ± SEM from three or more experiments. Cytotoxicity assays: Simvastatin mediated cytotoxicity was assessed by cell viability assay using 4 mM calcein-AM and 2 mM ethidium homodimer-1 applying fluorescent microscopy. Immunocytochemistry (ICC): Transformation of fibroblast (vimentin +) to myofibroblast (α-smooth muscle actin and vimentin +) and nuclear translocation of phosphoSMAD 2/3 was determined by immuno- fluorescence using confocal or fluorescent microscopy. RNA isolation, cDNA synthesis, Real-timeqPCR: Equal amounts of RNA prepared from cells by Trizol, treated with DNase and purified (RNaseasy, Qiagen) were reverse transcribed to cDNA (miScript RT II, Qiagen). qPCR were performed for cyclins mRNAs in an ABI 7300, using SYBR Green Master Mix (Applied Biosystems). Immunoblotting: Western immunoblot analysis was performed using anti α-SMA, anti GAPDH, anti-phospho and total SMAD2/3. Co-Immunoprecipitation: Interaction between SMAD2/3 and PP2A or PPM1A was determined by SMAD2/3 immunoprecipitation and immunoblotted with anti PP2A or anti PPM1A. ELISA: culture media was collected and estimated for the release of collagen synthesis markers, pro-collagen type I C-terminal peptide (PICP) and type III N-terminal peptide (PINP) from hVF using commercial kits. Data were analyzed by Student’s t-test.

RESULTS

CONCLUSIONS
Simvastatin prevents the pro-fibrotic cytokine, TGF-β1 induced differentiation of fibroblasts to myofibroblasts by SMAD2/3 dephosphorylation via phosphatases: PPM1A and PP2A.

REFERENCES
