Cardiac reprogramming factor Gata4 reduces postinfarct cardiac fibrosis through direct repression of the profibrotic mediator snail

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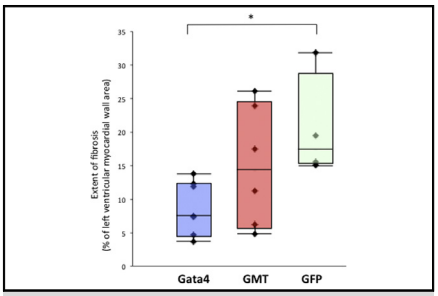
ABSTRACT

Objective: The administration of a variety of reprogramming factor cocktails has now been shown to reprogram cardiac fibroblasts into induced cardiomyocyte-like cells. However, reductions in ventricular fibrosis observed after reprogramming factor administration seem to far exceed the extent of induced cardiomyocyte-like cell generation in vivo. We investigated whether reprogramming factor administration might primarily play a role in activating antifibrotic molecular pathways.

Methods: Adult rat cardiac fibroblasts were infected with lentivirus encoding the transcription factors Gata4, Mef2c, or Tbx5, all 3 vectors, or a green fluorescent protein control vector. Gene and protein expression assays were performed to identify relevant antifibrotic targets of these factors. The antifibrotic effects of these factors were then investigated in a rat coronary ligation model.

Results: Gata4, Mef2c, or Tbx5 administration to rat cardiac fibroblasts in vitro significantly downregulated expression of Snail and the profibrotic factors connective tissue growth factor, collagen1a1, and fibronectin. Of these factors, Gata4 was shown to be the one responsible for the downregulation of the profibrotic factors and Snail (mRNA expression fold change relative to green fluorescent protein for Snail, Gata4: 0.5 ± 0.3, Mef2c: 1.3 ± 1.0, Tbx5: 0.9 ± 0.5, Gata4, Mef2c, or Tbx5: 0.6 ± 0.2, $P < .05$). Chromatin immunoprecipitation quantitative polymerase chain reaction identified Gata4 binding sites in the Snail promoter. In a rat coronary ligation model, only Gata4 administration alone improved postinfarct ventricular function and reduced the extent of postinfarct fibrosis.

Conclusions: Gata4 administration reduces postinfarct ventricular fibrosis and improves ventricular function in a rat coronary ligation model, potentially as a result of Gata4-mediated downregulation of the profibrotic mediator Snail. (J Thorac Cardiovasc Surg 2017;154:1601-10)



Gata4 administration alone inhibited postinfarct ventricular fibrosis.

Central Message

We propose a direct molecular mechanism by which Gata4 decreases cardiac fibrosis by downregulating the expression of the profibrotic transcription factor Snail in cardiac fibroblasts.

Perspective

Gata4 administration alone could significantly reduce postinfarct fibrosis and improve cardiac function, although Gata4 administration alone is incapable of generating iCMs. The ability of Gata4 to thereby independently inhibit postinfarct fibrosis may be an important new means of treating heart failure separate from the recent focus on the role of Gata4 as a cellular reprogramming factor.

See Editorial Commentary page 1611.

The recent discovery that cardiac fibroblasts could be transdifferentiated into induced cardiomyocyte-like cells (iCMs) led to the reporting by our group and others that administration of a transcription factor "cocktail" (eg, Gata4, Mef2c, and Tbx5 [GMT]) into areas of

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This study was funded by the National Heart Lung and Blood Institute (1R01HL121294-01A1 [to T.K.R.]) and supported in part by the Mouse Phenotyping Core at Baylor College of Medicine with funding from the National Institutes of Health (U54 HG006348).

Received for publication Dec 7, 2016; revisions received June 11, 2017; accepted for publication June 14, 2017; available ahead of print July 12, 2017.

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0022-5223/$36.00

http://dx.doi.org/10.1016/j.jtcvs.2017.06.035

Abbreviations and Acronyms

αSMA	= α-smooth muscle actin
ANOVA	= analysis of variance
bp	= base pair
ChIP	= chromatin immunoprecipitation
EF	= ejection fraction
EMT	= epithelial-mesenchymal transition
GFP	= green fluorescent protein
GMT	= Gata4, Mef2c, and Tbx5
HDAC2	= histone deacetylase 2
hpf	= high-power field
iCM	= induced cardiomyocyte-like cell
LV	= left ventricular
MET	= mesenchymal-epithelial transition
MF	= myofibroblast
qPCR	= quantitative polymerase chain reaction
qRT-PCR	= quantitative reverse transcription polymerase chain reaction

myocardial scar resulted in the apparent replacement of large areas of postinfarct scar with functional myocardium and up to a 37% accompanying increase in ventricular function.[1-5] We and others[1-4] also observed that reductions in infarct size appeared to far exceed the extent of scar repopulation with iCMs, suggesting that yet unknown antifibrotic mechanisms may underlie reprogramming efficacy.

Consistent with these observations, we have also noted that GMT administration significantly reduced the ventricular infarct population of myofibroblasts (MFs), which are thought to be differentiated from quiescent cardiac fibroblasts by inflammatory stimuli such as transforming growth factor-β and responsible for inducing tissue fibrosis.[6] In normal circumstances, MFs are dissipated through apoptosis after healing is complete, limiting the extent of fibrotic reaction to inflammatory stimuli. In pathologic states, MF activation persists and leads to excessive degrees of fibrosis, but the specific molecular mechanisms underlying this prolonged activation remain unclear.

Given these observations, we hypothesized that 1 or several of the transcription factors that have been used to induce cardiac cellular reprogramming might inhibit MF differentiation as an antifibrotic mechanism distinct from iCM generation. We accordingly sought to investigate the potential role of the cardiac reprogramming factors GMT in suppressing profibrotic processes in vitro and in vivo.

MATERIALS AND METHODS

Vectors and Cells

Lentivirus vectors encoding GMT and the marker gene green fluorescent protein (GFP) and a control lentivirus construct encoding GFP alone were prepared as previously described.[1] Adult cardiac fibroblasts were harvested from 8- to 10-week–old male Sprague Dawley rats (Harlan, Indianapolis, Ind) using standard cell isolation protocols that were approved by the Baylor College of Medicine Institutional Animal Care and Use Committee.[7] All animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals*.

Harvested cells were maintained in Iscove's Modified Dulbecco's Medium culture media (Life Technologies, Carlsbad, Calif) supplemented with 10% fetal bovine serum (Gibco, Gaithersburg, Md) at a concentration of 1×10^5 cells/mL. The cells were then seeded onto a 10-cm dish with a total of 1×10^6 cells for chromatin immunoprecipitation (ChIP) quantitative polymerase chain reaction (qPCR) and a 6-well dish with a total of 10^5 cells/well for Western blot and quantitative real-time polymerase chain reaction (qRT-PCR). Second passage cells were then infected with 1×10^5 TU of lentivirus encoding GMT, a combination of these vectors (1×10^5 TU dose of each vector; GMT); or an equivalent dose of GFP (negative control).[1] Cells were harvested for ChIP qPCR, analysis 5 days after the infection, and qRT-PCR and Western analyses 14 days after the infection.

Quantitative Polymerase Chain Reaction Real-Time Polymerase Chain Reaction

To perform qRT-PCR, total RNA was isolated from cultured cells or primary tissues using Trizol, and cDNA was generated from 1 μg RNA using the Superscript III first strand synthesis kit (Invitrogen, Carlsbad, Calif). qRT-PCR was then performed using SYBR Green Master mix (Life Technologies) on a ViiA7 (Life Technologies). Results were normalized by comparison with glyceraldehyde 3-phosphate dehydrogenase. Primer sequences are listed in Table 1.

Chromatin Immunoprecipitation-Quantitative Polymerase Chain Reaction

Cells designated for ChIP analysis were first cross-linked with formaldehyde, lysed, and sonicated, using ChromaFlash Chromatin Extraction kit (p-2001, Epigenetek, Farmingdale, NY). ChIPs were conducted with a polyclonal anti-Gata4 (sc-25310, Santa Cruz, Dallas, Tex), anti-HDAC2 (ab12169, abcam, Cambridge, Mass), or anti-histone 3 lysine 27 acetylation (4729, abcam) antibody.

To find the theoretic Gata4 binding sites, we analyzed the 6000 base pairs (bp) located before transcription starting point of the Snail gene using the following sequences that were previously reported bound by Gata4: TGATAA, AGATAA, AGATAG and TGATAG.[8] In addition, an extra potential binding sequence, AGATAT was analyzed with the Pattern Search for Transcription Factor Binding Sites program (http://www.gene-regulation.com/cgi-bin/pub/programs/patch/bin/patch.cgi). A region (Region I) that contains none of these sequences was included in this analysis (Figure E1).

For ChIP-qPCR assays, all primers were synthesized by Sigma (St Louis, Mo). These sequences are listed in Table 1. Fold enrichment of the PCR product was calculated after normalization with input (no antibody added) of all 3 types of infected cells.

Rat Coronary Artery Ligation Myocardial Infarction Model

After approval of the protocol by the Baylor College of Medicine Institutional Animal Care and Use Committee, myocardial infarction was created via proximal left anterior descending coronary artery ligation in adult male Sprague Dawley rats (n = 9/group).[1,2] Immediately after coronary ligation, animals received direct administration (total of 100 μL, 5 uniformly distributed 20 μL injections) into the myocardial infarct zone of lentivirus (1×10^5 TU) encoding Gata4, GFP, or a combination of GMT (3×10^5 total dose GMT).[1,2] Four animals that did not survive to study completion were excluded from the final analysis