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The TRPA1 channel is a cardiac target of mIGF-1/SIRT1 signaling

Valerio Pazienza,[1]* Cristoforo Pomara,[2,3]* Francesco Cappello,[4,5] Raffaele Calogero,[6] Matteo Carrara,[6] Gianluigi Mazzoccoli,[7]* and Manlio Vinciguerra[1,5,8]*

[1]*Division of Gastroenterology, Department of Medical Sciences, IRCCS Scientific Institute "Casa Sollievo della Sofferenza," San Giovanni Rotondo, Italy;* [2]*Department of Anatomy, University of Malta, Msida, Malta;* [3]*Department of Forensic Pathology, University of Foggia, Foggia, Italy;* [4]*Department of Experimental Medicine and Clinical Neuroscience, Section of Human Anatomy, University of Palermo, Palermo, Italy;* [5]*Euro-Mediterranean Institute of Science and Technology, Palermo, Italy;* [6]*Molecular Biotechnology Center, University of Turin, Turin, Italy;* [7]*Division of Internal Medicine and Chronobiology Unit, Department of Medical Sciences, IRCCS Scientific Institute "Casa Sollievo della Sofferenza," San Giovanni Rotondo, Italy; and* [8]*Division of Medicine, University College London, London, United Kingdom.*

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Pazienza V, Pomara C, Cappello F, Calogero R, Carrara M, Mazzoccoli G, Vinciguerra M. The TRPA1 channel is a cardiac target of mIGF-1/SIRT1 signaling. *Am J Physiol Heart Circ Physiol* 307: H939–H944, 2014. First published August 8, 2014; doi:10.1152/ajpheart.00150.2014.—Cardiac overexpression of locally acting muscle-restricted (m)IGF-1 and the consequent downstream activation of NAD^+-dependent protein deacetylase sirtuin 1 (SIRT1) trigger potent cardiac antioxidative and antihypertrophic effects. Transient receptor potential (TRP) cation channel A1 (TRPA1) belongs to the TRP ion channel family of molecular detectors of thermal and chemical stimuli that activate sensory neurons to produce pain. Recently, it has been shown that TRPA1 activity influences blood pressure, but the significance of TRPA1 in the cardiovascular system remains elusive. In the present work, using genomic screening in mouse hearts, we found that TRPA1 is a target of mIGF-1/SIRT1 signaling. TRPA1 expression is increased in the heart of cardiac-restricted mIGF-1 transgenic (Tg) mice, both in cardiomyocytes and noncardiomyocytes. In wild-type mice, SIRT1 occupied the TRPA1 promoter, inhibiting its expression, whereas in the presence of the cardiac mIGF-1 transgene, SIRT1 was displaced from the TRPA1 promoter, leading to an increase in its expression. Cardiac-specific ablation of SIRT1 (cardiac-specific knockout) in mIGF-1 Tg mice paradoxically did not increase TRPA1 expression. We have recently reported a systemic "hormetic" effect in mIGF-1 Tg mice, mild hypertension, which was depleted upon cardiac-specific knockout of SIRT1. Administration of the selective TRPA1 antagonist HC-030031 to mIGF-1 Tg mice restored blood pressure to basal levels. We identified TRPA1 as a functional target of the cardiac mIGF-1/SIRT1 signaling pathway, which may have pharmacological implications for the management of cardiovascular stress.

transient receptor potential cation channel, subfamily A, member 1; insulin-like growth factor-1; sirtuin 1; heart

CARDIOVASCULAR DISEASES are a leading cause of mortality, representing one-third of all global deaths. IGF-1 and sirtuin-1 (SIRT1) are crucial mediators of cell homeostasis and cardiovascular stress (7). In mammals, a complex IGF-1 signaling system with multiple alternative spliced isoforms displaying distinct effects on cardiovascular function is in place (7). These variants have a common core peptide, flanked by varying termini (class 1 and 2 NH_2-terminal peptides as well as E peptides). IGF-1 is both a systemic growth factor produced by the liver in response to growth hormone and a local growth factor acting in an autocrine/paracrine manner in skeletal and heart muscles. Muscle-restricted (m)IGF-1 is a locally acting isoform that comprises class 1 NH_2-terminal and Ea COOH-terminal peptides (7). mIGF-1 boosts antioxidative cell defenses by upregulating skeletal muscle or cardiac gene programs with regenerative, antioxidant, and antiapoptotic properties (21, 27, 28). Moreover, mIGF-1 repairs the heart from injury through the production of specific cytokines that recruit endothelium-primed cells for de novo vascularization of myocardial tissue, indicating that cardiomyocyte (CM)-specific overexpression of this transgene might have profound systemic effects (20).

The SIRT family of NAD^+-dependent protein deacetylases has been deeply implicated in the regulation of organism healthspan (9). SIRT1 is the largest and best-characterized member: its enzymatic activation triggers pleiotropic beneficial effects (9). In fact, pharmacological or nutritional interventions capable of activating SIRT1 have been shown to increase lifespan in most model organisms (9). In contrast, SIRT1 knockout (KO) mice die at birth or soon after due to developmental defects of the retina and heart (26). Moderate SIRT1 cardiac-specific overexpression has been shown to protect mice from cardiac oxidative stress and postponed the onset of age-dependent cardiac fibrosis and cell death, with increased expression of antioxidants, such as catalase, through forkhead box O-dependent mechanisms (1). In vitro and in vivo findings have reinforced these cardioprotective effects of SIRT1, suggesting that its activation might be of benefit for the treatment of cardiac diseases (9). IGF-1 and SIRT1 actually can impinge on the same signaling pathways (7, 25). In this respect, we have shown that the liver-produced circulating IGF-1 isoform and mIGF-1 display distinct effects in cardioprotection (29). Using hypertrophic (ANG II)/oxidative (paraquat) stressors,

* V. Pazienza and C. Pomara, and G. Mazzoccoli and M. Vinciguerra, contributed equally to this work.

Address for reprint requests and other correspondence: M. Vinciguerra, Univ. College London (UCL), Institute for Liver and Digestive Health, UCL Medical School, Royal Free Campus, London NW3 2PF, UK (e-mail: m.vinciguerra@operapadrepio.it).

we identified a signaling pathway that protects CMs and that relies on mIGF-1-dependent SIRT1 activation (29, 30). For in vivo studies in mice, we generated cardiac-specific mIGF-1 transgenic (Tg) mice in which SIRT1 was excised from adult CMs in an inducible and conditional fashion [mIGF-1 Tg × SIRT1 cardiac-specific KO (CKO)]. Functional and molecular analyses of these animals confirmed that mIGF-1-induced SIRT1 activity was required to protect the heart from oxidative stress-induced cell damage and lethality (30). Moreover, we uncovered unexpected systemic roles for the cardiac mIGF-1/SIRT1 pathway in mice, among which mild hypertension (3). The robust physiological responses obtained with mIGF-1-induced SIRT1 activity suggested a potential mechanistic basis for strategies to improve the outcome of heart disease. To explore this more in depth at the molecular level, using a high-throughput sequencing approach [chromatin immunoprecipitation (ChIP)-Seq], we identified new direct putative genomic binding targets of nuclear SIRT1 in the heart (3): among these, transient receptor potential (TRP) cation channel A1 (TRPA1). This molecule belongs to the TRP ion channel family of molecular detectors of thermal and chemical stimuli that activate sensory neurons to produce pain (12). TRPA1 is the smallest of the mammalian TRP superfamily of cation channels, which comprises 28 members assigned to 6 subfamilies based on sequence homology. These channels have been strongly linked to the pathogenesis of clinical disorders, such as cancer and inflammation (2, 13). TRPA1 activity might also be implicated in the pathogenesis of cardiovascular disorders: it is highly expressed in cerebral endothelial cells and smooth muscle cells, and pharmacological or genetic modulation of its activity influences blood pressure (BP) (6). However, the significance of TRPA1 in the cardiovascular system is not understood. For this reason, in this work, we explored the relevance of TRPA1 as a target of cardioprotective mIGF-1/SIRT1 signaling using mouse genetics as a tool and BP as a functional readout. We found that cardiac TRPA1 expression is modulated by mIGF-1 and can translate the effects of this growth factor on BP levels in a SIRT1-dependent manner.

METHODS

Animal models. Transgenic FVB mice carrying rat mIGF-1 cDNA driven by the mouse α-myosin heavy chain (MyHC) promoter (α-MyHC/mIGF-1) were generated and maintained as previously described (21). SIRT1 floxed (*Fl/Fl*) mice were as previously described (4) and acquired from The Jackson Laboratory. Tamoxifen-inducible α-MyHC/mER-CRE-mER Tg mice were crossed to SIRT1 *Fl/Fl* mice to deplete SIRT1 expression in adult CMs (22) upon tamoxifen administration. Mice were placed on tamoxifen-containing chow (Harlan Special Diet TD.55125) at 4 mo of age for 2 wk, leading to efficient and reproducible gene recombination (14). The mIGF-1 transgene was introduced by three-way crosses to generate α-MyHC/mIGF-1 Tg × α-MyHC/mER-CRE-mER; $SIRT1^{Fl/Fl}$ mice (referred to as mIGF-1 Tg × SIRT1 CKO mice) (30). PCR genotyping was performed using genomic DNA from tail biopsies. For in vivo inhibition of TRPA1 activity, mice were injected with the TRPA1 antagonist HC-030031 (20, 50, and 100 mg/kg ip dissolved in 10% DMSO, Sigma-Aldrich) for 30 min, an effective time for this drug adopted from previous studies and upon our preliminary experiments. All mouse procedures were approved by University College London (London, UK) and the European Molecular Biology Laboratory (Monterotondo, Italy) Ethical Committees and were in accordance with national and European regulations.

ChIP. Protein-DNA complexes were captured by fixing heart homogenates from adult wild-type (WT) or mIGF-1 TG mice at a resting state in 1% (vol/vol) formaldehyde (Sigma) for 10 min at room temperature with gentle shaking. The reaction was quenched by the addition of 0.125 M glycine for 5 min at room temperature. Cells were washed (3 times) with cold PBS and resuspended in cell lysis buffer (50 mM HEPES, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% Nonidet P-40, and 1× proteinase inhibitor; 1×10^7 cells/ml) on ice for 10 min to release nuclei. Nuclei were pelleted (4,000 rpm, 10 min, 4°C) and resuspended in nuclei lysis buffer [140 mM NaCl, 10 mM Tris·Cl (pH 8), 1% Nonidet P-40, and 1× proteinase inhibitor] on ice for 10 min. Cells were sonicated using a Bioruptor Sonicating Waterbath (Diagenode). Lysate (300 ml) was sonicated for 40 pulses of 30-s "on" 30-s "off" in 1.5-ml TPX Eppendorf tubes, with ice replaced regularly to minimize overheating of samples. Chromatin was pooled and centrifuged for at 13,000 rpm for 10 min at 4°C to remove debris, and single-use aliquots (300 μl) were used for immunoprecipitation or stored at −80°C. Chromatin was immunoprecipitated by bringing each aliquot of chromatin to 1 ml with nuclei lysis buffer proteinase inhibitor. Chromatin was precleared with 80 μl of ChIP-grade protein agarose A beads (Upstate) for 4 h at 4°C with rotation and incubated overnight with 4 μg of SIRT1 monoclonal antibody (10E04, Millipore) or unrelated rabbit IgG polyclonal antibody (Chemicon) at 4°C with rotation. Immunoprecipitated complexes were collected by incubation with 100 μl of ChIP-blocked protein A agarose beads (Upstate) for 4 h at 4°C with rotation, and nonspecific complexes were removed by washing beads twice with high-salt buffer [0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris·Cl (pH 8.1), and 500 mM NaCl], low-salt buffer [0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris·Cl (pH 8.1), and 150 mM NaCl], LiCl buffer [0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholic acid, 1 mM EDTA, and 10 mM Tris·Cl (pH 8.1)], and TE buffer, consisting of 10 mM Tris (pH 8.0) and 1 mM EDTA.. Washes were performed for 5 min at 4°C with rotation, and beads were collected by centrifugation at 3,000 rpm at 4°C for 3 min. DNA was eluted with 100 μl elution buffer (1% SDS and 100 mM $NaHCO_3$). Beads were vortexed for 30 s and incubated at room temperature with rotation for 15 min, the process was repeated, and eluates were combined (200 μl). Cross-links were reversed with 0.3 M NaCl by incubating at 65°C overnight in a hybridization oven, and RNA was removed with RNase A (Upstate) at 37°C for 30 min. Protein was removed by incubating samples with proteinase K (Upstate) for 1 h at 45°C. DNA was purified using a Qiagen PCR Purification Kit (Qiagen) and eluted in 30 μl distilled H_2O. For ChIP, individual SIRT1 ChIP samples were pooled and concentrated using a SpeedVac. PCR against regions 19–118, 77–176, and 285–384 from the transcription start site (TSS) was performed in SIRT1 pulled down samples and input and no template controls using *Taq* DNA Polymerase (catalog no. MO273, New England BioLabs) and processed for gel electrophoresis. Primer sequences were designed to amplify a region of 100 bp and are available upon request.

BP measurement. Noninvasive assessment of BP was performed by tail-cuff measurements (Visitech Systems, Apex, NC) as previously described (17).

Quantitative real-time PCR. Total RNA was isolated from hearts using TRIzol (Invitrogen). After RNA quality verification, 1–2 mg were used to prepare cDNA (Ready-To-Go, T-Primed First-Strand Kit, Amersham Bioscience). Quantitative PCR for SIRT1 was performed using SYBR green (Sigma) in a Light-Cycler (Roche) in a 25-μl volume with the following amplification program: *1*) 1 cycle for 50°C for 2 min; *2*) 1 cycle for 95°C for 10 min; *3*) 40 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s; and *4*) 1 cycle at 72°C for 10 min. Primer sequences for TRPA1 were as follows: forward 5′-AAGCGGAGACTTGGACATGA-3′ and reverse 5′-TAACGAGGCTCTGTGAAGCA-3′. UbiC, 18S rRNA (Rn18S), and GAPDH transcripts were used as internal controls, according to the GeNorm method (29). Primer sequences were as follows: UbiC, forward

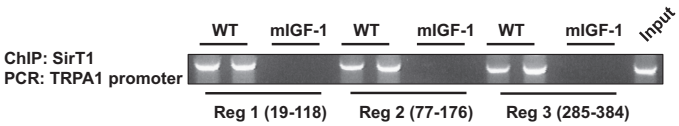

Fig. 1. Sirtuin 1 (SIRT1) occupancy of the transient receptor potential cation channel A1 (TRPA1) promoter in the myocardium of wild-type (WT) and muscle-restricted (m)IGF-1 mice. Chromatin was isolated from whole hearts of WT and mIGF-1 transgenic (TG) mice and processed for chromatin immunoprecipitation (ChIP) using a SIRT1 antibody. PCR products of three distinct regions of the TRPA1 gene promoter [19–118 (*Reg 1*), 77–176 (*Reg 2*), and 285–384 (*Reg 3*)], of 100 bp of length each, were separated by gel electrophoresis. Input was used as a control. Two representative WT and mIGF-1 TG mice (8 mice total) are shown.

5′-AGCCCAGTGTTACCACCAAG-3′ and reverse 5′-GCAAGAACTTTATTCAAAGTGCAA-3′; Rn18S, forward 5′-CGCGGTTCTATTTTGTTGGT-3′ and reverse 5′-AGTCGGCATCGTTTATGGTC-3′; and GAPDH, forward 5′-AACTTTGGCATTGTGGAAGG-3′ and reverse 5′-ACACATTGGGGGTAGGAACA-3′.

Primary CM isolation procedures. Excised mouse hearts were perfused using a Langendorff perfusion apparatus with Ca^{2+}-free Krebs-Ringer bicarbonate (KRB) solution containing collagenase (1 mg/ml) until they became flaccid. Hearts were then chopped finely, and the mince was agitated gently in the same medium to dissociate individual cells. The resulting cell suspension was filtered to remove undigested material, and CMs were separated from non-CMs (NCMs) by sedimentation of CMs at 500 *g* for 2 min. The supernatant containing NCMs was separated and further centrifuged at 1,500 *g* for 5 min to pellet cells. The Ca^{2+} tolerance of the sediment containing CMs was restored gently by resuspending it in KRB containing a progressively higher concentration of Ca^{2+} to a final concentration of 1 mM. Both CM and NCM sediments were stored in TRIzol (Invitrogen) for RNA analyses.

RNA in situ hybridization. In situ hybridization was performed as previously described using the coding region of TRPA1 to generate the RNA probe (10).

Statistical analysis. Results are expressed as means ± SE. Comparisons were made using Student's *t*-test. *P* values of <0.05 were considered as significant.

RESULTS

SIRT1 displacement from the TRPA1 promoter in the myocardium of mIGF-1 mice is associated with increased TRPA1 expression levels. ChIP-Seq screening for differential SIRT1 genomic binding sites in whole hearts from WT versus cardiac-restricted mIGF-1 Tg mice led to the identification of a few dozens of differential SIRT1 genomic binding sites (33 sites specific for WT mice and 32 sites specific for mIGF-1 Tg mice) (3). In this ChIP-Seq data set, SIRT1 showed threefold enrichment in the occupancy of the TRPA1 gene body in WT hearts compared with mIGF-1 Tg hearts (3). In the present study, we sought to validate these high-throughput sequencing data by canonical ChIP using a specific ChIP-grade anti-SIRT1 antibody followed by quantitative PCR amplification of three distinct selected promoter regions (19–118, 77–176, and 285–384) from the TSS, respectively. As shown in Fig. 1, in the presence of the mIGF-1 transgene, the binding of SIRT1 to these promoter regions was almost undetectable, indicating SIRT1 removal and suggesting that TRPA1 is a molecular target of the mIGF-1/SIRT1 pathway. TRPA1 is a cation channel involved in several clinical disorders; preliminary evidence links TRPA1 activity to vasodilation and vasoconstriction (18), although its cardiac expression levels and its role in cardiovascular biology are not clear. Here, using RNA in situ hybridization, we found that the TRPA1 transcript was massively upregulated in the hearts of mIGF-1 Tg versus WT mice (Fig. 2*A*). The TRPA1 signal localized to myocardial fibers (Fig. 2*A*). To understand if TRPA1 was differently

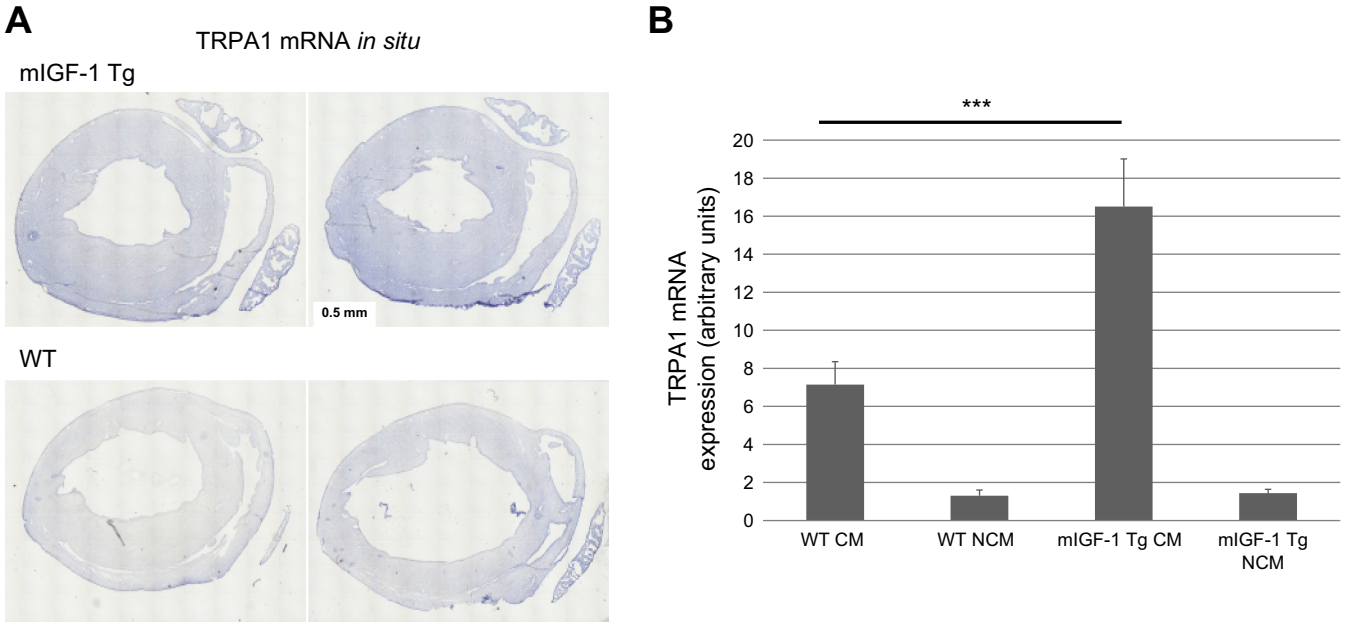

Fig. 2. TRPA1 mRNA expression in cardiac muscle of WT and mIGF-1 Tg mice. *A*: mRNA hybridization in situ to detect TRPA1 mRNA expression. Hearts from 4-mo-old adult animals were dissected, fixed overnight in 4% paraformaldehyde, and processed for whole mount in situ hybridization. Detection of transcripts on paraffin sections was performed using digoxigenin-labeled hybridization probes against the TRPA1 coding region. Two serial sections of a representative animal per group are shown. *B*: expression levels of TRPA1 mRNA were examined by quantitative RT-PCR in cardiomyocyte (CM) and non-CM (NCM) fractions of WT and mIGF-1 Tg mouse hearts. UbiC, 18S rRNA (Rn18S), and GAPDH transcripts were used as internal controls, according to the GeNorm method. Results are means ± SE of 10 animals. ***$P > 0.001$ vs. WT mice.

expressed in CMs or NCMs (endothelial cells, fibroblasts, macrophages, and smooth muscle cells, which comprises ≈50% of the total cardiac cell number), WT and mIGF-1 Tg hearts were fractionated to separate CMs from NCMs (14, 30), and TRPA1 mRNA levels were analyzed by quantitative PCR. TRPA1 mRNA was found ~5.5 times more expressed in the CM fraction compared with the NCM fraction (Fig. 2*B*) in WT hearts. A markedly higher expression in the CM fraction versus the NCM fraction for TRPA1 was also found in the presence of the cardiac mIGF-1 transgene (Fig. 2*B*). In mIGF-1 Tg hearts, an ~12.7-fold increase in TRPA1 mRNA expression in the CM fraction was observed, without evident changes in the NCM fraction, which comprised significantly higher expression values compared with WT hearts (Fig. 2*B*). These data are consistent with the imaging in situ (Fig. 2*A*) and demonstrate that *1*) TRPA1 is expressed more robustly in CMs compared with NCMs and *2*) TRPA1 is markedly upregulated by mIGF-1 in the heart, specifically in CMs and not in NCMs (Fig. 2, *A* and *B*).

CM-specific inducible genetic ablation of SIRT1 in mIGF-1 mice lowers TRPA1 expression. Since SIRT1 is displaced from the TRPA1 promoter in the myocardium in the presence of the mIGF-1 transgene, we investigated if genetic ablation of SIRT1 could elicit the same effects. As previously reported (30), to ablate SIRT1 activity in the CM compartment, we crossed CM-specific, tamoxifen-inducible α-MyHC/mER-CRE-mER Tg mice with conditional SIRT1$^{Fl/Fl}$ KO mice to produce SIRT1 CKO mice. Cardiac function and SIRT1 expression in these mice were unaltered during growth and adulthood (30). After 2 wk on a tamoxifen-enriched diet, 4-mo-old SIRT1 CKO mice displayed efficient CM-specific SIRT1 inactivation (30). This did not occur in tamoxifen-fed α-MyHC/mER-CRE-mER WT mice or SIRT1$^{Fl/Fl}$ mice and was cardiac specific. KO of cardiac SIRT1 in adult mice did not generate evident functional perturbations (30). Using a three-way crossing of mIGF-1 Tg mice with SIRT1 CKO mice, we next generated mIGF-1 Tg × SIRT1 CKO mice, which were born at the expected ratio and without any abnormalities (30). Thus, we further sought to analyze four groups of 4-mo-old mice for TRPA1 mRNA expression: WT, mIGF-1 Tg × SIRT1$^{Fl/Fl}$, SIRT1 CKO, and mIGF-1 Tg × SIRT1 CKO mice. Upon feeding these four groups of mice a tamoxifen-enriched diet for 2 wk, SIRT1 deletion occurred specifically in the heart of SIRT1 CKO and mIGF-1 Tg × SIRT1 CKO mice (30). Quantitative PCR analysis in CM and NCM compartments revealed that ablation of SIRT1 alone in CMs had no effects on TRPA1 expression in CMs and NCMs compared with WT mice (SIRT1 CKO mice; Fig. 3). Moreover, this gene expression analysis confirmed increased TRPA1 mRNA levels in CMs of mIGF-1 Tg × SIRT1$^{Fl/Fl}$ mice (similar to mIGF-1 Tg mice) compared with WT mice (Fig. 3); however, TRPA1 mRNA expression returned to basal levels in the CM compartment of mIGF-1 TG × SIRT1 CKO mice (Fig. 3). These data demonstrate that SIRT1 is required for the mIGF-1-dependent upregulation in TRPA1 expression in CMs.

mIGF-1/SIRT1-dependent mild hypertension is prevented by the systemic administration of the TRPA1 antagonist HC-030031. Consistent with previous studies, noninvasive measurements (17) of diastolic BP (DBP) and systolic BP (SBP) in WT, mIGF-1 Tg, and mIGF-1 Tg × SIRT1 CKO mice showed no differences between WT and mIGF-1 Tg × SIRT1 CKO mice, whereas an ~25% significant increase in both DBP and SBP in mIGF-1 Tg mice compared with WT littermates was observed in the basal state (Fig. 4) (3). In mIGF-1 Tg × SIRT1 CKO mice, SBP, and to a lesser extent DBP, was found restored to WT levels (Fig. 4). Given that TRPA1 activity can modulate DBP and SBP in rodents (6), we aimed to assess its potential impact on the mild hypertension induced by cardiac overexpression of the mIGF-1 transgene in a SIRT1-dependent fashion in mice. To this purpose, three cohorts of mice (WT, mIGF-1 Tg, and mIGF-1 Tg × SIRT1 CKO mice) were intraperitoneally administered vehicle (DMSO) or HC-030031, a selective TRPA1 channel blocker, for 30 min at different doses (20, 50, and 100 mg/kg) before DBP and SBP measurements. As shown in Fig. 4, administration of HC-030031 at 50 and 100 mg/kg blunted the high BPs, both DBP and SBP, induced by the cardiac mIGF-1 transgene in mice, whereas it had no significant inhibitory effects on the other groups. These findings suggest that increased TRPA1 expression is instrumental for mIGF-1-induced mild hypertension in mice, since pharmacological inhibition of this channel restored BP to basal levels.

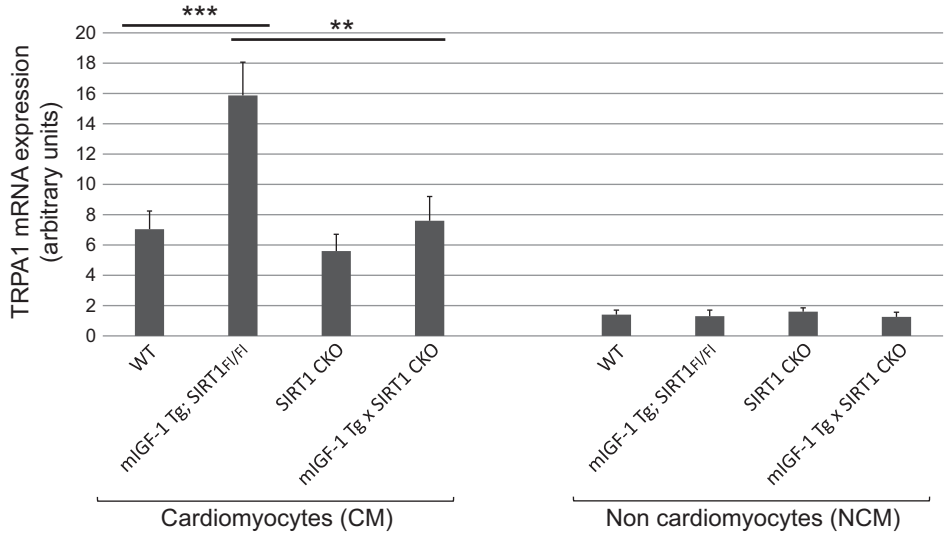

Fig. 3. Cardiomyocyte-specific inducible genetic ablation of SIRT1 in mIGF-1 mice lowers TRPA1 expression. All mice were 4 mo old and placed for 2 wk under a tamoxifen diet. Upon death, total RNA was extracted from CM and NCM fractions of the hearts of WT, mIGF-1 Tg; SIRT1 floxed (SIRT1$^{Fl/Fl}$), cardiac-specific SIRT1 knockout (SIRT1 CKO), and mIGF Tg × SIRT1 CKO mice. TRPA1 mRNA levels were determined by quantitative PCR. UbiC, Rn18S, and GAPDH transcripts were used as internal controls, according to the GeNorm method. Results are expressed as arbitrary units and are means ± SE of 8 animals. $^{**}P > 0.01$; $^{***}P > 0.001$.

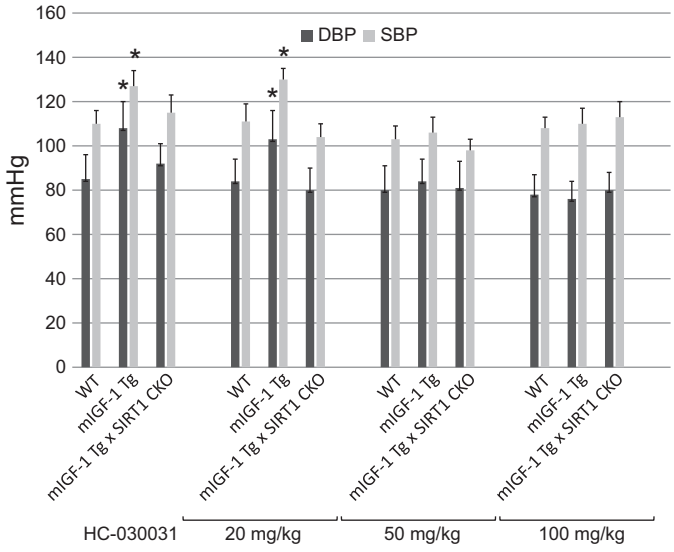

Fig. 4. mIGF-1/SIRT1-dependent mild hypertension is prevented by systemic administration of the TRPA1 antagonist HC-030031. Noninvasive blood pressure (BP) values in conscious mice were measured using the tail-cuff method [diastolic BP (DBP) and systolic BP (SBP)] in a cohort of WT, mIGF-1 Tg, and mIGF-1 Tg × SIRT1 CKO mice. Mice were intraperitoneally injected with vehicle (DMSO) or, for blockage of TRPA1 activity, HC-030031 was administered for 30 min at different doses (20, 50, and 100 mg/kg) before DBP and SBP measurements. Results are means ± SE of 8 animals for each genotype. *$P > 0.01$ vs. WT mice.

DISCUSSION

In several experimental models, activation of mIGF-1 and SIRT1 signaling pathways has proven effective in protecting against cardiovascular stresses and ischemic insults (3, 7, 21, 29, 30). SIRT1 pharmacological activators have been developed and showed preclinical efficacy in cardioprotection against infarct. We recently assessed that SIRT1 is located within the nuclei of CMs (3), in contrast with other reports (23, 24), whereas it is located in the cytoplasm of NCM cell types. ChIP-Seq offered us a powerful tool to determine how SIRT1 interacts with CM DNA (3), and we have provided the first SIRT1 genome-wide DNA binding data set reported, after the one obtained by Oberdoerferr et al. (16) in embryonic stem cells with a ChIP-on-ChIP approach (16). This report (16) described a few hundred genes bound by SIRT1 in the promoter region in embryonic stem cells under basal conditions, and under conditions of oxidative stress (H_2O_2 treatment), a massive displacement of SIRT1, which goes to occupy other promoters, was observed. In mouse hearts, we found that SIRT1 similarly bound to 302 gene promoters, but there were only a few dozen promoters exclusively bound by SIRT1 either in the WT heart or in the mIGF-1 Tg heart (3). This distribution indicates that the mIGF-1 transgene induces only subtle alterations in SIRT1 DNA-binding patterns. Most interestingly, these variations in mIGF-1/SIRT1 genomic (together with transcriptomic) effects in unchallenged mouse hearts were related to genes regulating functions beyond CM-specific homeostatic mechanisms and relating to global body functions. Interestingly, we found genes implicated in BP control (3). Among these, we focused on TRPA1, since its gene body was occupied abundantly by SIRT1 binding in WT hearts but not in mIGF-1 Tg hearts. TRPA1 was first isolated in 1999 in a screen for transformation-sensitive proteins in cultured fibroblasts (11), and it was subsequently shown to detect cold and chemical stimuli that activate sensory neurons to produce pain (12). More recent studies have extended our understanding of TRPA1 functions, and it appears that its activity might also be implicated in cardiovascular disorders and in modulating pressure (6), making it a novel target in cardiovascular research. TRPA1 is expressed in endothelial cells and smooth muscle cells, and, in the present study, we report for the first time its abundant expression at the level of mRNA in the CM cell fraction versus the NCM fraction of the murine heart. We found that TRPA1 mRNA is massively increased by the mIGF-1 cardiac transgene in a SIRT1-dependent manner, since CM-specific inducible genetic ablation of this deacetylase in the adult heart restored TRPA1 to basal levels. An outstanding question opened by our results is: "if displacement of SIRT1 from TRPA1 promoter in mIGF-1 Tg mice increased cardiac expression of this cation channel, why does genetic ablation of SIRT1 in the heart paradoxically not have similar effects?" The cardiac epigenome and transcriptome are intertwined in a very complex manner, and the increase in SIRT1 activity/expression and global changes in genome occupancy in the presence of mIGF-1 (3, 29, 30) might affect a myriad of unidentified transcriptional cofactors (5) that may contribute to activate TRPA1 transcription. Moreover, mIGF-1 has multiple downstream effectors, such as serum/glucocorticoid-regulated kinase, pyruvate dehydrogenase kinase isozyme 1, NF-κB, and others (7, 21), that are able to cross-talk with SIRT1: the disruption of this signaling interactome by genetic depletion of SIRT1 might be involved in the absence of TRPA1 upregulation in mIGF-1 Tg × SIRT1 CKO mice. The regulation of TRPA1 gene transcription by growth factors and stress-responsive signaling pathways is an untapped research field. In addition, mIGF-1 activates an antioxidant gene program in a SIRT1-dependent manner (1, 2). It has been demonstrated that the transcription factor hypoxia-inducible factor (HIF)-1α protects the heart against oxidative and hypoxic stresses (3) and, interestingly, activates TRPA1 gene transcription (4); HIF-1α activity is inhibited by SIRT1 through direct contact and deacetylation (5). Further studies should determine if in the absence of SIRT1 occupancy on the TRPA1 promoter in the heart of mIGF-1 Tg mice, as we have shown, active HIF-1α could be crucial in determining TRPA1 upregulation. It is surprising that mIGF-1 Tg mice are mildly hypertensive and that inhibition of TRPA1 can restore BP to basal levels, considering that BP is only partially determined by the heart and mostly by peripheral vascular resistance. The present study is limited by the lack of phenotyping the level of BP by telemetry; further studies will be necessary to assess the role of peripheral vascular resistance. The fact that the mIGF-1 cardiac transgene can elicit other systemic effects, such as an increase in the immune cell count and behavioral modification (better performance in a fear conditioning test) (3), underlie profound differences in the "circulome" of mIGF-1 Tg animals, depending on molecules secreted by CMs in a autocrine and/or paracrine fashion. In this respect, it has been shown that the cardiac mIGF-1 transgene is proangiogenic: it can trigger the production of cytokines that mobilize stem cells in the bone marrow to form new blood vessels (20). In the present study, we found that the effect of mIGF-1 on BP mediated by TRPA1 could be rescued by CM-specific depletion of SIRT1, which, in turn, was not associated with decreased cardiovascular performance (30). Of note, plasma measurement of vasoactive stress hormones associ-

ated with elevated BP, such as corticosterone and vasopressin, did not show any change (data not shown), indicating a lack of overt stress in the animal. This study does not rule out how the BP-lowering effect of inhibition of the mIGF-1/SIRT1/TRPA1 pathway is related to the development of cardio-/cerebrovascular diseases. However, although we lack an integrated physiological understanding of cardiac mIGF-1 systemic function on multiple distant target cell types, we propose that the moderate stress-inducing properties of the cardioprotective mIGF-1 pathway are coherent with the theory of hormesis, i.e., exposure to mild stress should result in an adaptive response with various benefits (8, 19).

The role of TRPA1 in cardiovascular aging is unknown. TRPA1 KO mice have been generated and characterized; homozygous mice display behavioral deficits in response to cold and mechanical stimuli (15). These animals have been used mostly for pharmacological and neurological studies, which showed that TRPA1 can also influence changes in BP in connection to alterations in blood flow, vascular reactivity, and autonomic system reflexes (18). This existing model could be valuable to study TRPA1 activity regulation by growth hormone-dependent signals and cardiac ischemic insults. Elucidating in detail the systemic effects of TRPA1 and of other targets of the mIGF-1/SIRT1 pathway may help to develop new cardiac and antiaging strategies.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: V.P., C.P., G.M., and M.V. conception and design of research; V.P., C.P., M.C., and M.V. performed experiments; V.P. and M.V. prepared figures; V.P., C.P., F.C., R.C., M.C., G.M., and M.V. approved final version of manuscript; F.C., R.C., M.C., G.M., and M.V. analyzed data; F.C., R.C., M.C., G.M., and M.V. interpreted results of experiments; F.C., G.M., and M.V. drafted manuscript; F.C., G.M., and M.V. edited and revised manuscript.

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