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Increased transient receptor potential vanilloid type 1 (TRPV1) channel expression in hypertrophic heart

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ABSTRACT

The aim of this study was to compare the expression of transient receptor potential vanilloid type 1 (TRPV1) channels in hypertrophic hearts from transgenic mice showing overexpression of the catalytic subunit alpha of protein phosphatase 2A alpha (PP2Ac alpha) with wild-type mice and with TRPV1−/− mice.

Transcripts of TRPV1, matrix metalloproteinase 9 (MMP9), discoidin domain receptor family, member 2 (DDR-2), atrial natriuretic peptide (ANP), GATA 4, and regulatory microRNA (miR-21) were analyzed using quantitative real-time PCR. Ventricle-to-body-weight-ratio was significantly higher in PP2Ac alpha transgenic mice compared to wild-type mice and TRPV1−/− mice (8.6 ± 1.3 mg/g; 5.4 ± 0.3 mg/g; and 5.4 ± 0.4 mg/g; respectively; $p < 0.05$ by Kruskal–Wallis test). TRPV1 transcripts were significantly higher in PP2Ac alpha transgenic mice compared to wild-type mice (1.7 ± 0.2 arbitrary units vs. 0.8 ± 0.1 arbitrary units; $p < 0.05$). TRPV1 protein expression was also significantly higher in PP2Ac alpha transgenic mice compared to wild-type mice. A significant linear correlation was observed between TRPV1 transcripts and the ventricle-to-body-weight-ratio (Spearman $r = 0.78$; $p < 0.05$). The expression of DDR-2 was significantly higher in PP2Ac alpha transgenic mice compared to wild-type mice and TRPV1 knockout mice. The expression of miR21 was significantly higher in PP2Ac alpha transgenic mice compared with TRPV1−/− mice (0.103 ± 0.018 (PP2Ac alpha transgenic mice); 0.089 ± 0.009 (wild-type mice); and 0.045 ± 0.013 (TRPV1−/− mice), respectively; $p < 0.05$). Masson Goldner staining revealed that PP2Ac alpha transgenic mice showed increased heart fibrosis compared with TRPV1 knockout mice.

The study suggests an important role of TRPV1 in the pathogenesis of genetically associated heart hypertrophy.

1. Introduction

The role of transient receptor potential (TRP) cation channels in cardiovascular function and disease is increasingly recognized. TRP-mediated calcium influx is thought to be essential for heart hypertrophy and specific TRP subtypes have been found to mediate receptor-stimulated and pressure-overload induced heart hypertrophy [1]. Heart hypertrophy may be mediated by increased formation of cardiac para- and/or autocrine factors including endothelin-1, norepinephrine or angiotensin II, the receptors of which are coupled to G-proteins [2]. Downstream signaling pathways involve activation of phospholipase C causing increased formation of diacylglycerol, which is known to activate transient receptor potential vanilloid type 1 (TRPV1) cation channels. TRPV1 channels have been described in sensory nerve fibers in the heart [3]. The activation state of the TRPV1 receptors has been shown to be associated with cardiac functions, i.e. heart rate and inotropy [4].

We investigated the role of TRPV 1 in respect to heart hypertrophy in two specific (different) genetically modified mice models. Since it has been shown by our group that overexpression of the catalytic subunit of protein phosphatase 2A impairs cardiac function, this model was chosen for research in the present study

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[5]. The basis for the investigated genetically modified mouse model is the fact that protein phosphatase 2A, which is a multifunctional protein phosphatase with critical roles in excitable cell signals, and which has been suggested to regulate key ion channels and transporters [6], dephosphorylates phospholamban, an intrinsic membrane protein of the cardiac sarcoplasmic reticulum. Dephosphorylation of phospholamban in turn reduces the activity of the cardiac sarcoplasmic reticulum calcium ATPase, causing reduced filling of intracellular stores. Hence, dephosphorylation of phospholamban leads to reduced heart inotropy. We tested the hypothesis that in this novel genetic model of impaired cardiac function induced by cardiac specific overexpression of the catalytic subunit of protein phosphatase 2A, TRPV1 expression correlates with heart hypertrophy. Wild-type mice as well as TRPV1 knockout mice served as controls. Transcripts of TRPV1 together with a marker of myocardial fibrosis (matrix metalloproteinase 9; MMP9), a marker of heart failure (atrial natriuretic peptide; ANP), the heart failure-associated transcription factor GATA 4, and regulatory microRNA (miR-21) associated with heart failure [7] were analyzed using quantitative real-time PCR.

2. Materials and methods

2.1. Animals

Transgenic mice showing overexpression of the catalytic subunit alpha of protein phosphatase 2A (PP2Ac alpha) were used as described by us previously [5]. The NotI–SalI restriction enzyme fragment encoding mouse PP2Ac alpha along with its 5′- and 3′-untranslated regions was excised from the reverse transcriptase–PCR product and ligated into the same sites of a mouse cardiac alpha-myosin heavy chain promoter expression cassette containing the SV40 transcriptional terminator. The transgene composed of the alpha-myosin heavy chain promoter, the entire protein coding region for mouse PP2Ac alpha (and 69 and 326 base pairs of 5′- and 3′-untranslated sequences, respectively) and the SV40 polyadenylation signal sequence was isolated from the parent plasmid as a 7-kb NruI fragment and used for microinjection of fertilized mouse eggs. Transgene-positive mice were identified by Southern blotting and PCR assay of tail genomic DNA. One founder lineage was established and used for further studies. TRPV1−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed under a 12 h/12 h day/night cycle, and food and water were given ad libitum. Animals were given the normal standard laboratory chow. The local Animal Care and Use Committee approved all animal protocols according to US National Institutes of Health guidelines.

2.2. RNA isolation and quantitative real-time PCR

RNA isolation and quantitative real-time PCR was performed as described recently by our group [8]. Total RNA was isolated from cardiac tissue using RNeasy mini kit including RNase-free DNase set (Qiagen, Hilden, Germany). RNA was used to synthesize first-strand cDNA using the Transcriptor first-strand cDNA synthesis kit (Roche Diagnostics), total RNA was reverse-transcribed with a RT mixture consisting of Oligo-dT and 5 U AMV reverse transcriptase at 50 °C for 60 min, followed by heating to 85 °C for 5 min.

mRNA for TRPV1, matrix metalloproteinase 9 (MMP-9), discoidin domain receptor family, member 2 (DDR-2), transcription factor GATA4, catalytic subunit alpha of protein phosphatase 2A alpha (PP2Ac alpha), atrial natriuretic peptide (ANP) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were quantified using quantitative real-time PCR (LightCycler, Roche Diagnostics). Gene specific PCR primers for mice were designed using Lasergene7 software (DNA-Star, Madison, WI) or the internet based primer design tool primer3 (http://frodo.wi.mit.edu/). Primer design was checked with the internet based tool Oligo calc (http://www.basic.northwestern.edu/biotools/oligocalc.html). GAPDH was used as an internal control (housekeeping gene). To avoid the amplification of genomic DNA, primers were chosen in different exons. The primers for real-time PCR were the following: TRPV1 (Reference Sequence (RefSeq) database accession number: NM_001001445.1), forward, CCCATTGTGCAGATTGAGCAT; reverse, TTCCTGCAGAAGAGCAAG AAGC; MMP-9 (NM_013599.2); forward, CTCAGAGATTCTCCGTGTC CTGTA; reverse, GACTGCCAGGAAGACACTTGGTTA; DDR-2 (NM_022563.1), forward, CGGATCCTGATTGGTTGCTT; reverse, GTGATGAGGAGCGGTTGTTATTG; GATA4 (NM_008092.3), forward, AATGCCTGTGGCCTCTATCA; reverse, CTGGTTTGAATCCCCTCCTT; PP2a (NM_019411.3), forward, CTCTCACTGCCTTGGTGGAT; reverse, AGGCCATTGGCATGATTAAA; ANP (NM_008725.2), forward, GAACCTGCTAGACCACCT; reverse, CCTAGTCCACTCTGGGCT; GAPDH (NM_008084.2), forward, ACCTCAACTACATGGTCTAC; reverse, TTGTCATTGAGAGCAATGCC. The PCR products were size fractionated on 1% agarose gels, and DNA was visualized by ethidium bromide staining using an imaging analyzer (Gel Doc 2000, Bio-Rad). Control PCR was performed in samples containing RNA (RNA sample), without RNA (−RNA sample), RNA without reverse transcriptase (−RT sample) and in samples containing no cDNA (−cDNA sample). These controls confirmed specific amplification of target and housekeeping gene.

Quantitative real-time PCR was performed using 2 μL of single stranded cDNA which was added to a final volume of 20 μL, which contained 4 μL of LightCycler-Fast Start DNA SYBR Green I mix (Roche Diagnostics), and 500 nmol/L of each primer. PCR was started with denaturation at 95 °C for 10 min. Then 45 cycles were performed under the following conditions: denaturation at 95 °C for 10 s, annealing at 68 °C (TRPV1), 60 ° (GAPDH), 62 °C (MMP9, DDR-2), 58 °C (GATA4), 56 ° (ANP) and 58 °C (PP2a) for 10 s, and extension at 72 °C for 15 s. Fluorescence data were acquired at the end of each extension phase. After amplification, a melting curve analysis from 65 °C to 95 °C with a heating rate of 0.1 °C per second with a continuous fluorescence acquisition was made to assure correct PCR amplification. Data were recorded on a LightCycler 2.0 Instrument and cycle threshold values (crossing points, Cp) for each reaction were determined using LightCycler Software Version 4.0 (Roche Diagnostics). The TRPV1, MMP9, ANP, GATA4 and PP2a expressions were determined relative to the housekeeping gene GAPDH and normalized ratios of gene expression were calculated including efficiency correction and calibrator normalization. Normalized ratios were expressed according to the following equation: $E_T^{CpT(C)-CpT(S)} \times E_R^{CpR(S)-CpR(C)}$ with E_T or E_R, efficiency of target/reference amplification; CpT or CpR, cycle number at target/reference detection threshold (crossing point); T, target; R, reference; S, unknown sample; C, calibrator. All substances were obtained from Sigma–Aldrich (Taufkirchen, Germany) or Merck Biosciences (Schwalbach, Germany) if not indicated otherwise.

2.3. Isolation of microRNA

microRNA from mouse heart was prepared using mirVana miRNA isolation kit (Ambion). Amplification and detection of miR21-were performed with the LightCycler instrument with the cycle profile according to the mirVana qRT–PCR miRNA detection kit (Ambion). As an internal control, U6 primers were used for RNA template normalization.

2.4. Immunoblotting of TRPV1 channels

Frozen tissue samples of mouse heart were pulverized by mortar and pestle in a mortar precooled in liquid nitrogen and afterward transferred to a tube containing 1 mL ice-cold phos-

phate-buffered saline and homogenized on ice for 30 s using an Ultraturrax instrument (IKA–Werke, Germany). The homogenate was centrifuged at 4 °C for 3 min at 3000 rpm and the supernatant discarded. Then 1 mL solution L2 containing 25 mmol/L Tris–HCl, pH 8; 1 mol/L NaCl; 200 mmol/L ethylenediaminetetraacetic acid; 1 mol/L β-mercaptoethanol; 1 mol/L sodium fluoride and complete mini protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany) was added. The homogenate was sonicated three times for 20 s and centrifuged at 4 °C for 10 min at 10 500 rpm. The proteins in the supernatant were concentrated using a centrifugal filter device (Amicon ultra, Millipore), mixed with loading buffer, heated up to 100 °C for 3 min, separated using a denaturing 10% sodium-dodecyl-sulfate–polyacrylamide-gel electrophoresis at 150 V for 90 min, and transferred to pure nitrocellulose membranes (Trans-Blot transfer medium, Bio-Rad Laboratories, CA, USA) at 14 V overnight. Membranes were blocked with Odyssey blocking buffer (Li-COR Biosciences, Bad Homburg, Germany) for 1 h at room temperature and incubated with primary rabbit anti-mice TRPV1 antibody (Alomone Labs) at a 1:500 solution containing 0.1% Tween 20, Odyssey blocking buffer, and phosphate-buffered saline for 1 h, then washed four times for 5 min. Afterward membranes were incubated with the secondary antibody (IRDye800-infrared fluorescent dye-conjugated sheep anti-rabbit antibody (Biomol, Hamburg, Germany)) at a 1:1000 solution containing 0.1% Tween 20, Odyssey blocking buffer, and phosphate-buffered saline for 1 h and washed four times for 5 min. Imaging was performed at wavelengths of 700 nm and 800 nm. The predicted molecular weight for TRPV1 was 95 kDa. These experiments confirmed the molecular mass of the TRPV1 channel and showed that the antibody can be used to identify TRPV1 channels in mouse cardiac tissue.

2.5. Histology

Masson Goldner staining of hearts were performed using conventional techniques.

2.6. Statistical methods

All data were expressed as mean ± SEM of at least four independent experiments and were compared using a two-tailed Mann–Whitney test or Kruskal–Wallis test with Dunn's multiple comparison post test, as appropriate. The null hypothesis was rejected at $p < 0.05$. Relations between variables were investigated using linear regression analysis. Where error bars do not appear on the figure, error was within the symbol size. All data were analyzed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Heart hypertrophy in PP2Ac alpha transgenic mice

Ventricle-to-body-weight-ratio was significantly higher in PP2Ac alpha transgenic mice compared to wild-type mice and TRPV1 knockout mice (8.6 ± 1.3 mg/g; 5.4 ± 0.3 mg/g; and 5.4 ± 0.4 mg/g; respectively; $p < 0.05$ by Kruskal–Wallis test), indicating heart hypertrophy in transgenic mice (Fig. 1A). The expression of PP2Ac alpha transcripts were significantly higher in PP2Ac alpha transgenic mice compared to wild-type mice and TRPV1 knockout mice (3.63 ± 1.26 arbitrary units; 0.18 ± 0.04 arbitrary units; 0.01 ± 0.01 arbitrary units; respectively; $p < 0.01$ by Kruskal–Wallis test, Fig. 1B). In TRPV1 knockout mice the expression of PP2Ac alpha transcripts was 0.008 ± 0.006. As expected, compared to wild-type mice, the expression of PP2Ac alpha transcripts was 20 times higher in PP2Ac alpha transgenic mice. It should be noted that compared to TRPV1−/− mice, the expression of PP2Ac alpha transcripts was 454 times higher in PP2Ac alpha transgenic mice.

3.2. Increased TRPV1 expression in PP2Ac alpha transgenic mice

Next, we evaluated TRPV1 transcripts in the heart. Fig. 1C shows the expression of TRPV1 in PP2Ac alpha transgenic mice and wild-type mice. The PCR product for TRPV1 is comprised of 179 bp. Furthermore, as expected, in TRPV1 knockout mice, TRPV1 could not be detected. TRPV1 transcripts were significantly higher in PP2Ac alpha transgenic mice compared to wild-type mice (1.7 ± 0.2 arbitrary units vs. 0.8 ± 0.1 arbitrary units; $p < 0.05$ by Mann–Whitney test; Fig. 1D). We observed a significant correlation between the expression of TRPV1 transcripts and ventricle-to-body-weight-ratio ($r^2 = 0.54$; $p < 0.05$; Fig. 1E). Compared to base, in PP2Ac alpha transgenic mice the apex contained 121 ± 43% of TRPV1 transcripts, whereas in wild-type mice the apex contained 67 ± 20% of TRPV1 transcripts. These results indicate that the increased TRPV1 transcripts observed in PP2Ac alpha transgenic mice is predominantly observed in the epicarp of the heart. Immunoblotting confirmed that TRPV1 protein expression was also significantly higher in PP2Ac alpha transgenic mice compared to wild-type mice (1.55 ± 0.01 arbitrary units vs. 1.00 ± 0.01 arbitrary units; $p < 0.05$ by Mann–Whitney test; Fig. 1F). However, we did not observe a significant linear correlation between TRPV1 protein expression and ventricle-to-body-weight-ratio ($r^2 = 0.35$; $p = 0.12$), probably indicating that protein expression is differentially regulated.

3.3. Heart failure and increased fibrosis in PP2Ac alpha transgenic mice

ANP and GATA4 are established markers of heart failure. ANP expression was significantly higher in PP2Ac alpha transgenic mice compared to wild-type mice and TRPV1 knockout mice (1.43 ± 0.56 arbitrary units; 0.76 ± 0.16 arbitrary units; and 0.08 ± 0.06 arbitrary units; respectively; $p < 0.05$ by Kruskal–Wallis test), indicating heart failure in transgenic mice (Fig. 2A). GATA4 expression was significantly higher in PP2Ac alpha transgenic mice compared to wild-type mice and TRPV1 knockout mice (1.36 ± 0.59 arbitrary units; 1.27 ± 0.25 arbitrary units; and 0.09 ± 0.03 arbitrary units; respectively; $p < 0.05$ by Kruskal–Wallis test; Fig. 2B). Furthermore, as a marker for myocardial fibrosis, the MMP9 expression was significantly higher in PP2Ac alpha transgenic mice compared to wild-type mice and TRPV1 knockout mice (1.41 ± 0.23 arbitrary units; 0.84 ± 0.20 arbitrary units; and 0.06 ± 0.02 arbitrary units; respectively; $p < 0.05$ by Kruskal–Wallis test; Fig. 2C). The specific fibroblast marker for myocardial fibrosis, discoidin domain receptor family, member 2 (DDR-2), was also investigated. The expression of DDR-2 was significantly higher in PP2Ac alpha transgenic mice compared to wild-type mice and TRPV1 knockout mice (2.60 ± 0.55 arbitrary units; 0.73 ± 0.26 arbitrary units; and 0.19 ± 0.10 arbitrary units; respectively; $p < 0.05$ by Kruskal–Wallis test; Fig. 2D).

3.4. Increased expression of miR21 in PP2Ac alpha transgenic mice

The expression of regulatory miR21 was significantly higher in PP2Ac alpha transgenic mice compared to TRPV1−/− mice (0.103 ± 0.018 arbitrary units (PP2Ac alpha transgenic mice); 0.089 ± 0.009 arbitrary units (wild-type mice); and 0.045 ± 0.013 arbitrary units (TRPV1−/− mice), respectively; $p < 0.05$ by Kruskal–Wallis test; Fig. 2E). Histologic evaluation using Masson Goldner staining revealed that hearts from PP2Ac alpha transgenic mice showed increased fibrosis compared to TRPV1 knockout mice (Fig. 2F).

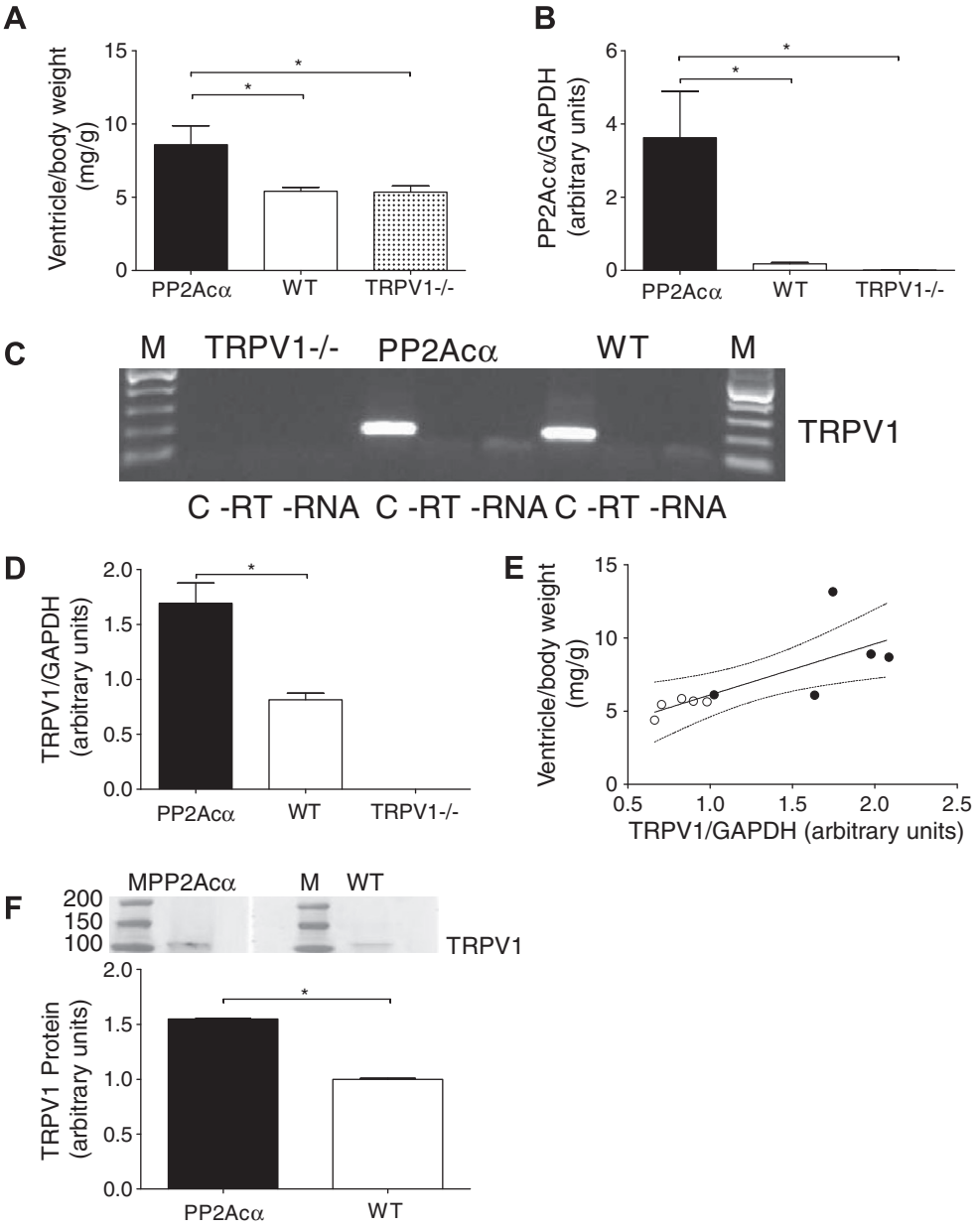

Fig. 1. Heart hypertrophy in PP2Ac alpha transgenic mice is associated with increased TRPV1. (A) Ventricle-to-body-weight-ratio in transgenic mice showing overexpression of the catalytic subunit alpha of protein phosphatase 2A (PP2Acα), in wild-type mice (WT), and in transient receptor potential vanilloid type 1 (TRPV1) knockout mice (TRPV1−/−). $^*p < 0.05$ by Kruskal–Wallis test with Dunn's multiple comparison post test. (B) PP2Ac alpha mRNA expression in heart from transgenic mice PP2Acα, WT, and TRPV1−/−. $^*p < 0.05$ by Kruskal–Wallis test with Dunn's multiple comparison post test. (C) Representative PCR products from mRNA of TRPV1 genes in heart from transgenic mice PP2Acα, WT, and TRPV1−/−. C indicates regular PCR, whereas control PCR was performed in samples with RNA without reverse transcriptase (−RT sample) or without RNA (−RNA sample). Marker denotes 100-bp ladder. (D) Increased TRPV1 mRNA expression in heart from transgenic mice PP2Acα compared to WT. $^*p < 0.05$ by Mann–Whitney test. TRPV1 mRNA could not be detected in TRPV1−/− mice. (E) Correlation between TRPV1 expression in heart and ventricle-to-body-weight-ratio in transgenic mice PP2Acα (filled circles) and WT (open circles; $r^2 = 0.54$; $p < 0.05$). (F) Increased TRPV1 protein expression in heart from transgenic mice PP2Acα compared to WT. Representative immunoblottings are shown on the top. M denotes marker. $^*p < 0.05$ by Mann–Whitney test.

4. Discussion

The present study showed an increased expression of TRPV1 cation channels in hypertrophic hearts. Transgenic mice had significantly elevated expression of both PP2Ac alpha transcripts and TRPV1 transcripts compared to wild-type mice and TRPV1 knockout mice, supporting the association between PP2Ac alpha and TRPV1 expression. We showed a significant correlation between TRPV1 expression and ventricle-to-body-weight-ratio in wild-type mice and transgenic mice showing an overexpression of the catalytic subunit of protein phosphatase 2A. These transgenic mice will develop severe heart hypertrophy within 3 months [5]. On the other hand, TRPV1−/− are viable, fertile, normal in size and do not display any gross physical or behavioral abnormalities. Rong et al. did not report a significant difference of litter size, growth rate, body weight or physical activity between TRPV1−/− mice and wild-type mice precluding overt heart failure in TRPV1−/− mice [9]. These data indicate that the development of heart hypertrophy is directly associated with increased TRPV1 expression.

Altered cytosolic calcium handling may contribute to hypertrophy of the heart and finally impaired pump function in heart failure. Furthermore, previous results indicated that reduced filling

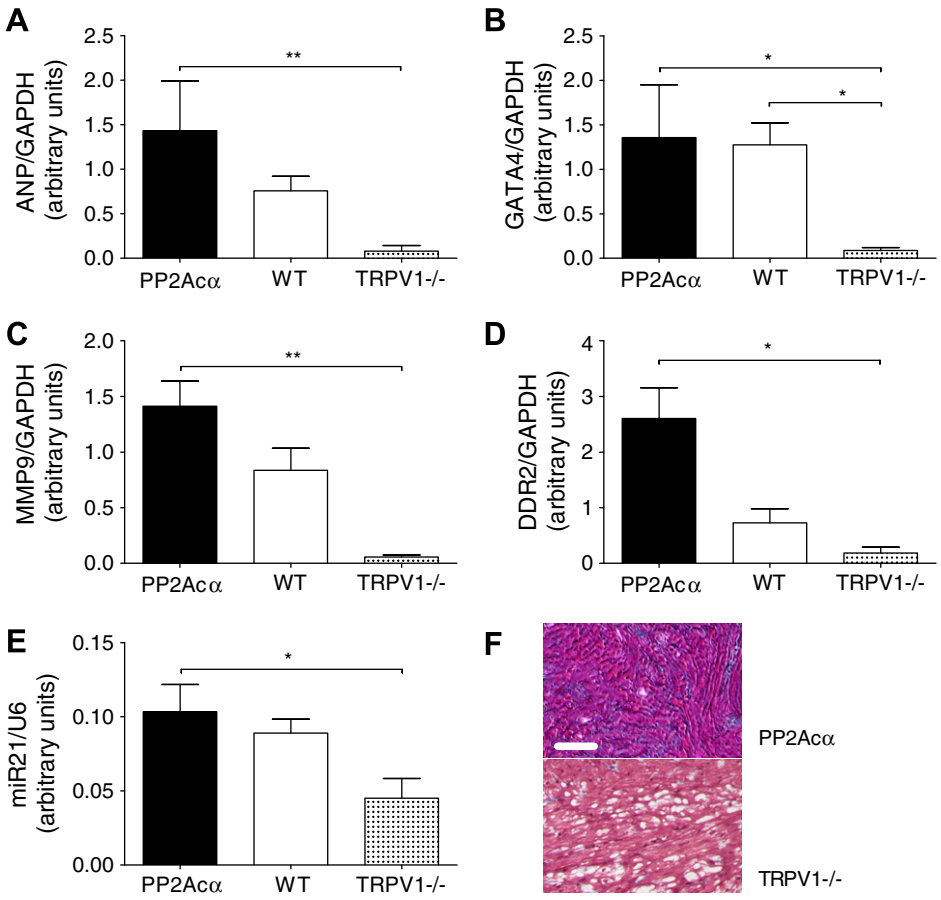

Fig. 2. Markers of heart failure and fibrosis in transgenic mice PP2Ac alpha are associated with increased TRPV1. Atrial natriuretic peptide (ANP) mRNA expression (A) transcription factor GATA4 mRNA expression (B) fibrosis marker MMP9 (C) discoidin domain receptor family, member 2 (DDR-2) (D) in heart from transgenic mice PP2Acα, WT, and TRPV1−/−. $^{*}p < 0.05$; $^{**}p < 0.01$ by Kruskal–Wallis test with Dunn's multiple comparison post test. (E) Regulatory microRNA mir21 in heart from transgenic mice PP2Acα, WT, and TRPV1−/−. $^{*}p < 0.05$ by Kruskal–Wallis test with Dunn's multiple comparison post test. (F) Masson Goldner staining of hearts from PP2Ac alpha transgenic mice and TRPV1 knockout mice. Blue color indicates fibrosis. Bar indicates 10 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of intracellular calcium stores may cause heart hypertrophy and deteriorate heart failure [10]. Seth et al. showed that the reduction of sarco-endoplasmic reticulum calcium ATPase expression was associated with upregulation of transient receptor potential canonical channels, sodium–calcium exchanger, and related transcriptional factors, including stimulating protein 1, myocyte enhancer factor 2, or nuclear factor of activated cells 4 [11]. Gergs et al. showed that increased dephosphorylation of phospholamban by overexpression of the catalytic subunit of protein phosphatase 2A in transgenic mice reduces the activity of the sarco-endoplasmic reticulum calcium ATPase leading to reduced filling of intracellular stores [5]. The present study indicated that overexpression of the catalytic subunit of protein phosphatase 2A in transgenic mice was associated with increased expression of TRPV1 compared to wild-type mice and TRPV1 knockout mice. These findings may indicate that increased calcium influx through TRPV1 channels may contribute to disturbed calcium homeostasis in PP2Ac alpha transgenic mice. An increased transmembrane calcium influx may aggravate excitation–contraction coupling, apoptosis of cardiac cells, and finally cardiac remodeling.

In the heart, TRPV1 expression has been observed to be restricted to epicardial and endothelial tissue and to sensory nerve fibers innervating the heart [3,4]. The TRPV1 expressed in the sensory nerves may modulate cardiac function directly by releasing several neurotransmitters including substance P and calcitonin gene-related peptide from peripheral nerve terminals [3]. It was shown that capsaicin-sensitive sensory nerves containing calcitonin gene-related peptide contribute to cardioprotective mechanisms since elimination of capsaicin-sensitive afferent nerves by systemic treatment with capsaicin promote the development and progression of myocardial dysfunction in an established adriamycin-induced experimental cardiomyopathy model in rats [12]. Wei et al. reported that in streptozotocin-induced diabetic mice a reduced expression of TRPV1 was related to the poor recovery of cardiac function after myocardial ischemia, further underscoring the importance of these channels for heart viability [13]. Furthermore in a DNA microarray study it was shown that selective chemoenervation by capsaicin pretreatment results in a cardiac dysfunction characterized by a significant elevation of left ventricular end-diastolic pressure and up-regulation of TRPV1 and other TRP-related genes [14]. Since we observed increased TRPV1 expression in the heart it may be hypothesized that increased activation of sensory nerves leading to increased release of these neurotransmitters finally aggravate heart hypertrophy and heart failure in transgenic mice showing overexpression of the catalytic subunit of protein phosphatase 2A. Recently, Huang et al. showed that in TRPV1−/− mice left anterior descending coronary ligation produced greater infarct size and lower survival rate compared to wild-type mice [4]. A severe ischemic challenge shall necessarily be more deleterious to hearts with of paucity of cardiac myofibrils and hence reduced cardiomyocyte contractility. Furthermore, as already indicated by Wang et al., the long-term absence the TRPV1

in TRPV1−/− mice or the long-term overexpression of TRPV1 in transgenic mice may elicit several additional compensatory changes [3].

In the present study we observed a significant association between TRPV1 expression and both ANP and MMP9 expression, indicating enhanced heart failure and enhanced fibrosis in hypertrophic hearts. Earlier studies indicated that an increased calcium influx can activate the calcneurin signaling pathway leading to increased ANP and MMP9 expression [15]. microRNAs are small non-coding RNAs which are thought to regulate gene-expression on a post-transcriptional level by degrading or translational repression of their correlating target mRNAs. Recently increased expression of miR-21 has been shown to be associated with heart failure via the stimulation of MAP kinase signaling in fibroblasts. [7] Furthermore it has been shown that miR-21 regulates fibroblast metalloproteinase-2 via phosphatase and tensin [16].

Conflict of interest

None.

References

[1] M. Nishida, H. Kurose, Roles of TRP channels in the development of cardiac hypertrophy, Naunyn Schmiedebergs Arch. Pharmacol. 378 (2008) 395–406.
[2] N. Wettschureck, H. Rütten, A. Zywietz, D. Gehring, T.M. Wilkie, J. Chen, K.R. Chien, S. Offermanns, Absence of pressure overload induced myocardial hypertrophy after conditional inactivation of Galphaq/Galpha11 in cardiomyocytes, Nat. Med. 7 (2001) 1236–1240.
[3] L. Wang, D.H. Wang, TRPV1 gene knockout impairs postischemic recovery in isolated perfused heart in mice, Circulation 112 (2005) 3617–3623.
[4] W. Huang, J. Rubinstein, A.R. Prieto, L.V. Thang, D.H. Wang, Transient receptor potential vanilloid gene deletion exacerbates inflammation and atypical cardiac remodeling after myocardial infarction, Hypertension 53 (2009) 243–250.
[5] U. Gergs, P. Boknik, I. Buchwalow, L. Fabritz, M. Matus, I. Justus, G. Hanske, W. Schmitz, J. Neumann, Overexpression of the catalytic subunit of protein phosphatase 2A impairs cardiac function, J. Biol. Chem. 279 (2004) 40827–40834.
[6] N. Bhasin, S.R. Cunha, M. Mudannayake, M.S. Gigena, T.B. Rogers, P.J. Mohler, Molecular basis for PP2A regulatory subunit B56alpha targeting in cardiomyocytes, Am. J. Physiol. Heart Circ. Physiol. 293 (2007) H109–H119.
[7] T. Thum, C. Gross, J. Fiedler, T. Fischer, S. Kissler, M. Bussen, P. Galuppo, S. Just, W. Rottbauer, S. Frantz, M. Castoldi, J. Soutschek, V. Koteliansky, A. Rosenwald, M.A. Basson, J.D. Licht, J.T. Pena, S.H. Rouhanifard, M.U. Muckenthaler, T. Tuschl, G.R. Martin, J. Bauersachs, S. Engelhardt, MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signaling in fibroblasts, Nature 456 (2008) 980–984.
[8] F. Thilo, A. Scholze, D.Y. Liu, W. Zidek, M. Tepel, Association of transient receptor potential canonical type 3 (TRPC3) channel transcripts with proinflammatory cytokines, Arch. Biochem. Biophys. 471 (2008) 57–62.
[9] W. Rong, K. Hillsley, J.B. Davis, G. Hicks, W.J. Winchester, D. Grundy, Jejunal afferent nerve sensitivity in wild-type and TRPV1 knockout mice, J. Physiol. 560 (2004) 867–881.
[10] G. Inesi, A.M. Prasad, R. Pilankatta, The Ca^{2+} ATPase of cardiac sarcoplasmic reticulum: Physiological role and relevance to diseases, Biochem. Biophys. Res. Commun. 369 (2008) 182–187.
[11] M. Seth, C. Sumbilla, S.P. Mullen, D. Lewis, M.G. Klein, A. Hussain, J. Soboloff, D.L. Gill, G. Inesi, Sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) gene silencing and remodeling of the Ca^{2+} signaling mechanism in cardiac myocytes, Proc. Natl. Acad. Sci. USA 101 (2004) 16683–16688.
[12] M. Katona, K. Boros, P. Sántha, P. Ferdinandy, M. Dux, G. Jancsó, Selective sensory denervation by capsaicin aggravates adriamycin-induced cardiomyopathy in rats, Naunyn Schmiedebergs Arch. Pharmacol. 370 (2004) 436–443.
[13] Z. Wei, L. Wang, J. Han, J. Song, L. Yao, L. Shao, Z. Sun, L. Zheng, Decreased expression of transient receptor potential vanilloid 1 impairs the postischemic recovery of diabetic mouse hearts, Circ. J. 73 (2009) 1127–1132.
[14] A. Zvara, P. Bencsik, G. Fodor, T. Csont, L. Hackler Jr., M. Dux, S. Fürst, G. Jancsó, L.G. Puskás, P. Ferdinandy, Capsaicin-sensitive sensory neurons regulate myocardial function and gene expression pattern of rat hearts: A DNA microarray study, FASEB J. 20 (2006) 160–162.
[15] K. Kuwahara, Y. Wang, J. McAnally, J.A. Richardson, R. Bassel-Duby, J.A. Hill, E.N. Olson, TRPC6 fulfills a calcineurin signaling circuit during pathologic cardiac remodeling, J. Clin. Invest. 116 (2006) 3114–3126.
[16] S. Roy, S. Khanna, S.R. Hussain, S. Biswas, A. Azad, C. Rink, S. Gnyawali, S. Shilo, G.J. Nuovo, C.K. Sen, MicroRNA expression in response to murine myocardial infarction: miR-21 regulates fibroblast metalloprotease-2 via phosphatase and tensin homologue, Cardiovasc. Res. 82 (2009) 21–29.