Cardiovascular Pathology 25 (2016) 478–482

Contents lists available at ScienceDirect

Cardiovascular Pathology

Original Article

Endoglin selectively modulates transient receptor potential channel expression in left and right heart failure☆,☆☆

Kevin J. Morine, Vikram Paruchuri, Xiaoying Qiao, Mark Aronovitz, Gordon S. Huggins, David DeNofrio, Michael S. Kiernan, Richard H. Karas, Navin K. Kapur *

Molecular Cardiology Research Institute and Division of Cardiology, Department of Medicine, Tufts Medical Center, 800 Washington Street, Boston, MA 02111, USA

ARTICLE INFO

Article history:
Received 5 May 2016
Received in revised form 4 August 2016
Accepted 18 August 2016
Available online xxxx

Keywords:
Heart failure
Transient receptor potential channels
Right ventricular failure
Endoglin

ABSTRACT

Introduction: Transient receptor potential (TRP) channels are broadly expressed cation channels that mediate diverse physiological stimuli and include canonical (TRPC), melastatin (TRPM), and vanilloid (TRPV) subtypes. Recent studies have implicated a role for TRPC6 channels as an important component of signaling via the cytokine, transforming growth factor beta 1 (TGFβ1) in right (RV) or left ventricular (LV) failure. Endoglin (Eng) is a transmembrane glycoprotein that promotes TRPC6 expression and TGFβ1 activity. No studies have defined biventricular expression of all TRP channel family members in heart failure.
Hypothesis: We hypothesized that heart failure is associated with distinct patterns of TRP channel expression in the LV and RV.
Methods: Paired viable LV and RV free wall tissue was obtained from human subjects with end-stage heart failure (n=12) referred for cardiac transplantation or biventricular assist device implantation. Paired LV and RV samples from human subjects without heart failure served as controls (n=3). To explore a functional role for Eng as a regulator of TRP expression in response to RV or LV pressure overload, wild-type ($Eng^{+/+}$) and Eng haploinsufficient ($Eng^{+/-}$) mice were exposed to thoracic aortic (TAC) or pulmonary arterial (PAC) constriction for 8 weeks. Biventricular tissue was analyzed by real-time polymerase chain reaction.
Results: Compared to nonfailing human LV and RV samples, mRNA levels of TRPC1, 3, 4, 6, and TRPV-2 were increased and TRPM2, 3, and 8 were decreased in failing LV and RV samples. TRPC1 and 6 levels were higher in failing RV compared to failing LV samples. After TAC, murine LV levels of TPRC1 and 6 were increased in both $Eng^{+/+}$ and $Eng^{+/-}$ mice compared to sham controls. LV levels of TRPC4, TRPM3 and 7, TRPV2 and 4 were increased in $Eng^{+/+}$, not in $Eng^{+/-}$ mice after TAC. After PAC, all TRP channel family members were increased in the RV, but not LV, of $Eng^{+/+}$ compared to sham controls. In contrast to $Eng^{+/+}$, PAC did not increase RV or LV levels of TRP channels in $Eng^{+/-}$ mice.
Conclusions: This is the first study to demonstrate that TRP channels exhibit distinct profiles of expression in the LV and RV of patients with heart failure and in murine models of univentricular pressure overload. We further introduce that the TGFβ1 coreceptor Eng selectively regulates expression of multiple TRP channels in the setting of LV or RV pressure overload.

1. Introduction

Heart failure is a major cause of morbidity and mortality for nearly 24 million individuals worldwide [1]. While much attention has focused on signaling mechanisms regulating left ventricular (LV) remodeling, the negative impact of right ventricular (RV) dysfunction on survival remains a significant problem for patients with left heart failure or lung disease [2–6]. However, several lines of evidence suggest that the RV and LV have distinct profiles of response to injury including the following: (a) the developmental origin of the RV from a heart field distinct from the LV, (b) a thin RV free wall with susceptibility to increased wall stress, (c) a greater dependence of RV stroke volume on afterload, and (d) enhanced RV contractile resilience to pressure overload [7–11]. Our understanding of the mechanisms governing RV remodeling stems primarily from data generated in models of LV failure. The identification of molecular targets that improve LV and RV function remains a significant unmet need for patients suffering from heart failure and lung disease.

Transient receptor potential (TRP) channels are broadly expressed cation channels that mediate diverse physiological stimuli and include

☆ Funding: This work was supported by a grant from the National Institutes of Health (R56HL118113-01A1) to N.K. and a Heart Failure Society of America Research Fellowship to K.M.
☆☆ There are no relevant financial relationships to disclose.
* Corresponding author at: Tufts Medical Center, 800 Washington Street, Box #80, Boston, MA 02111, USA. Tel.: +1 617 636 9371; fax: +1 617 636 1444.
E-mail address: Nkapur@tuftsmedicalcenter.org (N.K. Kapur).

http://dx.doi.org/10.1016/j.carpath.2016.08.004
1054-8807/

canonical (TRPC), melastatin (TRPM), and vanilloid (TRPV) subtypes [12]. TRP channels are emerging as key mediators of cardiac hypertrophy and fibrosis; however, little is known about TRP channel family expression in HF. Several recent studies implicate TRPC-6 as a central mediator of signaling via transforming growth factor beta 1 (TGFβ1). In these studies, TGFβ1 promotes expression of calcineurin, which increases levels of TRPC-6, which triggers calcium influx and subsequent calcineurin activation, thereby setting up a self-propagating mechanism for pathologic LV hypertrophy, fibrosis, and increased mortality in heart failure [13–16]. Expression of TRP channel family members in heart failure remains poorly understood.

Endoglin is a 180-kDA transmembrane glycoprotein that promotes TGFβ1 signaling in heart failure [17,18]. Endoglin null mice die at embryonic day 10.5 due to impaired cardiovascular development and extraembryonic angiogenesis [19]. In contrast, endoglin heterozygous mice ($Eng^{+/-}$) are viable and have reduced total body levels of endoglin. We recently reported that loss of the TGFβ1 coreceptor endoglin attenuates increased TRPC-6 expression, reduces RV fibrosis, and improves survival in murine models of RV failure [20]. Whether endoglin regulates expression of other TRP channel family members is not known. Based on these background data, the purposes of this study were to determine TRP channel expression in the LV and RV from patients with end-stage HF and to explore whether endoglin regulates expression of TRP channels in response to LV or RV pressure overload.

2. Methods

2.1. Human samples

Paired viable LV and RV free wall tissue was obtained from human subjects with end-stage HF (n=12) referred for orthotopic heart transplantation (OHT) or biventricular assist device implantation (BIVAD). Nonfailing LV and RV tissue obtained from the National Disease Research Interchange served as controls (n=3). All tissue was immediately frozen in liquid nitrogen and stored at −80°C until further processing as described below. All surgical procedures and tissue harvesting were performed in concordance with the National Institutes of Health and Tufts University Institutional Review Board guidelines.

2.2. Surgical models of heart failure

Animals were treated in compliance with the Guide for the Care and Use of Laboratory Animals (National Academy of Science). Animal protocols were approved by the Tufts Medical Center Institutional Animal Care and Use Committee. As described previously, adult, male, 12- to 14-week-old C57BL/6 wild-type (WT) ($Eng^{+/+}$) and $Eng^{+/-}$ mice underwent constriction of the thoracic aorta (TAC) or pulmonary artery (PAC) for 8 weeks to generate models of left HF or right HF, respectively [18,21]. Sham-operated mice served as controls (n=6). Ten weeks after TAC or PAC, terminal hemodynamics were recorded using biventricular conductance catheters as previously described [21], and both RV and LV tissue obtained for further analysis by real-time polymerase chain reaction (RT-PCR).

2.3. PCR

Total RNA was extracted from the LV and RV with Trizol (Life Technologies) and converted to cDNA with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). PCR was performed in triplicate using 40 cycles at 94°C for 15 s, 60°C for 30 s, and 72°C for 30 s with an ABI Prism 7900 Sequence Detection System. The primers used for detection are shown in Table 1.

2.4. Statistics

All statistical analyses were performed using Graph Pad Prism v6 (Graph Pad Software, Inc.). Comparison between two experimental groups was performed with the unpaired Student's t test and for three groups or more with a one-way analysis of variance. α Values less than .05 were accepted as statistically significant.

3. Results

3.1. TRP channel expression in human heart failure

To determine whether TRP channel expression is altered in patients with heart failure, LV and RV samples were obtained from patients with advanced heart failure referred for OHT (n=9) or BIVAD support (n=3; Table 2). Nonischemic cardiomyopathy was the primary etiology for heart failure in 11 subjects. Compared to nonfailing control LV and RV samples, levels of TRPC1, 3, 4, and 6 mRNA were increased in the failing LV and RV, respectively (Fig. 1). Levels of TRPC1 and 6 mRNA were higher in the failing RV than the failing LV. TRPV1 and 3 were not detectable in the human samples. TRPV2 levels were similarly increased in the failing LV and RV. Levels of TRPM 1, 6, and 7 mRNA were not detectable in the LV or RV. Levels of TRPM2, 3, and 8 were reduced to a similar extent in the failing LV and RV compared to nonfailing control LV and RV samples. TRPC2 is a pseudogene in humans and was not analyzed [12]. TRPC5 and 7 were not detectable in the human LV or RV. Compared to nonfailing human LV and RV samples, endoglin mRNA levels were increased in the LV and RV (Fig. 2).

3.2. TRP channel expression in LV pressure overload

To explore whether isolated LV failure altered TRP channel expression and the effect of endoglin haploinsufficiency on TRP channel expression, we employed the well-established model of LV pressure overload induced by TAC in WT and endoglin haploinsufficient mice ($Eng^{+/-}$). Compared to sham controls and WT mice after TAC, both RV and LV mRNA levels of endoglin are lower in $Eng^{+/-}$ mice (Fig. 2). In sham-operated controls, TRPC-1, 3, 4, and 6; TRPV-2 and 4; and TRPM-3, 4, 6, and 7 are expressed in the LV and RV. Compared to sham-operated controls, LV pressure overload increased LV mRNA levels of TRPC-1, 4, and 6; TRPM-3 and 7; and TRPV-2 and 4 levels (Fig. 3). TRPM-4 and TRPM-6 levels were unchanged with TAC in the LV and RV (data not shown). LV pressure overload only increased RV mRNA levels of TRPC-4 (Fig. 4). Compared to sham-operated controls, LV pressure overload increased LV mRNA levels of TRPC1 and TRPC 6 only in $Eng^{+/-}$ mice (Fig. 3). No change in RV mRNA levels of any TRP channels was observed after LV pressure overload in $Eng^{+/-}$ mice. Compared to WT mice subjected to TAC, LV mRNA levels of TRPC-4, TRPM-3, TRPV-2, and TRPV-4 were lower in $Eng^{+/-}$ mice after TAC. We observed

Table 1
Clinical characteristics of patients with advanced heart failure

Patient	Age (y)	Gender	Cardiomyopathy	Indication for surgery
1	20	F	NICM	OHT for postpartum cardiomyopathy
2	52	F	NICM	OHT for NICM post-LVAD
3	64	M	NICM	OHT for HCM
4	53	F	NICM	BIVAD for myocarditis
5	45	M	NICM	BIVAD for myocarditis
6	34	M	NICM	OHT for NICM post-LVAD
7	50	F	NICM	OHT for NICM
8	53	F	NICM	OHT for chemotherapy related NICM post-LVAD
9	44	F	NICM	OHT for giant cell myocarditis
10	64	M	ICM	OHT post-LVAD
11	56	M	NICM	BIVAD for myocarditis
12	67	F	NICM	OHT for chemotherapy related NICM