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Abnormal sodium channel mRNA splicing in hypertrophic cardiomyopathy

Adam M. Noyes [a,1], Anyu Zhou [a,1], Ge Gao [b], Lianzhi Gu [b], Sharlene Day [c], J. Andrew Wasserstrom [d], Samuel C. Dudley [a,*]

[a] Department of Cardiology, Warren Alpert School of Medicine at Brown University, Providence, RI, United States
[b] Cardiology Section, University of Illinois at Chicago, Chicago, IL, United States
[c] Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, MI, United States
[d] Feinberg Cardiovascular Research Institute, Feinberg School of Medicine, Northwestern University, Chicago, IL, United States

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ABSTRACT

Background: Our previous studies showed that in ischemic and nonischemic heart failure (HF), the voltage-gated cardiac Na^+ channel α subunit (SCN5A) mRNA is abnormally spliced to produce two truncated transcript variants (E28C and D) that activate the unfolded protein response (UPR). We tested whether SCN5A post-transcriptional regulation was abnormal in hypertrophic cardiomyopathy (HCM).
Material and methods: Human heart tissue was obtained from HCM patients. The changes in relative abundances of SCN5A, its variants, splicing factors RBM25 and LUC7A, and PERK, a major effector of the UPR, were analyzed by real time RT-PCR and the expression changes were confirmed by Western Blot.
Results: We found reduced full-length transcript, increased SCN5A truncation variants and activation of UPR in HCM when compared to control hearts. In these patients, real time RT-PCR revealed that HCM patients had decreased SCN5A mRNA to 27.8 ± 4.07% of control ($P < 0.01$) and an increased abundance of E28C and E28D (3.4 ± 0.3 and 2.8 ± 0.3-fold, respectively, $P < 0.05$). PERK mRNA increased 8.2 ± 3.1 fold ($P < 0.01$) in HCM patients. Western blot confirmed a significant increase of PERK.
Conclusions: These data suggested that the full-length SCN5A was reduced in patients with HCM. This reduction was accompanied by abnormal SCN5A pre-mRNA splicing and UPR activation. These changes may contribute to the arrhythmic risk in HCM.

1. Introduction

Hypertrophic cardiomyopathy (HCM) leading to sustained ventricular tachyarrhythmias is the most common cause of sudden cardiac death (SCD) in young patients. The cause of arrhythmias in HCM is unclear, however. Previous reports show that, in ischemic or nonischemic systolic heart failure (HF), SCN5A mRNA that encodes the cardiac Na^+ channel is abnormally spliced to produce two truncated transcript variants (E28C and D) that encode nonfunctional Na^+ channels that are trapped in the endoplasmic reticulum (ER), leading to activation of the unfolded protein response (UPR) [1–3].

The activity of SCN5A-encoded sodium channel determines cardiac depolarization and electrical conduction. Mutations in SCN5A genes, encoding the α-subunit of ion channels that conduct Na^+ current (I_{Na}), can be responsible for arrhythmogenic disorders. Sodium channel defects have been linked to the development of cardiac arrhythmias and to SCD [4]. In this work, we tested whether SCN5A post-transcriptional regulation was abnormal in HCM and whether the presence of truncated Na^+ channels was associated with activation of the UPR, potentially contributing to electrical remodeling and arrhythmias in HCM.

2. Material and methods

2.1. Human heart tissue samples

De-identified human heart tissue was obtained from myectomy samples from 7 patients with HCM, and 6 patient samples of normal human ventricular tissue obtained from unused transplant donor hearts were provided by Dr. J. Andrew Wasserstrom (Northwestern University, Chicago, IL). The control tissue samples were obtained from mid left ventricular free wall using a transmural section. All tissues were obtained with informed consent in accordance with the stipulations in the World Medical Association Declaration of Helsinki.

HCM tissue sample numbers obtained were 26, 32, 38, 46, 48, 61, 83. All patients had cardiac myosin binding protein C (MYBPC3) truncating mutations, except 46, which had a cardiac regulatory myosin light chain (MYL2) mutation. The control patients had no family history of SCD, arrhythmia or syncope. HCM, including specific gene mutations, and control patient demographics are reported in Table 1.

* Corresponding author at: Brown University, 593 Eddy Street, APC 814, Providence, RI 02903, United States.
E-mail address: samuel_dudley@brown.edu (S.C. Dudley).
[1] Co-first authors.

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2.2. Real-time PCR quantification

Total RNA was isolated from human ventricular tissue using the RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instruction. Total RNA was reverse transcribed into complementary DNA (cDNA) using random primer and SuperScript III (Termor Scientific, Waltham, MA) following the manufacturer's protocol. Quantitative real-time reverse-transcriptase polymerase chain reaction (qRT-PCR) was carried out using gene-specific primers described previously [1,2,5] Fast SYBR® Green Master Mix (Thermo Scientific, Waltham, MA) and 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The qRT-PCR reaction was activated with an initial denaturation step at 95 °C for 20 s, followed by cycles of denaturation at 95 °C for 3 s, and annealing and extension at 60 °C for 30 s. Samples were run in triplicate and averaged. Gene expression levels were normalized to the level of β-actin.

2.3. Antibodies and Western blot

Anti-RBM25 and anti-LUC7L3 antibodies were purchased from Novus Biologicals (Littleton, CO). Anti-PERK antibody was purchased from Cell Signaling Technologies (Danvers, MA). Anti *pan*-actin antibody was purchased from Thermo Fisher Scientific (Waltham, MA). Frozen cardiac samples were homogenized in triple-detergent lysis buffer (50 mmol/L Tris-HCl, 150 mmol/L NaCl, 0.1% SDS, 1% NP-40, and 1× Halt® Protease & Phosphatase Inhibitor Cocktail (Thermo Scientific, Waltham, MA), pH 7.5). The homogenates were centrifuged at 10,000*g* at 4 °C for 20 min and supernatants were collected for Western blot analysis. Equal amounts (30 μg) of proteins were separated on 4–20% mini-PROTEAN® TGX™ gels (Biorad, Hercules, CA) and were transferred on to PVDF membrane (EMD Millipore, Billerica, MA). Protein expression levels were detected by using specific primary antibodies and Clarity™ Western ECL Blotting Substrate (Biorad, Hercules, CA). Band intensities were be detected by ChemiDoc MP imaging System and analyzed by Image Lab software (Biorad, Hercules, CA).

2.4. Statistics

All data are presented as means and SD. Means were compared using unpaired Student's *t*-test. A probability value $P < 0.05$ was considered statistically significant.

3. Results

3.1. Cardiac Na^+ channel SCN5A pre-mRNA was abnormally spliced in HCM

We have shown previously that the pre-mRNA of SCN5A exon 28 is abnormally spliced in ischemic or nonischemic, acquired cardiomyopathy [3,4]. Here, we investigated whether the pre-mRNA splicing of SCN5A exon 28 was altered in HCM. Compared to that in the normal control human hearts, the full-length SCN5A transcript was substantially decreased in HCM heart tissues. The mRNA level of the full-length SCN5A in the HCM patient group was 27.8% ± 4.1% (Fig. 1, Panel A, $P < 0.01$) of that in the normal control group. Consistent with an inverse relationship of the full-length transcripts to the SCN5A splice variants, the expression levels of the truncated variants E28C and E28D were increased significantly in HCM hearts. SCN5A variants E28C and E28D increased 3.4 ± 0.3 fold (Fig. 1, Panel B, $P < 0.05$) and 2.8 ± 0.3 fold, (Fig. 1, Panel C, $P < 0.05$) in HCM patients, respectively.

3.2. Splicing factors RBM25 and Luc7A were upregulated in HCM

In dilated or ischemic cardiomyopathy, abnormal splicing of SCN5A pre-mRNA is mediated by splicing factors RBM25 and Luc7A. In HCM, the expression of RBM25 and Luc7A increased at both the mRNA and protein levels. Compared to that in the normal human heart tissue, RBM25 increased 3.17 ± 0.47 fold (Fig. 2, Panel A, $P < 0.05$) and Western blot confirmed an increase of RBM25 protein of 1.6 ± 0.05 fold (Fig. 2, Panel B, $p < 0.05$). The expression of Luc7A mRNA increased 2.16 ± 0.40 fold (Fig. 2, Panel C, $P < 0.05$) fold. Western blot confirmed the expression of Luc7A protein increased 1.90 ± 0.20 fold (Fig. 2, Panel D, $P < 0.05$) in patients with HCM.

3.3. UPR component PERK was upregulated in human HCM

In ischemic or dilated cardiomyopathy, abnormal SCN5A splicing variants are known to activate the UPR, and this activation further reduces SCN5A full-length mRNA transcript abundance [1]. To investigate whether increased SCN5A variants promote UPR activation in HCM, an UPR major component PERK was measured at both the mRNA and protein levels by qRT-PCR and Western blotting. Real time RT-PCR revealed that PERK mRNA increased 8.2 ± 3.1 fold (Fig. 3, Panel A, $P < 0.05$) in HCM patients compared to controls. Western blotting confirmed a significant increase of PERK protein of 2.3 ± 0.01 fold (Fig. 3, Panel B, $P < 0.05$) in HCM.

4. Discussion

Modification in cardiac sodium current alters cardiac conduction and is associated with arrhythmogenesis [6]. Hypertrophic cardiomyopathy is frequently associated with arrhythmic SCD [7,8], though the pathophysiological triggers remain poorly understood [9]. Ventricular arrhythmia is common in patients with HCM [10] with ventricular tachycardia proving to be a significant independent risk factor for SCD in HCM, especially in the young [11].

Previously, we have shown that ischemic and nonischemic heart failure are associated with increased abnormal SCN5A mRNA splicing, that the expression of SCN5A cardiac Na^+ channel mRNA splice variants in white blood cells (WBCs) correlates with levels in the heart, and that normalized WBC splicing levels predict increased risk of ventricular arrhythmias [12]. In dilated or ischemic cardiomyopathy, the two SCN5A mRNA splicing variants reach greater that >50% of the total SCN5A mRNA and do not produce functional Na^+ channels [2,5]. The expression of these variants in cells stably expressing the full-length Na^+ channel causes a dose-dependent reduction in full-length SCN5A transcripts, as well as the reduction in functional Na^+ current expression [2,5]. This dominant negative effect is mediated by the UPR [1].

Table 1
Demographics of study patients.

Subject	Sex	Age	Race	Mutation	Family History of SCD	Prior ventricular arrhythmia[a]	Prior Syncope	NYHA class	LVOT resting (mm Hg)
HCM	F	48	C	MYBPC3 c.2905 + 1G>A	Yes	Yes	Yes	II	163
HCM	F	43	C	MYBPC3 c.3330 + 2T>G	Yes	Yes	No	III	130
HCM	F	44	C	MYBPC3 c.772G>A	No	Yes	No	I	92
HCM	F	19	C	MYL2 c.482 A>G	No	Yes	No	III	55
HCM	M	35	C	MYBPC3 c.2308G>A	Yes	Yes	No	I	10
HCM	F	57	C	MYBPC3 c.1928-2 A>G	Yes	No	Yes	II	94
HCM	M	60	C	MYBPC3 c.927-9 G>A	No	Yes	No	III	44
CON	F	56	C	N/A	No	No	No	N/A	N/A
CON	M	58	AA	N/A	No	No	No	N/A	N/A
CON	F	76	C	N/A	No	No	No	N/A	N/A
CON	M	61	C	N/A	No	No	No	N/A	N/A
CON	M	65	C	N/A	No	No	No	N/A	N/A
CON	M	55	C	N/A	No	No	No	N/A	N/A

AA: African American; C: Caucasian; CON: control; HCM: Hypertrophic Cardiomyopathy; MYBPC: Myosin-Binding Protein C; MYL: Myosin Light Chain; LVOT: Left Ventricular Outflow Tract; N/A: Not Applicable; NYHA: New York Heart Association; SCD: Sudden Cardiac Death.

[a] Ventricular arrhythmic events were defined as >3 consecutive beats of ventricular ectopy at a heart rate equal to or above 120 beats per minute detected by ambulatory monitoring.