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BRG1 and BRM function antagonistically with c-MYC in adult cardiomyocytes to regulate conduction and contractility

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ABSTRACT

Rationale: The contractile dysfunction that underlies heart failure involves perturbations in multiple biological processes ranging from metabolism to electrophysiology. Yet the epigenetic mechanisms that are altered in this disease state have not been elucidated. SWI/SNF chromatin-remodeling complexes are plausible candidates based on mouse knockout studies demonstrating a combined requirement for the BRG1 and BRM catalytic subunits in adult cardiomyocytes. *Brg1*/*Brm* double mutants exhibit metabolic and mitochondrial defects and are not viable although their cause of death has not been ascertained.
Objective: To determine the cause of death of *Brg1*/*Brm* double-mutant mice, to test the hypothesis that BRG1 and BRM are required for cardiac contractility, and to identify relevant downstream target genes.
Methods and results: A tamoxifen-inducible gene-targeting strategy utilizing αMHC-Cre-ERT was implemented to delete both SWI/SNF catalytic subunits in adult cardiomyocytes. *Brg1*/*Brm* double-mutant mice were monitored by echocardiography and electrocardiography, and they underwent rapidly progressive ventricular dysfunction including conduction defects and arrhythmias that culminated in heart failure and death within 3 weeks. Mechanistically, BRG1/BRM repressed *c-Myc* expression, and enforced expression of a DOX-inducible *c-MYC* trangene in mouse cardiomyocytes phenocopied the ventricular conduction defects observed in *Brg1*/*Brm* double mutants. BRG1/BRM and c-MYC had opposite effects on the expression of cardiac conduction genes, and the directionality was consistent with their respective loss- and gain-of-function phenotypes. To support the clinical relevance of this mechanism, BRG1/BRM occupancy was diminished at the same target genes in human heart failure cases compared to controls, and this correlated with increased *c-MYC* expression and decreased *CX43* and *SCN5A* expression.
Conclusion: BRG1/BRM and c-MYC have an antagonistic relationship regulating the expression of cardiac conduction genes that maintain contractility, which is reminiscent of their antagonistic roles as a tumor suppressor and oncogene in cancer.

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1. Introduction

Heart failure is a major cause of morbidity and mortality worldwide. Its complexity arises from abnormalities in many aspects of cardiac function, from metabolism to electrophysiology, resulting in impaired contraction and sudden cardiac death [1]. Electrophysiological remodeling is among the most common and important alterations in human heart failure. Heart failure patients suffer from both bradyarrhythmias and tachyarrhythmias, and sudden cardiac death is 6–9-fold more common in heart failure patients than the general population [1,2]. While some genes associated with the proarrhythmic state in heart failure are known, the epigenetic mechanisms regulating their expression are unknown, as are the functional connections between metabolic and arrhythmogenic conduction that occurs in human heart failure [1]. Epigenetic mechanisms are capable of long-term regulation of gene expression, which is highly relevant to cardiomyocytes where the expression of some genes must be maintained in the same cell over an individual's lifetime.

SWI/SNF chromatin-remodeling complexes are recruited by transcription factors to the enhancers and promoters of target genes where they reposition nucleosomes in an ATP-dependent manner to epigenetically regulate transcription [3–6]. Target genes are either activated or repressed in a context-dependent manner. Although SWI/SNF complexes are heterogeneous, BRG1 (also known as SMARCA4) and BRM (also known as SMARCA2) are the only catalytic subunits with ATPase activity. SWI/SNF complexes physically interact with cardiogenic transcription factors such as NKX2.5, TBX5, and GATA4, which make them a plausible candidate for regulating cardiomyocyte development in the embryo and contractile function in adults. Mouse knockout studies have demonstrated that BRG1 is required for cardiomyocyte development but is dispensable in adult cardiomyocytes [7–9], whereas BRM is completely dispensable [10]. To test the hypothesis that BRG1 and BRM functionally compensate in the adult cardiomyocyte, we generated *Brg1*$^{fl/fl}$ mice carrying an inducible, cardiomyocyte-specific *αMHC-Cre-ERT2* transgene that were also *Brm*$^{-/-}$. Indeed, these *Brg1*/*Brm* double mutants died within 3 weeks following the loss of *Brg1*, and they exhibited metabolic perturbations and mitochondrial defects in the heart [11,12]. However, their cause of death is unclear, and it is not known whether SWI/SNF is required for cardiac conduction or if this model is relevant to the study of heart failure. We, therefore, focused our efforts in the current study to address these issues and to identify downstream target genes that provide mechanistic insight.

2. Materials and methods

2.1. Mice

The αMHC-Cre-ERT mice [also known as B6.Cg-Tg(Myh6-cre/Esr1)1JmkJ or αMHC-MerCreMer] were obtained from The Jackson Laboratory (#005657, Bar Harbor, ME) and genotyped as previously described [13]. The *Brg1* conditional mutant mouse line and *Brm* constitutive mutant mouse line have been described previously [10,14,15]. Genotyping of the *Brg1* floxed and Δfloxed alleles and the *Brm* mutation were performed by PCR as previously described [10,14,15]. To induce the *Brg1* conditional mutation in adult cardiomyocytes, 3–6 month old male and female mice were provided rodent chow containing tamoxifen (Sigma-Aldrich #T5648, St. Louis, MO) over a 7-day period. 500 mg of tamoxifen was mixed with 1 kg of ground-up rodent chow and then mixed with water, kneaded into pellets, and dried in a hood. Provided to mice ad libitum, the dose was estimated to be 80 mg/kg/day. After the 7-day treatment period, the tamoxifen-fortified chow was removed and replaced with the same chow lacking tamoxifen. A similar number of male and female mice were used in the study, and no phenotypic differences were observed between the two sexes as indicated in Supplemental Fig. 2.

The bi-transgenic mouse line that inducibly overexpresses the human *c-MYC* cDNA in cardiomyocytes under the control of the αMHC promoter has been previously described [16]. Mice were raised in the absence of doxycycline (DOX) to prevent developmental consequences from c-*MYC* overexpression. c-*MYC* was induced by feeding mice Dox-containing rodent chow (200 mg/kg, Bio-Serve, Frenchtown, NJ) ad libitum. Dox had no effect on single transgenic littermates, which were used as controls in analyses performed in this study.

All mouse experiments were approved by the Institutional Animal Care and Use Committees (IACUC) review boards at the University of North Carolina at Chapel Hill and Case Western Reserve University and were performed in accordance with federal guidelines.

2.2. Echocardiography

Conscious cardiac transthoracic echocardiography was performed on mice at the indicated time points using a VisualSonics Vevo 2100 ultrasound biomicroscopy system (VisualSonics, Inc., Toronto, Ontario, Canada) as previously described [17,18]. Two-dimensional M-mode echocardiography was performed in the parasternal long-axis view at the level of the papillary muscle on loosely restrained mice. Anterior and posterior wall thickness was measured as distance from epicardial to endocardial leading edges. Left ventricular internal diameters were also measured. Left ventricular systolic function was assessed by ejection fraction (LV EF% = [(LV Vol; d-LV Vol; s/LV Vol; d) × 100]) and fractional shortening (%FS = [(LVEDD − LVESD)/LVEDD] × 100). Investigators were blinded to mouse genotype from collection through waveform measurements. Each measurement represents the average of three cardiac cycles from each mouse.

2.3. Electrocardiography

Continuous electrocardiographies (ECGs) were monitored by surgically implanting a TA10ETA radiotelemetry device (Data Sciences International (DSI), St. Paul, MN) into the abdomen of mice anesthetized with isoflurane and transmitting the information to APR-1 receivers under the cages that were coupled to the Ponemah v.5.0 Physiology Platform for data analysis (DSI).

2.4. RNA isolation

Cardiac tissues were homogenized using a TissueLyser LT (Qiagen N.V. #69980, Venlo, The Netherlands) according to the manufacturer's protocols. Approximately 20–40 mg of apical ventricle was homogenized in 1 mL of Trizol (Life Technologies #15596-026, Carlsbad, CA) using a 5-mm stainless steel bead (Qiagen N.V. #69989). Chloroform (200 μL) was added, centrifuged at 12,000*g* (15 min at 4 °C), isopropanol (0.5 mL) was then added to the aqueous phase, centrifuged at 12,000*g* (10 min at 4 °C) and the resulting RNA pellet was washed with 1 mL of 75% ethanol, centrifuged at 7500*g* (5 min at 4 °C). The resulting pellet was dried and resuspended in RNase-free water. RNA concentrations were then determined by UV spectroscopy (absorbance of 260–280 nm).

2.5. Transcriptome profiling and RT-qPCR

RNAs were reverse-transcribed using iScript reverse transcription supermix (Bio-Rad Laboratories #170-8841, Hercules, CA). Transcriptome profiling was performed at the UNC Lineberger Comprehensive Cancer Center (LCCC) Genomic Core Facility using the Agilent Once Color 80k60k Sure Print G3 Mouse Gene Expression Array (G4858A-028005). TaqMan gene expression assays (Life Technologies) were performed using universal TaqMan master mix (Life Technologies #4304437). The method for RNA isolation and Real-time RT-PCR was