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MITF interacts with the SWI/SNF subunit, BRG1, to promote GATA4 expression in cardiac hypertrophy

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ABSTRACT

The transcriptional regulation of pathological cardiac hypertrophy involves the interplay of transcription factors and chromatin remodeling enzymes. The Microphthalmia-Associated Transcription Factor (MITF) is highly expressed in cardiomyocytes and is required for cardiac hypertrophy. However, the transcriptional mechanisms by which MITF promotes cardiac hypertrophy have not been elucidated. In this study, we tested the hypothesis that MITF promotes cardiac hypertrophy by activating transcription of pro-hypertrophy genes through interactions with the SWI/SNF chromatin remodeling complex. In an *in vivo* model of cardiac hypertrophy, expression of MITF and the BRG1 subunit of the SWI/SNF complex increased coordinately in response to pressure overload. Expression of MITF and BRG1 also increased *in vitro* when cardiomyocytes were stimulated with angiotensin II or a β-adrenergic agonist. Both MITF and BRG1 were required to increase cardiomyocyte size and activate expression of hypertrophy markers in response to β-adrenergic stimulation. We detected physical interactions between MITF and BRG1 in cardiomyocytes and found that they cooperate to regulate expression of a pro-hypertrophic transcription factor, GATA4. Our data show that MITF binds to the E box element in the GATA4 promoter and facilitates recruitment of BRG1. This is associated with enhanced expression of the GATA4 gene as evidenced by increased Histone3 lysine4 tri-methylation (H3K4me3) on the GATA4 promoter. Thus, in hypertrophic cardiomyoctes, MITF is a key transcriptional activator of a pro-hypertrophic gene, GATA4, and this regulation is dependent upon the BRG1 component of the SWI/SNF complex.

1. Introduction

Heart failure due to pathological cardiac hypertrophy is a leading cause of mortality worldwide [1,2]. Pathological cardiac hypertrophy is an intricate process that involves transcriptional changes in the cardiomyocyte. It can be triggered in response to pressure overload or stimulation with neurohormonal factors such as endothelin-1 (ET-1), angiotensin II, α_1 and β-adrenergic agonists which activate signaling cascades that then promote a shift from the expression of adult proteins to expression of fetal genes. The fetal gene expression program is executed by the transcription factors GATA4, Nkx2.5, TBX5, and MEF2, whose roles in embryonic cardiac development are recapitulated in the hypertrophic cardiomyocyte [3,4]. Recent studies show that the full spectrum of changes in the gene expression profile requires the activities of many additional transcription factors as well as chromatin remodeling enzymes [5–7]. Among these newly identified regulators of hypertrophy is the Microphthalmia-Associated Transcription Factor (MITF), which is required for the hypertrophic response to β-adrenergic signaling [8].

MITF is a basic helix-loop-helix leucine zipper transcription factor that regulates gene expression by binding to E box elements in the promoter region of its target genes [9]. The MITF gene is transcribed from alternative promoters that give rise to splice isoforms which differ at the amino terminus and have cell and tissue specific distribution [9]. MITF-M is the most well studied isoform, expressed abundantly in neural crest derived melanocytes and melanoma [10]. Other MITF isoforms are critical for the development and function of diverse cell lineages including mast cells and osteoclasts. Interestingly, the different MITF isoforms regulate unique and overlapping gene sets, demonstrating some degree of cell specificity as well as biological redundancy [11,12]. Mice with mutations at the MITF locus have pigmentation defects, deafness, osteopetrosis, decreased heart to body weight ratio, and a dampened hypertrophic response to β-adrenergic signaling [8,9].

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The heart specific isoform, MITF-H, is highly expressed in cardiomyocytes and is essential for the hypertrophic response [8]. At present, only three genes that are directly regulated by MITF-H in cardiomyocytes have been identified [13–15]. Furthermore, little is known about how MITF-H activity is coordinated with the activities of other transcriptional regulators or with chromatin remodeling enzymes that regulate cardiomyocyte specific gene expression.

SWI/SNF enzymes are ATP dependent multi-subunit chromatin remodeling complexes that are required for heart development during mouse embryogenesis [16–18]. Heterogeneous complexes composed of a catalytic subunit, BRG1 or BRM, and 9-12 BRG1/BRM associated factors (BAFs) have distinct functions in mammalian cells [19–21]. BRG1 [16,17], Baf60C [22,23], Baf250A [24,25], Baf180 [26], Baf45C/DPF3 [27,28], and BAF200 [29] are all essential for cardiomyocyte differentiation and/or heart morphogenesis.

Components of the SWI/SNF complex have also been implicated in the regulation of cardiac hypertrophy [16,30–32]. In mice, BRG1 is predominantly expressed during embryogenesis, then down-regulated in adult hearts, and re-activated in adult hearts that are subjected to stress. Conditional BRG1 deletion in adult mice prevents cardiac hypertrophy from developing after trans-aortic constriction (TAC) [16]. Furthermore, BRG1 and other SWI/SNF subunits are highly enriched on fetal cardiac gene promoters in hypertrophic hearts [30,31].

Prior studies demonstrated independent roles for MITF and BRG1 in the regulation of cardiac hypertrophy [8,13,16]. However, the transcriptional mechanisms utilized by MITF to promote cardiac hypertrophy remain largely undefined. We previously showed that interactions between MITF and BRG1 promote MITF target gene expression in melanoma cells and drive aspects of melanoma tumorigenicity [21,33]. These observations prompted us to test the hypothesis that MITF interacts with BRG1 in cardiomyocytes to activate pro-hypertrophy genes in response to hypertrophic stimulation.

We discovered that MITF and BRG1 cooperate to induce cardiomyocyte hypertrophy. In an *in vivo* model of cardiac hypertrophy, expression of MITF increased coordinately with that of BRG1 in response to pressure overload. MITF and BRG1 levels also increased in primary cardiomyocytes that were treated with angiotensin II or with isoproterenol and in H9c2 immortalized cardiomyocytes that were stimulated with isoproterenol. Depletion of MITF or BRG1 by transfection with short interfering RNAs (siRNAs) attenuated the hypertrophic response as evidenced by decreased cell size and decreased expression of two cardiac hypertrophy genes, brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP). GATA4 is a transcriptional activator of both BNP and ANP. Importantly, the upstream region of the GATA4 gene has a conserved E box that is important for its expression and is potentially regulated by MITF [34]. We determined that depletion of MITF decreased GATA4 mRNA levels and that MITF can bind to the E box in the GATA4 promoter and trans-activate a GATA4 reporter containing an intact E box. Furthermore, MITF was detected on the endogenous GATA4 promoter, required to recruit BRG1, and to promote H3K4 trimethylation. Thus, in stimulated cardiomyocytes, a key transcriptional activator of pro-hypertrophic gene expression, GATA4, is directly activated by MITF and this regulation is dependent upon the BRG1 component of the SWI/SNF complex.

2. Results

2.1. MITF and BRG1 levels increase after transverse aortic constriction (TAC) induced cardiac hypertrophy and in cardiomyocytes treated with angiotensin II

BRG1 expression is silenced in adult myocardium and re-activated in hearts subjected to pressure overload [16], but it is not known if MITF expression also increases under these conditions. To determine if MITF is activated coordinately with BRG1 when cardiac hypertrophy is induced by pressure overload, we subjected mice to trans-aortic constriction (TAC) and examined the expression of MITF and BRG1 in hypertrophic hearts. The development of cardiac hypertrophy was confirmed by an increase in heart weight (HW) to body weight (BW) ratio (Fig. 1A) and increased expression of two markers of hypertrophy, brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) (Fig. 1B). Under these conditions, there was a significant increase in the expression of MITF and BRG1 at both the mRNA (Fig. 1C) and protein (Fig. 1D) levels in TAC mice compared to sham operated animals, two weeks after surgery.

Pressure overload is associated with increased levels of angiotensin II [35]. To determine whether MITF and BRG1 expressions increase coordinately in response to angiotensin II stimulation, we isolated adult mouse cardiomyocytes and cultured them in the presence and absence of angiotensin II. We detected an increase in MITF and BRG1 protein levels in angiotensin II treated cardiomyocytes compared to controls (Fig. 1E). We also detected increased MITF and BRG1 protein levels when adult mouse cardiomyocytes were stimulated with isoproterenol, a β-adrenergic receptor agonist (Fig. 1F).

2.2. MITF and BRG1 levels increase in H9c2 cardiomyocytes after β-adrenergic stimulated hypertrophy

H9c2 cells are immortalized rat cardiomyocytes that respond similarly as primary rat cardiomyocytes to hypertrophy signals [36]. In order to determine if BRG1 levels increase coordinately with MITF, we treated H9c2 cells with 10 μM isoproterenol to activate β-adrenergic receptors. The activation of hypertrophy was confirmed by significant increases in the mRNA levels of BNP and ANP four hours post β-adrenergic stimulation (Fig. 2A) and further increases after 24 h (Fig. 2B). There was a coordinate increase in MITF and BRG1 mRNA levels at both of these time points (Figs. 2A, B) and also at the protein level (Fig. 2C). Thus, MITF and BRG1 are coordinately activated by multiple hypertrophic signals. Expression of these two proteins increases *in vitro* by β-adrenergic stimulation of immortalized cardiomyocytes (Figs. 2A, B, C). MITF and BRG1 are also coordinately activated in primary cardiomyocytes from adult mice by β-adrenergic stimulation (Fig. 1F) and by treatment with angiotensin II (Fig. 1E) as well as *in vivo* by pressure overload (Fig. 1A, B, C, D).

2.3. Depletion of MITF and BRG1 attenuates the β-adrenergic induced increase in cell size and expression of cardiac hypertrophy markers but not the expression of the β1-adrenergic receptor

To determine if MITF and BRG1 contribute to the activation of hypertrophy by β-adrenergic signaling, we transfected H9c2 cells with scrambled siRNA (siC) or siRNA that targets MITF or BRG1 and then induced hypertrophy with isoproterenol (Fig. 3A). Depletion of MITF or BRG1 did not affect cell size in untreated H9c2 cells. Consistent with previous findings, there was a significant increase in cell size after treatment with isoproterenol for 96 h in cells that were transfected with a control siRNA [37]. However, isoproterenol treated cells that were depleted of MITF or BRG1 did not increase in size (Fig. 3B).

We further tested if MITF and BRG1 are both required for activation of BNP and ANP upon induction of hypertrophy. We observed a significant increase in BNP and ANP mRNA levels in control cells (siC) that were treated with isoproterenol compared to untreated cells. However, BNP and ANP mRNA levels were significantly reduced in cells depleted of MITF or BRG1 (Fig. 3C).

In order to identify which steps of the hypertrophic response to β-adrenergic stimulation require MITF and BRG1, we investigated the effects of MITF and BRG1 knockdown on the expression of two key regulators of the pathway, the β1-adrenergic receptor 1 (β1-AR) and GATA4, a critical pro-hypertrophic transcription factor that activates ANP and BNP expression. Knockdown of either MITF or BRG1 did not affect β1-AR expression but abrogated the expression of GATA4, a transcriptional regulator of both ANP and BNP (3D). Interestingly,