37
MicroRNA-494 reduces ATF3 expression and promotes heart failure in cardiac hypertrophic remodeling in vivo

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Background: Heart failure is a leading cause of death in industrialized nations especially in the aging populations. Novel approaches to treat the heart after acute injury and to improve cardiac hypertrophic remodeling and heart failure processes remain unsatisfactory. Activating transcription factor 3 (ATF3) is a member of the ATF/CREB family of transcription factors that can be induced by stress in a variety of tissues, including cardiac hypertrophy. However, the role of ATF3 in miR-494 regulated ATF3 as therapeutic approaches for cardiac hypertrophic remodeling and heart failure is still unclear.

Methods and Results: Our recent study revealed that ATF3 can protect against pressure overload-induced heart failure. We demonstrated that ATF3 KO mice have rapid progression to cardiac dilation without proper hypertrophic remodeling after trans-aortic banding (TAB). We then fused tert-butylhydroquinone, a selective ATF3 inducer, which inhibited TAB induced cardiac dilatation and increased left ventricular contractility thus rescue heart failure. We used IPA database analysis to identify the miR-494 regulated ATF3 as therapeutic approaches for cardiac hypertrophic remodeling. One specific miRNA, miR-494, initially found in human retinoblastoma tissue, was identified and confirmed to have direct interaction with ATF3 in our H9C2 cells. We then generated miR-494 transgenic mice and demonstrated that miR-494 can be specifically expressed in the heart tissue in our miR-494 transgenic mice. We found that ATF3 expression were markedly decrease in miR-494 transgenic mice receiving TAB treatment as compared to wild type (WT) mice, at 8 weeks and 12 weeks periods after TAB treatment. In addition, echocardiography data showed impaired LV contractility with worsen left ventricular chamber dilatation and wall thinning in miR-494 transgenic mice as compared to WT TAB mice, 12 weeks after TAB treatment. Cardiac hypertrophic markers, including atrial natriuretic factor (ANF), brain natriuretic peptide (BNP), and β-MHC, were decreased in miR-494 transgenic mice 8 weeks after TAB, but not in the WT mice. In addition, down regulation of SIRT1 was observed in miR-494 mice after 8 week, post TAB treatment compared to WT.

Conclusions: These results suggest that miR-494 repressed ATF3 expression in cardiac hypertrophic remodeling via suppressing beneficial hypertrophic markers in vivo. Therefore, suppression of this specific miR-494:ATF3 signaling pathway thus to activate ATF3 expression may ameliorate heart failure in cardiac hypertrophic remodeling and can be a potential therapeutic target.

38
A novel recessive plakophilin-2 gene mutation causes severe arrhythmogenic dilated cardiomyopathy and sudden cardiac death at young age

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Background: Dilated cardiomyopathy (DCM) is the main cause for heart transplantation (HTx). DCM has a heterogeneous etiology with genetic factors becomes increasingly known. Recently, it was shown that 30-50% of DCM cases might be genetic. However, private gene variants in more than 100 different genes lead to variable disease expressivity and penetrance. Mutations in genes coding for desmosomal proteins are frequently associated with arrhythmogenic right ventricular cardiomyopathy (ARVC). However, isolated cases of DCM were also reported. Candidate gene sequencing analysis in families of (in)significant familial DCM carriers. It can be induced by stress in a variety of tissues, including cardiac hypertrophy. However, the role of ATF3 in miR-494 regulated ATF3 as therapeutic approaches for cardiac hypertrophic remodeling and heart failure is still unclear.

Methods and Results: Our recent study revealed that ATF3 can protect against pressure overload-induced heart failure. We demonstrated that ATF3 KO mice have rapid progression to cardiac dilation without proper hypertrophic remodeling after trans-aortic banding (TAB). We then fused tert-butylhydroquinone, a selective ATF3 inducer, which inhibited TAB induced cardiac dilatation and increased left ventricular contractility thus rescue heart failure. We used IPA database analysis to identify the miR-494 regulated ATF3 as therapeutic approaches for cardiac hypertrophic remodeling. One specific miRNA, miR-494, initially found in human retinoblastoma tissue, was identified and confirmed to have direct interaction with ATF3 in our H9C2 cells. We then generated miR-494 transgenic mice and demonstrated that miR-494 can be specifically expressed in the heart tissue in our miR-494 transgenic mice. We found that ATF3 expression were markedly decrease in miR-494 transgenic mice receiving TAB treatment as compared to wild type (WT) mice, at 8 weeks and 12 weeks periods after TAB treatment. In addition, echocardiography data showed impaired LV contractility with worsen left ventricular chamber dilatation and wall thinning in miR-494 transgenic mice as compared to WT TAB mice, 12 weeks after TAB treatment. Cardiac hypertrophic markers, including atrial natriuretic factor (ANF), brain natriuretic peptide (BNP), and β-MHC, were decreased in miR-494 transgenic mice 8 weeks after TAB, but not in the WT mice. In addition, down regulation of SIRT1 was observed in miR-494 mice after 8 week, post TAB treatment compared to WT.

Conclusions: These results suggest that miR-494 repressed ATF3 expression in cardiac hypertrophic remodeling via suppressing beneficial hypertrophic markers in vivo. Therefore, suppression of this specific miR-494:ATF3 signaling pathway thus to activate ATF3 expression may ameliorate heart failure in cardiac hypertrophic remodeling and can be a potential therapeutic target.

39
Investigation of titin expression in explanted hearts with familial dilated cardiomyopathy and TTN truncating variants

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Introduction: Dilated cardiomyopathy (DCM) has estimated 30-40% prevalence for heart failure and is a leading cause for heart transplantation. Approximately 40% of DCM cases are inherited with marked locus and allelic heterogeneity and titin truncating variants (TTNtv) cause 25% of familial DCM (fDCM) cases. However, with over 300 exons and 34,000 amino acids, titin has the largest coding sequence in human genome with up to 3% of general population also carrying mutations in titin gene. Therefore studies of functional consequences of DCM-causing mutations have been limited.

Purpose: The aim of this study was to investigate titin expression in explanted hearts diagnosed with DCM.

Methods: We performed whole exome sequencing of explanted heart muscle samples from 30 DCM patients and screened for potentially disease-causing mutations in 58 HCM and DCM-related genes. We then characterised titin protein expression and levels of phosphorylation in myofibrils and whole tissue homogenates. We further investigated the impact of titin mutations on sarcomere function and regulation of filament proteins by myofibril contractility and in vitro motility (IVMA) assays.

Results: TTNtv were detected in 6 samples (22%) and confirmed by direct sequencing. One mutation was located at the Z-disk and 5 in the A band region of titin. We also found 4 mutations in OBSC, 3 mutations in MYH7, 2 in DSP and one each in TNNC1, TNNI3, MYOM1, VCL, GLA, PLB, PKP2 and LAMA4. We quantified titin protein expression in the whole heart tissue and myofibril fraction and showed no evidence of titin haploinsufficiency or presence of predicted truncated protein. We further looked at the total level of titin phosphorylation and found no differences between donor hearts, DCM with and without titin mutations. We also characterised the contractility of myofibrils with TTNtv by measuring maximum force, rate of force generation and passive stiffness. Finally, we investigated the properties of troponin extracted from hearts with TTNtv by IVMA. Three TTNtv-bearing heart samples showed normal troponin function while two with the most distal mutations showed deregulated relationship of troponin phosphorylation and calcium sensitivity.

Conclusions: Our results suggest that TTNtv do not affect total titin protein expression and phosphorylation in the heart tissue but may impact on the function of the heart muscle through different mechanisms.