The Role Played by Transcription Factor E3 in Modulating Cardiac Hypertrophy

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Summary

Transcription factor E3 (TFE3), which is a key regulator of cellular adaptation, is expressed in most tissues, including the heart, and is reportedly overexpressed during cardiac hypertrophy. In this study, TFE3’s role in cardiac hypertrophy was investigated. To understand TFE3’s physiological importance in cardiac hypertrophy, pressure-overload cardiac hypertrophy was induced through transverse aortic constriction (TAC) in both wild-type (WT) and TFE3 knockout mice (TFE3−/−). Eleven weeks after TAC induction, cardiac hypertrophy was observed in both WT and TFE3−/− mice. However, significant reductions in ejection fraction and fractional shortening were observed in WT mice compared to TFE3−/− mice. To understand the mechanism, we found that myosin heavy chain (Myh7), which increases during hemodynamic overload, was lower in TFE3−/− TAC mice than in WT TAC mice, whereas extracellular signal-regulated protein kinases (ERK) phosphorylation, which confers cardioprotection, was lower in the left ventricles of WT mice than in TFE3−/− mice. We also found high expressions of TFE3, histone, and MYH7 and low expression of pERK in the normal human heart compared to the hypertensive heart. In the H9c2 cell line, we found that ERK inhibition caused TFE3 nuclear localization. In addition, we found that MYH7 was associated with TFE3, and during TFE3 knockdown, MYH7 and histone were downregulated. Therefore, we showed that TFE3 expression was increased in the mouse model of cardiac hypertrophy and tissues from human hypertensive hearts, whereas pERK was decreased reversibly, which suggested that TFE3 is involved in cardiac hypertrophy through TFE3-histone-MYH7-pERK signaling.

Key words: Mice, Transverse aortic constriction, ERK signaling, Cardioprotection

Transcription factor E3 (TFE3) is a basic helix-loop-helix leucine zipper (bHLH-Zip) DNA-binding protein that regulates transcription by either binding to E-box elements in the 5′-flanking regions or acting as a functional enhancer of TFE3-responsive genes.1 TFE3 belongs to the MiT/TFE family of transcription factors, which includes microphthalmia-associated transcription factor (MITF), transcription factor EB (TFEB), and transcription factor EC (TFEC).2 TFE3 and MITF form homodimers or heterodimers and have partially redundant roles in the differentiation of osteoclasts3 and mast cells.4 It serves as a key player in cellular adaptation to stress,5 regulates autophagy, lysosomal biogenesis,6 mitophagy,7 and the Golgi stress response.8,9 We have previously demonstrated that MITF is a key regulator of cardiac hypertrophic response to β-adrenergic stimulation. We have also observed a much smaller heart mass in middle-aged MITF-mutated mice than in wild-type (WT) mice and these mice have significantly decreased cardiac function and cardiac output, demonstrating MITF’s key role in the development of cardiac hypertrophy.10 It is of high importance to identify and elucidate the role of transcription factors like TFE3 that promote pathological changes in the heart by influencing the key signaling pathways. Among the various molecules activated dur-

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EXPERIMENTAL STUDY

Cardiac Hypertrophy

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ing cardiomyocyte stretching due to pressure overload, extracellular signal-regulated protein kinases (ERKs), in particular, are important for protein synthesis. ERK phosphorylation was shown to be increased in the initial stages following pressure-overload induction, but once functional decompensation occurs and during the late detrimental eccentric hypertrophy, increased cardiomyocyte apoptosis with the downregulation of ERK can be seen. However, a previous study showed that upregulating MEK1-ERK1/2 signaling in the heart protects the myocardium from ischemia-reperfusion injury. Although TFE3 knockout mice (TFE3−/−) are functionally and phenotypically normal, no data describe the effect of TFE3 knockout on cardiac function. We hypothesize that TFE3 deletion may confer cardiac protection against pathological cardiac remodeling.

In this study, TFE3’s role in cardiac hypertrophy was investigated, examining both functionality and signaling. We report for the first time that TFE3 along with pERK is involved in human hypertension and in TAC-induced pressure-overload cardiac hypertrophy in the mouse through the TFE3-histone-myosin heavy chain (MYH7)-pERK pathway.

Methods

Human heart tissue sections, heart lysate, and immunohistochemistry ethics statement: Human heart tissues and heart lysate were purchased from Novus Biologicals (USA), and the tissues were collected in compliance with their own Institutional Review Board guidelines. Ethical approval for this study was granted by the National University of Singapore (reference number: NUS-IRB: 12-405). We purchased paraffin-embedded human left ventricular heart sections from normal and hypertensive donors. The donors were females, aged between 72 and 86 years. The clinical diagnosis of normalcy and hypertensive donors was approved by the Institutional Animal Welfare Committee. All experiments were performed in compliance with the Israeli Prevention of Cruelty to Animals Law and previously. Animals: All experiments were performed in compliance with the Israeli Prevention of Cruelty to Animals Law and were approved by the Institutional Animal Welfare Committee (NIH approval number OPRR-A01-5011). All mice lines were held and propagated in a specific pathogen-free environment at the Faculty of Medicine, Hebrew University, under a 12-hour light/dark cycle, and provided with food and water ad libitum. TFE3−/− mice were kindly provided by David Fisher (Massachusetts General Hospital, USA). Mice were genotyped as described previously.

Protein extraction, cell culture, western blot, and immunoprecipitation: Heart tissues were lysed using RIPA lysis buffer. H9c2 cells were cultured in DMEM (Sigma-Aldrich) high-glucose medium, supplemented with 10% fetal bovine serum (Biological Industries, USA). The primary culture of adult mouse heart cardiomyocytes were isolated from the mouse heart’s left ventricle using the direct needle perfusion technique and were separated from non-myocytes, as described previously. Mouse heart lysate and H9c2 cell lysate were immunoprecipitated with TFE3, histones 1, 2A, and 3, and MYH7 antibodies (Santa Cruz Biotechnology, USA) separately using Dynabeads, according to the manufacturer’s instructions (Thermo Scientific, USA). The beads were then rinsed with PBS and subjected to SDS-PAGE. Protein bands were detected by western blot (WB) using TFE3, histone, and MYH7 antibodies. For siRNA knockdown experiments, H9c2 cells were transfected with TFE3 siRNA (siTFE3; Integrated DNA Technologies Pte. Ltd., Singapore) and the NC for 48 hours. Then, the cells were lysed with RIPA (Thermo Scientific) lysis buffer and the protein concentration was determined using a BCA assay kit (Thermo Scientific). Western blot of human heart lysate, mouse lysate, and cell lysate was performed with antibodies against TFE3, histones, pERK, and MYH7, as described previously. For WB, we used 1:1000 dilutions of all the primary antibodies.

Immunofluorescence analyses: H9c2 were cultured on glass coverslips, then fixed with 4% paraformaldehyde (Sigma-Aldrich) in PBS for 20 minutes, followed by Triton X100 (Sigma-Aldrich) permeabilization. Fixed cells were incubated with 0.5% BSA/PBS and 0.5% Triton X 100 for 1 hour. The anti-TFE3 antibody was incubated at 4°C overnight (dilution 1:100). Washing by PBS was followed by incubation with the secondary antibody conjugated to Alexa Flour Cy3 (Jackson Laboratories, USA) for 1 hour. After secondary antibody incubation, coverslips were dipped rapidly in PBS with DAPI (Bio-Rad, USA) for nuclei staining. Samples were mounted using Vectashield mounting medium (Vector Laboratories, USA). Images were taken using a Zeiss LSM710 confocal microscope. The control sample was processed as above, but without adding anti-TFE3 and incubating with only the secondary antibody.

MAPK/ERK inhibition and subfractionation of H9c2 cells: MAPK/ERK phosphorylation was inhibited using its selective inhibitor, U0126 (Sigma-Aldrich). Briefly, H9c2 cells were serum-starved for 2 hours prior to the addition of 10 μM U0126. Subsequently, cells were fractionated into cytoplasmic and nuclear extracts, as described previously. Animals: All experiments were performed in compliance with the Israeli Prevention of Cruelty to Animals Law and were approved by the Institutional Animal Welfare Committee (NIH approval number OPRR-A01-5011). All mice lines were held and propagated in a specific pathogen-free environment at the Faculty of Medicine, Hebrew University, under a 12-hour light/dark cycle, and provided with food and water ad libitum. TFE3−/− mice were kindly provided by David Fisher (Massachusetts General Hospital, USA). Mice were genotyped as described previously.
generate these mice, a homologous recombination method was used to replace a PGK-neo cassette instead of bHLH-Zip domains (7 exons extended in a 5.5-kb genomic fragment localized on the proximal short arm of the X chromosome).30

TAC animal model and echocardiography: TAC was performed in 12-week-old male C57BL/6 mice as described previously.16 Mice were divided into four groups: TFE3 WT sham, TFE3 WT TAC, TFE3−/− sham, and TFE3−/− TAC. In brief, mice were anesthetized with isoflurane (3-4% induction and 1.5-2.5% maintenance), the chest was then opened, and a 6-0 Prolene silk suture was placed around the aorta using the blunt end of a 27-gauge needle. The needle was removed and the chest was closed. Subsequently, 2.5 mg/mL of Carprofen, an analgesic drug (Norocarp, NorBrook), was infused subcutaneously to reduce mice suffering. The same procedure, but without the aortic ligation, was used for sham surgeries. After the surgery, the animals were monitored twice daily. Echocardiography was performed at baseline and 11 weeks after TAC surgery using the Vvo2100 digital imaging system (Visual Sonics, USA). Mice were then sacrificed by CO2 inhalation, followed by cervical dislocation; subsequently, the heart and body weights were measured. Left ventricles were isolated and frozen immediately in liquid nitrogen. The TAC surgery and echocardiography measurements were performed single blind, with the person performing the surgery and measurements unaware of whether the mice were WT or TFE3−/−.

PCR and construction of mouse TFE3 plasmids and isoforms: To amplify mouse TFE3 isoforms, WT mouse RNA was extracted from the heart left ventricle (TRizol® Reagent, Thermo Fisher); then, cDNA was synthesized using SuperScript™ III Reverse Transcriptase (Thermo Fisher). The full-length mouse TFE3 gene was amplified using the following primers: F, 5′-ATATGGGATCCATGTC TCATGCCAGCGACCAGCT-3′ and R, 5′-AGGCTTAG CATGGAAGAGGATCTCTTACA-3′. The full-length and short TFE3 isoforms were identified using mouse cDNA by mtTFE3 Exon4 F, 5′-ACTCTTCTGCCCCCT-GAA ACTG-3′ and mtTFE3 Exon6 R, 5′-CTGGACAGGAATT GCTGACG-3′, which gave two distinct bands at 295 and 185 bp. The mtTFE3 S245A and mtTFE3 S245D plasmids were constructed using the Q5® Site-Directed Mutagenesis Kit (New England Biolabs, USA), according to the manufacturer’s instructions. Plasmids were transfected into H9c2 cells. The cells were fractionated into cytoplasmic and nuclear components, as described previously.15 For real-time PCR, total RNA was extracted from the heart of WT TAC and TFE3−/− TAC mice using the Quick-change miniprep RNA extraction kit (Zymo, USA), and reverse transcribed using the qScript cDNA synthesis kit (QuantaBio, USA). The primers used for gene amplification for real-time PCR were as follows: GAPDH F, 5′-TA TGACTCCACTCGGCCA-3′, GAPDH R, 5′-TGAAA GATGTGGATGGCCCT-3′; Myh7F, 5′-CCTGGAGAATG ACAAGACGC-3′, Myh7R, 5′-AGGGCTTGCTCATCCT CAAT-3′. Quantitative real-time PCR was performed with the Fast SYBR™ green master mix (Applied Biosystem, Thermo Fisher Scientific) using the Step-One Plus Real-Time PCR system (Applied Biosystem, Thermo Fisher

Statistics: All comparisons between the two groups were performed using the exact Mann-Whitney U test or the Wilcoxon signed rank test as appropriate. All multiple comparisons were analyzed by the Kruskal-Wallis and two-tailed tests with the Conover post hoc test. Analyses were performed using SPSS, and graphs were plotted using GraphPad Prism. A null hypothesis was rejected at the P<0.05 level for all tests. All data are reported as the mean ± SEM.

Results

TFE3, pERK, MYH7, and histone expression in the human heart: Immunohistochemical staining of TFE3 and pERK in normal and hypertensive human heart ventricles demonstrated that TFE3 was upregulated and pERK was downregulated in ventricles from hypertensive hearts compared to normal heart tissue. TFE3 was localized in the cytoplasm of the cardiomyocytes (Figure 1A, B). This result is consistent with previously published data in which TFE3 was localized mainly in the cytoplasm under normal conditions.31 pERK was found to be higher in the normal human heart than in the hypertensive heart (Figure 1C, D). Upregulation of TFE3, histone, and MYH7 and downregulation of pERK were demonstrated in ventricle lysates from hypertensive hearts compared to normal heart lysates by western blotting (Figure 1E).

TFE3 expression in mouse hearts and rat cardiomyoblast cell line (H9c2): TFE3 was found to be expressed in a primary culture of adult mouse cardiomyocytes (Figure 2A), in which two distinct isoforms were observed in both cardiomyocytes and heart lysates (Figure 2A, B). As described previously, mouse TFE3 has two known spliced isoforms, a low-molecular-weight TFE3S, in which exon 5 is skipped, and the high-molecular-weight TFE3L.18 To identify the spliced variants of TFE3, PCR was carried out from exons 4 to 6. The mRNA of TFE3S and TFE3L isoforms were detected in both the mouse heart (Figure 2C) and the H9c2 rat cell line (Figure 2D). Following the fractionation of H9c2 cells into cytoplasmic and nuclear components, TFE3 was detected predominantly in the cytoplasm of H9c2 cells (Figure 2E), Localization of TFE3 in cytoplasm of H9c2 cells was further confirmed using confocal imaging (Figure 2F).

Characterization of TFE3 cardiac function in untreated WT and TFE3−/− mice: Western blot analyses were performed in TFE3−/− mice and their normal littermates (Figure 3A), confirming that TFE3−/− mice do not express TFE3. Morphometric analyses (Figure 3B) of the heart weight to body weight (HW/BW) ratio in 10-week-old mice did not reveal statistically significant differences between normal littermates and TFE3−/− mice (5.04 ± 0.2 versus 4.9 ± 0.1 mg/g, respectively, n=7-8, P=0.681). Echocardiography was performed on 10-week-old mice (Figure 3C-E). No significant differences were noted in the heart rate (529 ± 15 versus 531 ± 31 bpm, P=0.865), fractional shortening (41.2 ± 1.7% versus 40.6 ± 2.4%, P=0.952), and left ventricular end diastolic diameter (3.7 ± 0.2 versus 3.7 ± 0.1 mm, P=0.952).

Pressure-overload cardiac hypertrophy of TFE3−/− mice
and normal littermates: As no major pathology was observed in TFE3−/− mice at baseline, we employed the transverse aortic constriction (TAC) model of pressure-overload cardiac hypertrophy to study the effect of TFE3 expression. Cardiac hypertrophy was observed after 11 weeks of TAC (Figure 4A-D) in both TFE3−/− mice and the normal littermates. The HW/BW ratio was significantly higher in TAC than in sham WT littermates by approximately 31% (5.6 ± 0.1 versus 7.4 ± 0.6 respectively, n = 5-7, P = 0.0073). The HW/BW ratio was also significantly higher by approximately 23% in TFE3−/− TAC mice than in sham mice (6.4 ± 0.4 versus 5.2 ± 0.2 respectively, n = 5, P = 0.0476) (Figure 4A). The cardiomyocyte diameter was measured by microscopic analyses of fixed LV sections (Figure 4B). It was observed that the left ventricular mass to body weight (LV mass/BW) ratio was 40% higher in WT TAC mice than in sham mice (n = 5-7, P = 0.048) (Figure 4C) and 33.1% higher in TFE3−/− TAC mice than in sham mice (n = 5, P = 0.0317). TAC induced a 12% increase in myocyte diameter in WT mice (n = 5-7, P = 0.0256) and an increase of 16% in TFE3−/− mice (n = 5, P = 0.047). Interestingly, the cardiomyocyte diameter in WT sham mice was 8% higher than that in TFE3−/− sham mice (n = 5-7, P = 0.0058); this difference was not observed after TAC. TAC resulted in greater thickening of the anterior wall (Figure 4D) in both WT (0.9 ± 0.04 versus 1.2 ± 0.09 mm, n = 5-7, P = 0.037) and TFE3−/− mice (0.8 ± 0.08 versus 1.1 ± 0.07 mm, n = 5, P = 0.031) than in the corresponding sham mice. The effect of TAC on TFE3 expression in mice hearts was evaluated (Figure 4E). TFE3 expression was 76% higher in WT mice following TAC than in sham mice (n = 5-7, P = 0.0232).

Analysis of heart function using echocardiography showed that WT mice developed left ventricular dysfunction at 11 weeks after TAC (Figure 5A-E). The left ventricle ejection fraction (EF%) was significantly lower in WT mice (42.4 ± 1.8% versus 31.1 ± 2.8%, n = 5-7, P = 0.007), but it was unchanged in their TFE3−/− littermates (45.3 ± 2.9% versus 45.5 ± 2.5%, n = 5, P = 0.156). In addition, the EF% was significantly lower in WT mice subjected to TAC than in TFE3−/− mice (31.1 ± 2.8% versus 45.5 ± 2.5%, n = 5, P = 0.012) (Figure 5B). Interestingly, left ventricle fractional shortening (FS%) was simi-
lar in TAC and sham TFE3−/− mice, whereas it was significantly lower in WT mice 11 weeks after TAC than in sham surgery mice (26.7 ±2.5 versus 19.5 ± 1.99, \(P = 0.037\)) (Figure 5C). After TAC, WT mice had a significantly higher (12.4%) left ventricular end diastolic volume (EDV; 110.1 ± 2.2 μL) than sham surgery mice (97.9 ± 4.6 μL; \(n = 5-7, P = 0.0431\)), whereas no significant difference was observed in TFE3−/− mice (Figure 5D). End systolic volume (ESV) was 29.1% higher in WT mice 11 weeks post TAC than in the WT sham group (51.1 ± 4.5 versus 65.98 ± 2.7, \(P = 0.0206\)), whereas there was no significant increase in TFE3−/− mice (Figure 5E). The difference in EDV post TAC between WT mice and their knockout siblings was statistically significant (110.1 ± 2.2 versus 88.2 ± 7.4 μL, \(P = 0.0057\)). Similarly, the difference in ESV post TAC between the same two groups was significant (65.98 ± 2.7 versus 43.4 ± 6.5, \(P = 0.0047\)). Notably significant differences in EF\% and FS\% were observed after TAC in the TFE3−/− groups, which are important findings, as these two factors are widely used to assess left ventricular dysfunction. Collectively, these results indicated that the absence of TFE3 can confer cardiac protection against negative cardiac remodeling.

**Cardiac hypertrophy biomarker in TFE3−/− mice in the pressure-overload TAC model:** The impact of TFE3 knockout on the expression of cardiac hypertrophy markers and modulators during pressure-overload cardiac hypertrophy was evaluated. The expression of Myh7 was measured, as well as the expression and phosphorylation of ERK, which is the main pathway studied in cardiac hypertrophy.\(^{19,20}\) Myh7 mRNA levels were significantly lower in TFE3−/− TAC mice than in WT TAC mice (\(P = 0.0396\), Figure 6A). ERK phosphorylation (Figure 6B, C), as measured by the ratio of phosphorylated ERK1 to total ERK1 (pERK1/ERK1), was significantly lower (approximately 32%) in TAC WT mice than in sham WT mice (\(n = 5-7, P = 0.0496\)). In contrast, phosphorylation of ERK was similar in the TFE3−/− TAC and the TFE3−/− sham mice. The relative ERK phosphorylation level was also observed to be higher in TFE3−/− mice than in their WT littermates 11 weeks after TAC (1.2 ± 0.2 versus 0.4 ± 0.02; \(n = 5-7, P = 0.001\)).

**ERK is responsible for TFE3 phosphorylation and cytoplasmic retention:** It has previously been reported that TFE3 was found to be phosphorylated by MAPK/ERK.\(^{21}\) In mouse cardiac TFE3, serine 245 was found to be phosphorylated by MAPK/ERK. To find out whether TFE3 is phosphorylated by ERK in H9c2 cells, a selective MAPK kinase inhibitor (U0126) was used. Phosphorylation by ERK and the effect of dephosphorylation on TFE3 subcellular translocation was investigated in the cytoplasm and the nucleus. TFE3 was translocated into the nucleus 15 minutes after ERK inhibition (Figure 7A). To confirm the effect of ERK activity on TFE3 nuclear translocation, a substitution mutation of serine 245 with alanine (S245A; mimics phosphorylation) was performed in TFE3 plasmids. The mutated TFE3 plasmids were transfected into H9c2 cells (Figure 7B). As can be seen, cells transfected with the alanine-substituted TFE3 (mTFE3 S245A) had less cytoplasmic S245A TFE3 than cells transfected with WT TFE3 and/or aspartic acid-substituted TFE3 (mTFE3
Figure 3. Phenotype comparison between WT and TFE3<sup>−/−</sup> mice at baseline. A: Western blot analyses of TFE3 expression in the hearts of normal littermates (WT) and TFE3<sup>−/−</sup> mice. Three representative mice are presented for each group. B: The HW/BW ratio in TFE3<sup>−/−</sup> mice and normal littermates (n = 7–8). C–E: Echocardiography was performed on 10-week-old mice; bpm: beat per minute. LVEDD indicates left ventricular end diastolic diameter.

Expression patterns of TFE3 in mouse heart and H9c2 cells, and TFE3 interaction with MYH7 and histones:
The interactions of TFE3 with histone and MYH7 were analyzed using immunoprecipitation. Mouse heart lysate and H9c2 cell lysates were immunoprecipitated with TFE3 and histone antibodies separately and blotted with TFE3 and MYH7 antibodies. Results showed that TFE3 co-precipitated with histones 2A and 3, indicating interactions between these proteins (Figure 8A, B). Likewise, the interaction between TFE3 and MYH7 was confirmed through immunoprecipitation, indicating the association of TFE3-histone-MYH7 as a complex in H9c2 cells and the mouse heart. Further characterization of this association...
Discussion

We have postulated a cardiac role of TFE3, as TFE3 signaling is involved in cardiac pathophysiology, and MITF, its close family member, is known as a regulator of cardiac hypertrophy. This study is the first to elucidate how TFE3 is involved in heart function.

High expressions of TFE3, histone, and MYH7 and low expression of pERK were found in human hypertensive hearts compared to normal hearts, which suggested a pathophysiological role of TFE3 in the human heart. We examined the expression of TFE3 in healthy human cardiac tissue and in cardiomyopathic human (HCM) tissue and found upregulation of TFE3 in HCM heart tissue (unpublished data). Our findings are in agreement with a previous study by Sato, et al. reporting that TFE3 and Gα16 are upregulated under pathological conditions.

TFE3−/− mice have a normal heart under basal conditions, suggesting that TFE3 is not required for the baseline regulation of cardiac growth and function. A similar finding was observed previously, whereby TFE3 full KO mice appeared phenotypically healthy. However, we noticed that both WT mice and TFE3−/− mice exhibited an augmented hypertrophic response in response to chronic...
Figure 5. Ejection fraction and fractional shortening in WT and TFE3−/− mice. Ejection fraction and fractional shortening are preserved in TFE3−/− mice but not in normal littermates after chronic pressure overload. A: Representative long axis views of a TFE3−/− mouse and a wild-type littermate (WT) 11 weeks after sham (left panels) or TAC surgery (right panels). B, C: Ejection fraction (B) and fractional shortening (C) of TFE3−/− (KO) mice and normal littermates (WT) 11 weeks after either sham or TAC procedure. D, E: Left ventricle end diastolic (D) and end systolic volume (E) of WT and KO mice are also shown (n = 5–7). *P < 0.05. **P < 0.01.

pressure overload. Although increases in HW/BW and LV mass/BW ratios were observed in all TAC mice, only the TFE3−/− mice preserved their cardiac function. We also found a significant decrease in left ventricular EF% and FS%, and an increase in the EDV and ESV in WT mice but not in the TFE3−/− littermates.

We found a lower expression of histone in H9c2 cells after silencing with TFE3 siRNA and found a direct association between TFE3, MYH7, and histone by immunoprecipitation, suggesting that the reduced expression of histone after TFE3 silencing may involve the histone acetyltransferase pathway. The dysregulation of posttranscriptional modifications of histones in chromatin is thought to be associated with the pathology of many diseases, including cardiovascular disease,25) and activating GATA-4 increases its binding to its downstream hypertrophy target gene β-MYH7 under conditions of induced left ventricle hypertrophy.26) Thus, such an interaction suggests a possible link between TFE3, histone modification, and pathological cardiac hypertrophy. In addition, MYH7 was found to be a direct interacting partner of TFE3 and was found to be downregulated during TAC in TFE3−/− mice and in
TFE3 gene silencing. We also found that silencing of TFE3 reduced the expression of histones 1, 2A, and 3. Taken together, these results suggest a functional correlation among TFE3 expression, histone, and MYH7 in cardiomyocytes. As we found a lower expression of Myh7 in TFE3−/− TAC mice than in WT TAC mice, and Izumo, et al.27) reported the high expression of Myh mRNA during cardiac hypertrophy, we propose a cardioprotective role of

Figure 6. Evaluation of cardiac hypertrophy biomarkers in WT versus TFE3−/− mice after TAC. A: Real-time PCR quantification of Myh7 mRNA level in hearts of TFE3−/− and WT mice after TAC surgery. Results represent the mean ± SEM (n = 5–7). B: Western blot analysis of ERK1, phosphorylated ERK1 (pERK1), and protein levels in hearts from WT and TFE3−/− mice 11 weeks after either sham or TAC surgery. GAPDH was used as the loading control. C: Densitometry results of ERK1 phosphorylation are depicted as the pERK1/ERK1 ratio (n = 5–7), *P < 0.05. **P < 0.01.

Figure 7. Inhibition of MAPK/ERK in H9c2 cells. A: TFE3 nuclear accumulation 15 minutes after ERK inhibition by MAPK/ERK inhibitor U0126. B: MAPK/ERK phosphorylates TFE3 at ser245 in plasmid-transfected H9c2 cells.
TFE3 through the histone-MYH7 pathway, based on these observations.

TFE3 upregulation changes the myocyte’s membrane, affecting the total cardiac structure and function, which might lead to a pathological condition in WT mice. However, this mechanism is totally absent in TFE3−/− mice, which could attenuate the negative remodeling of pathological cardiac hypertrophy, and may activate the pERK signaling pathway. ERK was relatively dephosphorylated in WT littermates 11 weeks after TAC, whereas it remained phosphorylated in TFE3−/− mice. This result is consistent with a previous result that showed that phosphorylation of ERK was increased during the early phase of cardiac hypertrophy and then decreased significantly 12 weeks after TAC.11)

Although the role of ERK in cardiac hypertrophy is not completely understood, data from several mouse models suggest that ERK signaling plays a protective, anti-apoptotic role in the heart. For example, inhibition of ERK1/2 with DUSP6 overexpression in a model of long-term pressure overload predisposed the myocardium to decompensation.20) MEK1 transgenic mice were profoundly protected from ischemia-reperfusion injury to the heart, showing reduced myocardial damage.20) Furthermore, loss-of-function experiments in ERK2 heterozygote gene-targeted mice showed enhanced myocardial injury.21)

In the rat cardiomyoblast cell line H9c2, we found that ERK phosphorylates TFE3 at serine 245. ERK inhibition causes the translocation of TFE3 from the cytoplasm to the nucleus after 15 minutes of inhibition. Dephosphorylated-mimicking S245A mTFE3 had reduced cytoplasmic TFE3 with increased nuclear retention. This result indicates that TFE3 is phosphorylated by ERK and the translocated TFE3 may participate in the modulation of genes involved in hypertrophy. Thus, we can conclude that TFE3 under regular conditions is phosphorylated at S245, ensuring its cytoplasmic retention. As a result of prolonged stress conditions, ERK phosphorylation was reduced, which subsequently caused TFE3 dephosphorylation at S245 and then nuclear translocation, along with the induction of cardiac hypertrophy genes. This effect was absent in TFE3−/− mice after TAC surgery. Considering all our findings, we propose that the absence of TFE3 may confer myocardial protection against pressure-overload-induced maladaptive cardiac hypertrophy because of changes in ERK signaling along with the histone pathway.

As TFE3 is a transcription factor and histone is expressed in the nucleus, we think that TFE3 in association with other proteins like MYH7 and histone might be involved in human stress conditions. Future research should focus on the involvement of TFE3 and its associated proteins in hypertrophy. Finally, our current work suggests a previously unknown role of TFE3 in cardiac hypertrophy and demonstrates that its absence during pressure-overload cardiac hypertrophy improves cardiac remodeling and could protect from decompensation and heart failure.

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Disclosure

Conflicts of interest: The authors confirm that there are no competing interests.
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