Increased FOG-2 in Failing Myocardium Disrupts Thyroid Hormone–Dependent SERCA2 Gene Transcription


Abstract—Reduced expression of sarcoplasmic reticulum calcium ATPase (SERCA)2 and other genes in the adult cardiac gene program has raised consideration of an impaired responsiveness to thyroid hormone (T3) that develops in the advanced failing heart. Here, we show that human and murine cardiomyopathy hearts have increased expression of friend of GATA (FOG)-2, a cardiac nuclear hormone receptor corepressor protein. Cardiac-specific overexpression of FOG-2 in transgenic mice led to depressed cardiac function, activation of the fetal gene program, congestive heart failure, and early death. SERCA2 transcript and protein levels were reduced in FOG-2 transgenic hearts, and FOG-2 overexpression impaired T3-mediated SERCA2 expression in cultured cardiomyocytes. FOG-2 physically interacts with thyroid hormone receptor-α1 and abrogated even high levels of T3-mediated SERCA2 promoter activity. These results demonstrate that SERCA2 is an important target of FOG-2 and that increased FOG-2 expression may contribute to a decline in cardiac function in end-stage heart failure by impaired T3 signaling. (Circ Res. 2008;103:493-501.)

Key Words: friend of GATA-2 • thyroid hormone receptor • sarcomplasmic reticulum calcium-activated ATPase-2 • heart failure

Congestive heart failure (CHF) is a lethal condition and represents the final common end point of many forms of heart disease. A hallmark feature of failing hearts is a downregulation of sarcoplasmic reticulum calcium ATPase (SERCA2) and α-myosin heavy chain (αMHC), which are critical components of the myocyte excitation–contraction coupling machinery.1,2 Although the regulatory mechanisms that affect these changes are poorly understood, a similar pattern of gene expression seen in the hypothyroid heart suggests that impaired thyroid hormone (T3) responsiveness may contribute to this transcriptional switch.3 Indeed, T3 is required for the physiological increase of SERCA2 and αMHC after birth.4 Low SERCA2 and αMHC expression levels in the hypothyroid or failing heart are recovered following T3 replacement.5,6 In heart failure models, decreased circulating T3 levels,7 accelerated myocardial T3 turnover,8 and altered thyroid hormone receptor (TR) isoform expression9 have been implicated mechanisms of cardiac-restricted T3 resistance.

T3 regulates transcription through interaction with the ligand-binding domain of high-affinity nuclear TR proteins that recognize T3 response elements (TREs) on target genes.10 Three major TR isoforms, TRα1, TRα2, and TRβ1, are expressed in the heart. TRα1 and TRα2 are abundantly expressed in the ventricular compact zone, which contributes the greatest force output from the heart, whereas TRβ1 expression is limited to the peripheral ventricular conduction system.11 TRα1, but not TRβ1, is required for normal SERCA2 and αMHC expression in vivo, and mouse TR gene deletion models support a predominant role for TRα1 in the regulation of cardiac contractile and electrophysiological functions.12 Without T3, TR proteins bind TREs and decrease basal gene expression through the recruitment of corepressor complexes. On T3 binding to TR, these complexes are displaced and coactivator proteins are recruited that modulate chromatin structure and enhance gene expression.13 Because an imbalance favoring TR corepressor binding impairs T3 responsiveness,14–16 we considered whether a cardiac-enriched corepressor protein may mediate local cardiac T3 resistance in CHF.

FOG-2 (friend of GATA-2) is a multi–zinc finger nuclear corepressor protein necessary for normal development of the heart and, in particular, the left ventricular (LV) compact zone.17,18 FOG-2 was originally identified as a GATA4-interacting protein19,20 but also interacts with nuclear hormone receptors, chicken ovalbumin upstream protein transcription factor (COUP-TF)21 and retinoid-X receptor-α (RXRα).22 In models of load-induced heart failure, GATA4, COUP-TF2, and RXRα regulate components of the cardiac gene program that affect natriuretic peptide expression.21,22

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cardiac metabolism,23 and myocyte architecture.24 Although FOG-2 represses the transcriptionsal activities of GATA4,19 COUP-TF2,21 and ligand-bound RXRs22 in cultured cardiomyocytes, the role of FOG-2 in the adult heart remains undefined. Here, we report that FOG-2 levels are increased in human end-stage heart failure and in a mouse model of dilated cardiomyopathy. Transgenic (Tg) mice with targeted overexpression of FOG-2 in the heart have reduced systolic and diastolic function. Diminished expression of SERCA2 is a primary finding associated with the cardiomyopathy created by FOG-2 overexpression. We demonstrate that FOG-2 binds TRα1 and impairs T3-mediated transcription of the SERCA2 promoter. Taken together, we identify a novel mechanism of cardiac resistance to T3 signaling caused by increased FOG-2 expression that may contribute directly to heart failure progression.

Materials and Methods

Mice

αMHC-CREB-S133A Tg mice25 and Fog-2−/− (heterozygous null) mice26 were described previously. αMHC-FOG-2 Tg mice were produced following injection of a transgene including the full-length mouse FOG-2 cDNA (base pairs 132 to 4770) downstream of the murine αMHC promoter26 into newly fertilized ICR (Taconic) mouse embryos. Founders identified by Southern blot analysis with FOG-2 probe (nucleotides 132 to 1207) were bred with ICR mice. Histological analysis was performed on hearts fixed in 4% paraformaldehyde and processed into paraffin blocks. Cardiac functional analysis was performed by echocardiography and cardiac catheterization (expanded Materials and Methods section). HEK-293 cells were transfected with either pcDNA3-FOG-219 with either pCR3-TRα1 or pCR3-myc-TRα1 using Polyfect (Qiagen). Cellular extract was diluted in an immunoprecipitation buffer and precipitated with FOG-2 antiserum,26 myc epitope antibody (Tufts University Core Facility), or no antibody (online data supplement, expanded Materials and Methods section) and immunoblotted with FOG-2 antiserum.26 Glutathione S-transferase (GST) pull-down assays were performed with fusion proteins containing either zinc fingers 5 and 6 (amino acids 497 to 831) or zinc fingers 7 and 8 (amino acids 752 to 1151)21 and recombinant rat TRα1 and TRα2 proteins labeled with [35S]-methionine (Amersham) using the TrT T7 Coupled Reticulocyte System (Promega) (online data supplement, expanded Materials and Methods section).

Transgenic Mice

FOG-2 cDNA template (nucleotides 132 to 1207) and 18S probes

Quantitative real-time RT-PCR (qRT-PCR) was performed using the AdEasy Adenoviral Vector System (Stratagene).27 A FOG-2 and enhanced green fluorescent protein (EGFP) (Ad-FOG-2) subcloned to generate a recombinant adenovirus expressing both FOG-2 and EGFP. Embryonic chick ventricular myocytes were prepared as described previously28 and grown to 70% to 80% confluence. Medium was replaced with DMEM/2% charcoal-stripped horse serum supplemented with vehicle or T3. After 24 hours the cells were lysed and luciferase (Promega) activity were measured using a luminometer (Thermo Labsystems). Fold activation is expressed as a ratio of luciferase activity to β-galactosidase activity and normalized across experiments to either the empty vector or FOG-2 reference condition.

Statistical Analysis

Microarray expression differences were determined by significance analysis of microarrays.31 Statistically significant differences between Tg and nontransgenic (NTg) groups were determined by an unpaired t test or log-rank survival analysis when appropriate. All other statistical analyses were performed by 2-way ANOVA, followed by Bonferroni post test using GraphPad Prism version 4.0 software. Data are expressed as means ± SEM. A minimum value of P<0.05 was accepted as statistically significant.

Results

FOG-2 Protein Is Increased in Failing Hearts

To explore FOG-2 expression in the setting of heart failure, we analyzed microarray data (Gene Expression Omnibus GDS1362) from 37 human cardiac tissue samples taken from nonfailing (NF) (n=6), nonischemic cardiomyopathy (n=21), and ischemic dilated cardiomyopathy (n=10).32 Significance analysis of microarrays identified FOG-2 as a differentially expressed gene among cardiomyopathy samples with a local false discovery rate of 0.06%. FOG-2 expression was increased 1.8-fold in nonischemic cardiomyopathy and...
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Figure 1. Increased FOG-2 protein in end-stage heart failure. a, Means±SEM log-transformed FOG-2 probe intensities in non-failing (NF) (n=6), ischemic dilated cardiomyopathy (ICM) (n=10), and nonischemic cardiomyopathy (NICM) (n=21) and human heart samples. One-way ANOVA followed by Holm–Sidak analysis. *P<0.01. b, Immunoblot analysis of ventricular FOG-2 expression in 12-week CREB-S133A Tg mice and NTg littermate controls. GAPDH and calsequestrin (CSQ) are protein loading controls.

FOG-2 Tg Mice Develop Heart Failure

To observe whether increased FOG-2 expression is associated with heart failure independent of concomitant therapy, we studied CREB-S133A mice, a well-characterized model of human dilated cardiomyopathy. These mice develop progressive chamber dilation and contractile dysfunction by 8 weeks of age and begin to die from heart failure by 10 weeks of age.25 Whereas 6-week-old CREB-S133A mice did not have significant differences in FOG-2 expression compared with NTg littermate controls (1.51±0.39-fold difference, \(P=0.19\)), a 2.53±0.45-fold increase in FOG-2 protein (\(P<0.05\)) was observed at 12 weeks of age (Figure 1b). Furthermore, increased myocardial FOG-2 protein expression was also observed in mice subjected to 14 days of transverse aortic constriction, at which time point, LV fractional shortening was significantly depressed (Figure 1 in the online data supplement). Thus, increased FOG-2 expression is associated with decompensated heart function.

Figure 2. Generation and characterization of FOG-2 Tg mice. a and b, Northern (a) and immunoblot (b) analysis comparing organ and ventricular FOG-2 expression in FOG-2-med Tg and NTg littermate control mice. 28S is an RNA loading control. c, Kaplan–Meier survival analysis of FOG-2-med Tg (●) (n=75), FOG-2-high Tg (○) (n=46), and NTg littermate control (▲) (n=44) mice. Differences in survival rates between the Tg and NTg groups were significant by the log rank test (\(P<0.05\)). d, Representative coronal sections of hearts from NTg littermate control and FOG-2-med Tg mice showed no gross hypertrophy of the ventricles. Scale bar, 100 μm. e, Hemodynamic analysis of FOG-2-med Tg and NTg littermate control mice. The means±SEM maximum (top) and minimum (bottom) first derivative of LV pressure tracings (dP/dt) measured at baseline and at indicated time points following stimulation with isoproterenol. Reductions in both maximum and minimum dP/dt in the Tg (●) (n=11) compared with NTg (▲) (n=9) were noted following isoproterenol. Two-way ANOVA with repeated measures followed by Bonferroni analysis. \(*P<0.05\) vs NTg.
**ECHOCARDIOGRAPHIC DATA IN FOG-2 Tg AND NTG MICE**

<table>
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<th>NTg (n=16)</th>
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Data are means ± SEM. Mice were 8–10 weeks of age. LVEDD indicates LV end-diastolic diameter; LVESD, LV end-systolic diameter. Significance of differences between means were determined by unpaired t test. P=0.05 was accepted as significant.

Hearts from FOG-2 mice displayed grossly normal-sized ventricles (Figure 2d) and enlarged atria with mural thrombi (supplemental Figure IIA). Cardiac valve leaflet structure was unremarkable (supplemental Figure IIB). A trend toward greater whole heart mass relative to body weight in Tg versus NTg hearts (4.21±0.08 versus 3.97±0.09 mg/g, P=0.06) was attributable to enlarged atria. The ventricular to body weight (3.5±0.1 versus 3.6±0.1 mg/g, P=NS) and tibia length (5.9±0.2 versus 6.0±0.2 mm/mm, P=NS) ratios were similar, as were cardiomyocyte cross-sectional areas (data not shown). Pico Sirius staining showed increased interstitial fibrosis (supplemental Figure III), consistent with findings in cardiomyopathy25 and hypothyroidism.34

To evaluate the functional cardiac phenotype underlying the susceptibility to heart failure in FOG-2 mice, we assessed contractile performance in 8- to 10-week-old FOG-2-med Tg and NTg mice. FOG-2 mice had mildly dilated LV systolic dimensions by echocardiography with significantly reduced fractional shortening (Table). Invasive pressure measurement showed reduced LV systolic pressure in FOG-2 mice (2750±288 versus 3866±615 mm Hg/sec, P=0.09) and diastolic pressures were similar. At baseline, FOG-2 mice revealed a trend toward depressed systolic function (dp/dt max 2750±288 versus 3866±615 mm Hg/sec, P=0.09) and diastolic relaxation (dp/dt min, −2242±258 versus −3471±578 mm Hg/sec, P=0.05) that became significant following isoproterenol challenge (Figure 2d).

**SERCA2 Is a Downstream Target of FOG-2**

Because FOG-2 mice demonstrated findings consistent with cardiomyopathy, gene expression was evaluated in 4- to 6-week-old FOG-2-high mice. FOG-2 mouse ventricles had increased B-type natriuretic peptide, β-myosin heavy chain (βMHC), and atrial natriuretic factor (supplemental Figure III), almost significantly decreased αMHC expression (P=0.06), and significantly decreased SERCA2 mRNA levels (Figure 3a) and protein levels (Figure 3b), which were similarly observed in FOG-2-med mice (mRNA, P<0.01).

To address potential concerns of overexpression artifact, we also measured SERCA2 protein levels in FOG-2/−/− (heterozygous null) mice. In this setting of reduced FOG-2 gene dosage, SERCA2 protein levels were increased nearly 2-fold (Figure 3c).

To examine the effects of FOG-2 on endogenous SERCA2 expression independently from the potential secondary effects of heart failure, we infected cultured neonatal rat ventricular cardiomyocytes with a recombinant adenovirus expressing FOG-2. Ad-FOG-2 infection resulted in a dose-dependent increase in FOG-2 protein and a significant dose-dependent decrease in SERCA2 protein with maximal suppression of SERCA2 to levels that were half of that quantified in uninfected cells (Figure 4a and 4b). T3 treatment increased SERCA2 expression; however, FOG-2 overexpression repressed this effect at the protein (Figure 4a and 4b) and transcript (Figure 4d) levels. Importantly, FOG-2 did not alter expression of calsequestrin, another calcium-handling protein specific to cardiomyocytes (Figure 4c). These results are consistent with SERCA2 being a downstream target of FOG-2 and show that FOG-2 abrogates T3-mediated transcription of the endogenous SERCA2 gene.

**FOG-2 Physically Interacts With TRα1**

Because FOG-2 can bind nuclear hormone receptors,21,22 we explored the interaction of FOG-2 with TRα1, the predominant regulator of SERCA2 expression in vivo.11 In HEK-293 cells, FOG-2 was overexpressed with either TRα1 or myc-tagged TRα1. A FOG-2 antisense recovered similar amounts of FOG-2 from both extracts (Figure 5a, right). By comparison, the myc antibody recovered FOG-2 only when coexpressed with the myc-tagged TRα1 (Figure 6a, middle), thus identifying a specific association between FOG-2 and myc-TRα1. Furthermore, in vitro–translated TRα1 was retained by GST-FOG-2-Zn7–8, but not by FOG-2-Zn5–6 or GST alone, in the presence or absence of T3 (Figure 5b). We also compared the retention of TRα1 with TRα2, an alternative product of the *Thra* gene that has a distinct C terminus (CT).35 GST-FOG-2-Zn7–8 selectively retained the TRα1-CT in the presence or absence of T3, but not the TRα2-CT (Figure 5b).

A functional analysis of the interaction of FOG-2 with TRα1 was performed in transient transfection studies of cultured cardiomyocytes using a reporter plasmid regulated by 2 tandem repeats of the consensus TRE sequence (Figure 5c). Transfection of TRα1 alone significantly repressed basal activity of the consensus TRE×2-tk reporter by >50%, whereas T3 treatment increased activity by ~8-fold. Although FOG-2 did not further repress activity beyond the level produced by TRα1, FOG-2 significantly abrogated transactivation by TRα1 following T3 treatment. Immunoblot analysis of cotransfected COS-7 cell lysates showed preserved, if not greater, TRα1 protein expression when coexpressed with FOG-2, suggesting that the diminished reporter activity was not caused by lower
TR/H9251 protein levels (data not shown). Furthermore, whereas the FOG-2-R3A mutant, which lacks repressive activity, permitted continued repression of basal reporter activity by TR/H9251 in the absence of T3, the FOG-2-R3A mutant did not abrogate T3-mediated TR/H9251 transactivation (Figure 5c). Taken together, these results show a ligand-independent interaction between the CT of FOG-2 including zinc-fingers 7 to 8 (amino acids 752 to 1151) and the CT of TR/H9251 and suggest that FOG-2 abrogation of T3-dependent TR/H9251 transactivation requires the FOG-2 N-terminal repressive domain.

FOG-2 Abrogates T3-Mediated SERCA2 Promoter Activity

Further transient transfection studies of cultured cardiomyocytes were performed using a T3-responsive fragment of the SERCA2 promoter that includes multiple TREs. To isolate the effect of FOG-2 on T3-mediated activation, we analyzed relative luciferase activity of the SERCA2-tk reporter with respect to the basal effects of FOG-2 in the absence of T3. Similar to its effects on the consensus TRE×2-tk reporter, FOG-2 did not further repress activity beyond the level produced by TRα1 in the absence of T3 and significantly abrogated T3-mediated transactivation to basal levels (Figure 6a).

Similar abrogation of T3-mediated activity was observed using heterologous tk promoters consisting of 3 tandem repeats of 2 SERCA2 TREs (TRE1 and TRE2) that preferentially bind TRα1 (Figure 6b). Furthermore, although increasing doses of T3 activated the SERCA2-tk reporter in a dose-dependent manner, doses of T3 as high as 500 nmol/L could not overcome the negative effects of FOG-2 (Figure 6c). These results confirm that FOG-2 disrupts T3-mediated TRα1 transactivation of the SERCA2 promoter.

Discussion

Our work demonstrates a novel role for FOG-2 in both adult heart disease and tissue-restricted T3 resistance. Increased FOG-2 expression in end-stage human heart failure, and at a time when CREB-S133A mice are susceptible to dying from heart failure, and wild-type mice subjected to transverse aortic constriction have decreased systolic function, attests to the fact that FOG-2 upregulation is a novel heart-failure associated feature. We demonstrate that FOG-2 disrupts T3-mediated transcription of the SERCA2 gene, which in cultured cardiomyocytes, cannot be overcome by high doses of T3. By lowering SERCA2 expression, increased FOG-2 expression may therefore contribute to the impaired systolic function observed in FOG-2 mice.
and diastolic function characteristic of end-stage heart failure. These observations have clinical implications because thyroid hormone therapy, long considered for treating heart disease, has been limited by its cardiotoxic and peripheral side effects.\textsuperscript{38}

Downregulation of SERCA2 is a critical event that accompanies the transition to decompensated heart failure.\textsuperscript{39–41} We observed a novel physical interaction between FOG-2 and TR\textsubscript{H9251}/H9251, the principal TR-regulating SERCA2 in the myocardium. Furthermore, this interaction occurred in the absence and presence of T3, thus distinguishing FOG-2 from other corepressors whose binding affinities to nuclear hormone receptors are reduced by ligand.\textsuperscript{42} Because the FOG-2-R3A inactive mutant did not abrogate T3-mediated TR-dependent transactivation but permitted intact TR-dependent silencing of a TRE-driven reporter, these results implicate that FOG-2 abrogation occurs via its highly conserved N-terminus repression motif, which has been shown to exert its effects via interactions with a nucleosome-associated complex.\textsuperscript{36,43} Alternatively, FOG-2 may impair T3-dependent transcriptional activity through competition with TR\textsubscript{α1} for limiting amounts of p300,\textsuperscript{44} a coactivator required for T3-dependent transcriptional activity.\textsuperscript{45}

Repression of endogenous SERCA2 in the absence of T3 suggests that FOG-2 also represses SERCA2 through mechanisms unrelated to T3 signaling. Sequences further upstream of the proximal SERCA2 promoter studied in this report that positively regulate the SERCA2 promoter\textsuperscript{46,47} are enriched with GATA4 consensus binding sites\textsuperscript{48} and have severely reduced transcrip
tional activity in severe LV pressure overload.\textsuperscript{47,48} Multiprotein complexes between FOG-2, GATA4, and other nuclear hormone receptors can cooperatively repress transcription\textsuperscript{21,22} and may also play a role in SERCA2 repression. Experiments are currently underway to address this question.

Consistent with our in vitro observations, in vivo cardiac overexpression of FOG-2 resulted in decreased expression of SERCA2 mRNA and protein. Furthermore, FOG-2 mouse hearts spontaneously developed multiple features of cardiomyopathy and a reversion to a fetal pattern of gene expression.

**Figure 4.** SERCA2 is a downstream target of FOG-2. a, Immunoblot analysis of neonatal rat ventricular cardiomyocytes lysates following infection with adenoviruses encoding FOG-2 (Ad-FOG2) or LacZ (Ad-LacZ), at the indicated moi, and stimulation with vehicle (black bars) or T3 (shaded bars). b, Densitometry analysis of SERCA2 band density normalized to GAPDH. Increased SERCA2 protein seen in T3-treated cells was reduced with increasing Ad-FOG2. c, Calsequestrin band density normalized to GAPDH did not change with Ad-FOG-2 infection. d, qRT-PCR of SERCA2 mRNA normalized to GAPDH was significantly reduced by Ad-FOG-2. Two-way ANOVA followed by Bonferroni analysis. *P < 0.05 vs moi 0/−T3 control; †P < 0.05 vs moi 0/+T3 control. All data from at least three independent experiments.
without gross or histological evidence of hypertrophy, a commonly accepted precursor to load-induced heart failure.49 The development of hypertrophy-independent cardiomyopathy in FOG-2 mice is reminiscent of several previously described mouse models of nonhypertrophic cardiomyopathy, including the Tg mutant CREB mouse50 and human idiopathic restrictive cardiomyopathy.51 Our findings suggest that FOG-2 may be a key downstream effector of signaling pathways responsible for mediating the transcriptional changes observed with heart failure via mechanisms independent of those which lead to pathological hypertrophy.

Recent studies supporting the necessary role of GATA4 for normal cardiac function and protection against load-induced heart failure51,52 may also shed light on the role of FOG-2 in cardiomyopathy. FOG-2 is a powerful repressor of GATA4-mediated gene expression19 and of cardiac myocyte hypertrophy in vitro.44 Similar to FOG-2 mice, heterozygous mutant GATA4 mice have systolic and diastolic dysfunction and blunted dP/dt max and dP/dt end responses to β-adrenergic stimulation. However, in contrast to FOG-2 mice, they do not develop progressive dysfunction and death, despite a nearly 50% reduction of GATA4 protein.52 FOG-2 mice also have more severe cardiac dysfunction and higher mortality than GATA4-deficient mice.52 These results suggest that the effects of FOG-2 overexpression cannot be explained simply by a loss of GATA4 activity and implicate GATA4-independent functions of FOG-2. We speculate that although GATA4 is necessary for compensatory hypertrophy in response to pathological stress,51 increased FOG-2 expression may directly trigger the transition to decompensated heart failure observed in end-stage cardiomyopathy.

Our study indicates that increased cardiac expression of FOG-2 promotes resistance of SERCA2 to T3 stimulation and may render the heart more susceptible to decompensation. This effect may extend broadly to other T3-regulated genes and pathways critical to efficient excitation–contraction coupling. Although it remains to be determined whether additional cardiac pathologies are associated with increased FOG-2 expression, our model suggests that therapies designed to decrease FOG-2 activity in the failing heart might slow the progression of heart failure. Defining the signaling pathways that regulate FOG-2 activity and the downstream targets of FOG-2 in cardiac myocytes will provide insight into mechanisms of heart failure progression.

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Disclosures

None.

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FOG-2 significantly abrogates T3-mediated SERCA2 promoter activity. Luciferase reporter assays performed in cultured cardiomyocytes cotransfected with empty vector or TRα1 with empty vector or FOG-2 followed by treatment for 24 hours with vehicle or T3. Fold activation is expressed as relative luciferase activity normalized to either the vehicle-treated, empty vector control, or the vehicle-treated FOG-2 control when appropriate. Two-way ANOVA followed by Bonferroni was used for statistical analysis. a, Cotransfection of FOG-2 expression plasmid abrogated TRα1 transactivation of the 0.6-kb SERCA2 luciferase reporter following T3 stimulation. *P<0.05 vs vector control (color-matched bar). b, Assays using reporter constructs driven by tk alone or with three tandem repeats of TRE1 transactivation. FOG-2 abrogated T3-mediated TRα1 transactivation. *P<0.05 vs vector control (black bar). c, Transactivation of 0.6-kb SERCA2 reporter by TRα1 with indicated doses of T3 (nmol/L) was abrogated by FOG-2. *P<0.05 vs vector control (black bar). Data are from at least 3 independent experiments done in triplicate.

Figure 6. FOG-2 significantly abrogates T3-mediated SERCA2 promoter activity.


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