Inhibition of the Hypoxia-Inducible Factor 1α–Induced Cardiospecific HERNA1 Enhance-Templated RNA Protects From Heart Disease

BACKGROUND: Enhancers are genomic regulatory elements conferring spatiotemporal and signal-dependent control of gene expression. Recent evidence suggests that enhancers can generate noncoding enhancer RNAs, but their (patho)biological functions remain largely elusive.

METHODS: We performed chromatin immunoprecipitation–coupled sequencing of histone marks combined with RNA sequencing of left ventricular biopsies from experimental and genetic mouse models of human cardiac hypertrophy to identify transcripts revealing enhancer localization, conservation with the human genome, and hypoxia-inducible factor 1α dependence. The most promising candidate, hypoxia-inducible enhancer RNA (HERNA)1, was further examined by investigating its capacity to modulate neighboring coding gene expression by binding to their gene promoters by using chromatin isolation by RNA purification and AN–BoxB tethering–based reporter assays. The role of HERNA1 and its neighboring genes for pathological stress–induced growth and contractile dysfunction, and the therapeutic potential of HERNA1 inhibition was studied in gapmer-mediated loss-of-function studies in vitro using human induced pluripotent stem cell–derived cardiomyocytes and various in vivo models of human pathological cardiac hypertrophy.

RESULTS: HERNA1 is robustly induced on pathological stress. Production of HERNA1 is initiated by direct hypoxia-inducible factor 1α binding to a hypoxia-response element in the histoneH3-lysine27acetylation marks–enriched promoter of the enhancer and confers hypoxia responsiveness to nearby genes including synaptotagmin XVII, a member of the family of membrane-trafficking and Ca2+-sensing proteins and SMG1, encoding a phosphatidylinositol 3-kinase–related kinase. Consequently, a substrate of SMG1, ATP-dependent RNA helicase upframeshift 1, is hyperphosphorylated in a HERNA1- and SMG1-dependent manner. In vitro and in vivo inactivation of SMG1 and SYT17 revealed overlapping and distinct roles in modulating cardiac hypertrophy. Finally, in vivo administration of antisense oligonucleotides targeting HERNA1 protected mice from stress-induced pathological hypertrophy. The inhibition of HERNA1 postdisease development reversed left ventricular growth and dysfunction, resulting in increased overall survival.

CONCLUSIONS: HERNA1 is a novel heart-specific noncoding RNA with key regulatory functions in modulating the growth, metabolic, and contractile gene program in disease, and reveals a molecular target amenable to therapeutic exploitation.
Clinical Perspective

What Is New?

- Chromatin immunoprecipitation–coupled sequencing of histone marks combined with RNA sequencing of ventricular biopsies from various mouse models of human cardiac hypertrophy were performed to identify hypoxia-inducible factor 1α–dependent transcripts from enhancer regions, showing conservation with the human genome.
- In following in vivo and in vitro screening approaches, HERNA1 was identified as robustly upregulated in cardiac ventricles on pressure overload, featuring characteristics of an intergenic enhancer RNA.
- HERNA1 binding to the promoter region of the neighboring genes Syt17 and Smg1 triggers a hypoxia-responsive transcriptional response leading to pathological growth, a glycolytic phenotype and a Syt17–driven contractile dysfunction corresponding to pressure-overload heart disease.

What Are the Clinical Implications?

- Suppression of stress-induced Herna1 production in mice resolved established cardiomyopathy through repression of Syt17 and Smg1 transcription, indicating that a tight coupling of enhancer transcription and successive induction of promoters in their vicinity is disease relevant.
- Data obtained from left ventricular biopsies of patients experiencing pressure overload–induced heart failure recapitulate the expression profile of translational mouse models and, hence, indicate a potential disease-driving role for the HERNA1–SMG1–SYT17 axis in humans as well.
- The beneficial effects of HERNA1 depletion in induced pluripotent stem cell–human cardiomyocytes protecting from structural, metabolic, and functional remodeling suggests HERNA1 as a novel RNA target for the treatment of heart failure.

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hronic heart failure is characterized by clinical symptoms of cardiac dysfunction and represents the culmination of prolonged left ventricular growth in response to pathological stressors including ischemia, hypertension, aortic stenosis, and genetic mutations. Despite being the leading cause of hospitalization and mortality worldwide, therapeutic strategies remain limited. Of particular concern is the fact that current treatment modalities have limited potential to treat the underlying cause of heart disease. Hence, targeting key causative drivers of disease development and progression have the potential to lead to more personalized and effective therapies.

Recent developments in transcriptomics and epigenetics has led to a deeper understanding of the mechanistic basis of gene regulation and revealed novel species of noncoding RNA. Of these, enhancer-templated RNAs (eRNAs) represent a novel class of gene-modulating noncoding RNAs that are templated at genomic enhancers. Enhancers are regulatory DNA elements that bind transcription factors to induce gene transcription through the formation of secondary structures that mediate the interaction of the enhancer with the promoter. Enhancers are activated in a cell-type and context-dependent manner that arises from the range of transcription factors that are active in specific cell types or on a particular stimulus. Transcription at enhancer elements positively correlates with enhancer activity, and is characterized by high histone 3 lysine 4 monomethylation (H3K4me1) and low H3K4 trimethylation (me3). In addition to H3K4 methylation, enrichment of H3K27 acetylation and absence of the repressive H3K27me3 signature also serve as indicators of transcriptional activity at enhancer regions. eRNA transcription has been described to induce transcription of either one or both neighboring coding 5′ and 3′ genes. Recent studies have identified eRNAs that are differentially expressed and correlate with the expression of nearby coding genes in mouse models of cardiomyopathy, and human patients experiencing heart disease in comparison with healthy subjects. Thus, the specific modulation of cell type and stress-responsive eRNAs has the potential to very precisely influence pathophysiologic gene networks.

Hypoxia-inducible factors (HIFs) are heterodimeric transcription factors composed of HIFα and HIF1β subunits that occupy central roles in oxygen homeostasis and the pathogenesis of human disease including cancer and cardiovascular disease. They are activated in hypoxic tissue to induce a transcriptional program embracing coding and noncoding RNA transcripts that are entrusted to modulate both the supply and consumption of oxygen. However, it is unclear if HIFs also activate transcription of eRNAs to afford cell and signal specificity of select HIF output responses. In the present study, we identify hypoxia-inducible factor 1α–activated eRNA (HERNA1), as critical determinant in pressure-overload heart disease and demonstrate how in vivo antisense oligonucleotides (ASO)–mediated inactivation of HERNA1 prevents stress-induced cardiac pathogenesis and dramatically improves overall survival in diseased mice.

METHODS

The data and analytic methods will be made available to other researchers. Some study material can be made available on request. Methods are expanded in the online-only Data Supplement.

Study Approval

Left ventricular samples were obtained from subjects with hypertrophic cardiomyopathy, aortic stenosis, or dilated...
cardiomyopathy and from healthy subjects. Institutional review board approval was obtained and subjects provided informed consent. Maintenance and animal experimentation were in accordance with institutional and Swiss Federal Veterinary Office guidelines.

Statistics
Statistical analyses of dependent samples were performed by paired t test (Excel), of unpaired samples, by unpaired (multiple) t tests, and, if not normally distributed, by Mann–Whitney U test (Prism 5.0). For multiple group comparisons, 1- or 2-way ANOVA analyses followed by a Dunnett or Tukey multiple comparison posttest were used as indicated in the respective figure legends. If measurements were taken on the same experimental units, repeated-measures ANOVA was used. P values of <0.05 were considered as significant. Unless otherwise indicated, n indicates the number of individual experiments.

RESULTS
HERNA1 Identification and Correlation With Pathology
To identify potential HIF-regulated eRNAs, we performed genome-wide epigenetic and transcriptomic analysis of cardiac left ventricular biopsies of 2 distinct mouse models of cardiac hypertrophy: surgery-induced aortic stenosis (transaortic constriction [TAC]) and ventricular-specific deletion of the von Hippel Lindau (Vhl) gene (referred to as Vhl cKO). Vhl protein is a negative regulator of oxygen-sensitive Hifα subunits and its deletion leads to constitutive activation of Hif1α and spontaneous cardiac hypertrophy. Chromatin immunoprecipitation–coupled sequencing was performed on biopsies of these models to identify enhancer domains as marked by monomethylated histone H3 lysine 4 (H3K4me1) signals, and regions of active or poised transcription as indicated by trimethylated histone H3 lysine 4 (H3K4me3) marks. RNA sequencing was also performed to detect differentially expressed transcripts. If measurements were taken on the same experimental units, repeated-measures ANOVA was used. P values of <0.05 were considered as significant. Unless otherwise indicated, n indicates the number of individual experiments.

Inhibition of HERNA1 Protects From Heart Disease

HERNA1 Is a HIF1α-Dependent Noncoding RNA With Enhancer Function
Because Herna1 transcription correlated with Hif1α activation, we investigated if Herna1 is a direct target of Hif1α. In silico analysis of the HERNA1 promoter revealed a conserved hypoxia response element (HRE) at position –121 bp and –195 bp upstream of the transcription start site in the mouse and human genome, respectively (Figure 2A). To assess Hif1α binding at this HRE, we performed Hif1α chromatin immunoprecipitation from nuclear extracts of NMCs subjected to normoxia (20% O2) or hypoxia (3% O2). Hif1α associated at the HRE of the HERNA1 promoter in native chromatin specifically in NMCs subjected to hypoxia (Figure 2B). Moreover, mutation of the conserved HERNA1 HRE resulted in blunted HERNA1 promoter-luciferase reporter activity on ectopic expression of HIF1α lacking the oxygen-dependent degradation domain (HIF1αODD) or stimulation with phenylepinephrine (PE; Figure 2C). HIF1α binding at the HERNA1 promoter and HIF1α-dependent promoter activation were confirmed in left ventricular biopsies of Hif1α fl/fl and Hif1α cko mice and in Vhl fl/fl and Vhl fl/fl cko mice, respectively, in isolated NMCs of these lines (Figure IV through IVH in the online-only Data Supplement). Conserved HRES were not detected in promoters of Smg1 or Syt17, and luciferase reporter assays with their respective promoters failed to demonstrate hypoxia or HIF1α sensitivity (Figure IV and IVJ in the online-only Data Supplement). To assess enhancer function of HERNA1, we cloned a 2.9-kb fragment containing HERNA1 and additional flanking sequences including the HRE, downstream of the SV-40 promoter driving luciferase expression, and quantified luciferase activity in response to HIF1αODD expression (Figure 2D). Reporter assays revealed a Hif1α dose-dependent increase in enhancer activity of the luciferase reporter promoter, whereas negligible effects on luciferase activity were observed with a 3-kb control genomic fragment encompassing the peroxisome proliferator activated receptor γ (Pparγ)
promoter void of enhancer features. Finally, to confirm the innate capacity of \textit{Herna1} to increase target gene expression, we used the \textit{AX}-BoxB tethering-based reporter assay.\textsuperscript{21} A chimeric RNA containing \textit{Herna1} fused to BoxB RNA was engineered to facilitate recruitment of the BoxB-\textit{Herna1} RNA fusion to the RNA binding domain.
estrogen receptor α (ERα) and acts with the functional mechanism of a documented ERα fact that the Herna1 (HERNA1) alone (Figure 2F and Figure IVK and IVL in the paper).

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Figure 2. Herna1 Is a HIF1α-dependent noncoding RNA with enhancer function.

A. Sequence of the human and mouse Herna1 promoter. Conserved hypoxia responsive element (HRE) is shown in red, with the core HRE motif capitalized. B. NMCs cultured at 20% O2 or 3% O2 and assessed for chromatin immunoprecipitation of the Herna1 promoter with a HIF1α-specific antibody (IP: HIF1α) or with a control isotype-matched antibody (IP: IgG control). C. HIF1α-dependent Herna1 promoter activity was determined by transient transfection of wild-type or HRE-mutated Herna1 promoter, respectively, fused to luciferase and with either an empty vector control, HIF1αODD or PE stimulation, and cotransfection with a β-galactosidase construct for normalization of luciferase signal. *P<0.05 in comparison with mock/Herna1 WT promoter transfected NMCs; %P<0.05 in comparison with HIF1αODD treated and Herna1 WT promoter transfected NMCs; &P<0.05 in comparison with PE treated and Herna1 WT promoter transfected NMCs. Two-way ANOVA with Tukey post hoc test. D. 3 kb of the genomic region flanking the Herna1 transcript was assessed for enhancer activity in response to HIF1α by transient transfection of Herna1 fused to SV-40 luciferase, and transfection with an empty vector control or HIF1αODD at increasing concentrations, and cotransfection with a β-galactosidase construct for normalization of luciferase signal. The Ppaα promoter serves as control. *P<0.05 in comparison with empty-vector control, 1-way ANOVA with Dunnett post hoc test. mRNA expression was assessed by qPCR after cotransfection of HIF1α Herna1-BoxB αΔ N–Srf; Figure 2E). Thus, Herna1 can be artificially tethered to serum response factor transcription factor αΔ promoter with the promoters of Smg1 and Syt17 to drive transcription. Hence, we quantified Smg1 and Syt17 expression parallels that of Smg1 and Syt17, we asked if the HIF1α promoted Smg1 and Syt17 transcript interac-tions of the respective RNAs by ChIRP-quantitative polymerase chain reaction (Figure 3C). Gapdh and Neat1 ChIRP pull-down products served as specificity controls for Herna1 interactions. A clear signal was detected for Herna1 at the Smg1 and Syt17 promoters between −1000 and −500 bp and −1000 and 0 bp upstream of the respective transcription start site (Figure 3C). ChIRP pull-down of Gapdh and Neat1 RNA did not reveal such robust interactions at the Smg1 (Figure 3C, upper) or Syt17 promoters (Figure 3C, lower). Fluorescence in situ hybridization for Herna1 and its flanking genes revealed coregulation and colocalization of all transcripts from the Smg1-Herna1-Syt17 gene cluster in isolated cardiomyocytes cultured at 3% O2 (Figure 3D). These results suggest that Herna1 acts at nearby genes to promote their transcriptional activation in a hypoxia-dependent manner.

HERNA1 Is Necessary for Pathology-Induced Smg1 and Syt17 Activity

We generated short-hairpin RNAs (shRNAs) targeting mouse Herna1 (shHerna1), of which 2 individual clones efficiently inhibited hypoxia-induced Herna1 expression, of N protein fused to the serum response factor (Srf) gene (N–Srf; Figure 2E). Thus, Herna1 can be artificially tethered to serum response factor transcription factor response elements within the early growth response gene 1 (Egr1) promoter (Figure 2E).22 We observed that the BoxB-Herna1 RNA fusion increased Egr1 mRNA expression in comparison with transfection of either BoxB or Herna1 alone (Figure 2F and Figure IVK and IVL in the online-only Data Supplement). These data support the fact that the Herna1 transcript itself directs flanking gene transcription on HIF1α-induced activation, and is consistent with the functional mechanism of a documented estrogen receptor α eRNA.10

Given that Herna1 expression parallels that of Smg1 and Syt17, we asked if the HERNA1 transcript interacts with the promoters of Smg1 and Syt17 to drive transcription. Hence, we quantified Herna1 binding at the Smg1 and Syt17 promoter by chromatin isolation by RNA purification (ChiRP),23 using glyceraldehyde-3-phosphate dehydrogenase (Gapdh) and nuclear paraspeckle assembly transcript 1 (Neat1), established coding and noncoding hyoxia targets,24,25 as promoter controls for the efficiency and specificity of Herna1 interaction (Figure 3A and 3B). Despite pronounced hypoxia-induced Gapdh and Neat1 expression (Figure 3A), Herna1 binding was not detected at the Gapdh or Neat1 promoters but mainly at the Smg1 and Syt17 promoters (Figure 3B), suggestive of selective Herna1 interaction at these promoters. Next, we subdivided the Smg1 and Syt17 promoters into three 500-kb domains and assessed interactions of the respective RNAs by ChiRP-quantitative polymerase chain reaction (Figure 3C). Gapdh and Neat1 ChIRP pull-down products served as specificity controls for Herna1 interactions.
resulting in the concordant downregulation of Smg1 and Syt17 in NMCs on the RNA and protein level (Figure 4A and 4B). In line with Smg1 repression, phosphorylation of its substrate Upf1 was abolished (Figure 4B). Next, we assayed for Herna1-mediated cell growth by [3H]leucine incorporation and cell size quantification in NMCs subjected to hypoxia, dimethyloxaloylglycine (a chemical inhibitor of prolyl hydroxylases), PE stimulation, or ectopic HIF1αΔODD expression. Herna1 depletion suppressed pathological stress-induced cell growth (Figure 4C and Figure VA through VC in the online-only Data Supplement), suggesting that Herna1 mediates the pathological growth response through HiFi1α. Transition to pathology occurs concomitantly with increased glucose utilization and dependence, reduced oxidative phosphorylation capacity, and repressed cardiomyocyte contractility.16 To interrogate Herna1 function in these contexts, NMCs expressing ectopic HIF1αΔODD were transduced with shHerna1 and kinetic analysis of glycolytic and fatty acid oxidation rates was determined. As noted in Figure 4D and 4E, ectopic HIF1αΔODD expression led to increased glycolysis, and repression of fatty acid oxidation and maximal respiratory capacity, as readout for mitochondrial respiratory function. However, on simultaneous Herna1 inactivation, the shift to glycolysis at the expense of fatty acid oxidation and mitochondrial function was severely attenuated (Figure 4F and 4G). A similar response was observed in dimethyloxaloylglycine, isoproterenol, or PE-treated NMCs on shHerna1 infection (Figure VD and VE in the online-only Data Supplement). shHerna1 treatment
also reverted the contractile defects associated with pathological transition, revealing a beneficial positive inotropic and lusitropic effect of Herna1 inhibition both at the basal state and on HIF1αΔODD expression (Figure 4H through 4K). These data reveal a requirement of Herna1 function in mediating the maladaptive growth, metabolic, and contractile changes associated with pathology.

Smg1 and Syt17 Drive Distinct Aspects of Pathological Transition

Little is known about Smg1 and Syt17 function in cardiomyocytes. We characterized the subcellular localization of the respective proteins in NMCs. Smg1 is primarily localized in the nuclei of cardiomyocytes, whereas Syt17 colocalizes with Atp5a1 (a core component of ATP Synthase/Complex V), indicative of mitochondrial localization (Figure 5A and 5B). Based on these findings and the fact that Smg1 and Syt17 are targets of Herna1, we identified shRNAs inhibiting Smg1 and Syt17 expression effectively at the RNA and protein level (Figure 5C and 5D and Figure VIA and VIB in the online-only Data Supplement). These shRNAs were transduced into cells subjected to hypoxia, ectopic HIF1αΔODD expression, or PE stimulation. Depletion of Smg1 and Syt17 in these contexts prevented
a hypertrophic cell growth response (Figure 5E and Figure VIC through VIF in the online-only Data Supplement), indicating that HIF1α-Herna1-Smg1/Syt17 axis promotes pathological stress-induced cardiomyocyte growth. Moreover, metabolic analysis of NMCs depleted for Smg1 or Syt17 in combination with either HIF1α dood overexpression or treatment with di-methylxaloylglycine, isoproterenol, or PE revealed a requirement of Smg1 or Syt17 for efficient reprogramming of cardiomyocyte metabolism toward glycolysis, in the context of stressors activating HIF signaling (Figure 5F and 4G and Figure VIG through VII in the online-only Data Supplement). In contrast to the function of both Smg1 and Syt17 in pathological growth and metabolism, only Syt17 regulates contractile function. Cardiomyocytes treated with shRNAs against Syt17 abolished the HIF1α dood-induced decrease in contractile amplitude, maximal velocity of contraction and relaxation (Figure 5H and 5I and Figure VIJ and VIK in the online-only Data Supplement), whereas Smg1 inactivation did not affect HIF1α dood-mediated contractile repression.
To identify downstream molecular changes dependent on Smg1 or Syt17, we performed RNA sequencing and analyzed differential gene expression on cardiomyocytes cultured in normoxia or hypoxia, concomitant to Smg1 or Syt17 inactivation. To simplify the data, we isolated gene subsets that were induced or repressed in hypoxia (relative to normoxia nS RNA controls) but normalized by either Smg1 or Syt17 inactivation. In categorizing these differentially expressed genes, we identified specific gene subsets that directly inhibit the hypoxia-driven growth, metabolic, and contractile maladaptation. As noted in Figure VIIA in the online-only Data Supplement, expression of a larger gene subset was normalized to control levels by Smg1 inactivation (in comparison to Syt17). Functional clustering of genes normalized by both Smg1 and Syt17 inactivation revealed an enrichment for genes implicated in growth and metabolic control (Figure VII B in the online-only Data Supplement), whereas individually, Smg1 and Syt17 inactivation also led to the normalization of specific metabolism and growth genes (Figure VII C and VII D in the online-only Data Supplement). Consistent with our in vitro contractility analysis, Syt17 knockdown led to expression normalization of genes linked to cardiomyocyte contractility (Figure VII E in the online-only Data Supplement). To confirm these effects, Serca2 and Phospholamban phosphorylation was analyzed in NMCs cultured in normoxia or hypoxia and transduced with shNs or shSyt17. Hypoxia led to a clear downregulation of Serca2 expression and reduced Phospholamban phosphorylation in shSyt17. Hypoxia led to a clear downregulation of Serca2 expression and reduced Phospholamban phosphorylation in shSyt17.
in normoxia and hypoxia (Figure VIIIF in the online-only Data Supplement). Thus, Herna1-dependent Smg1 and Syt17 expression in response to stressors engages, at a minimum, 2 pathways with distinct roles in cardiomyocyte growth, metabolism, and contractility regulation.

Herna1 Inactivation Attenuates Disease Development In Vivo

Enhancers modulate spatiotemporal gene expression and enhancer-associated RNAs can exhibit tissue- and context-specific gene expression.

Thus, we assessed Herna1 tissue distribution in mice subjected to aortic stenosis–induced hypertrophy (TAC) mimicking pressure overload as seen in aortic stenosis or hypertrophic obstructive cardiomyopathies. As noted in the quantitative polymerase chain reaction analysis, Herna1 expression was elevated in the left ventricle of mice only on TAC, whereas expression in other tissues remained low both in sham and TAC-treated mice (Figure 6A). To define Herna1 function in vivo, we screened for ASO gapmers that would efficiently target Herna1 for degradation in NMCs ectopically expressing HIF1αODD (Figure VIII A in the online-only Data Supplement). Gapmers are chimeric ASOs that contain a central block of deoxynucleotide monomers to induce RNaseH cleavage.

Herna1 gapmers inhibited hypoxia-induced Smg1 and Syt17 expression in NMCs, suppressed hypertrophy on ectopic HIF1αODD or PE stimulation, and rescued Hif1α-mediated contractile inhibition (Figure VIIIB through VIIID in the online-only Data Supplement). These gapmers were then applied in TAC experiments to investigate potential contributions of Herna1 to hypertrophic heart disease development. Scrambled and Herna1-targeting gapmers were delivered before TAC surgery and for the next 3 consecutive days as depicted in Figure 6B. The mice were assessed for 42 days post-TAC with echocardiography performed at regular 14-day intervals. TAC surgery led to a comparable increase in aortic flow velocity in the respective TAC groups (Figure 6C). Consistent with effects in vitro, Herna1GM3 delivery in mice resulted in blunted Herna1 levels, reduced Smg1 and Syt17 expression, and attenuated phosphorylation of Upf1 (Figure 6D and Figure VIII E in the online-only Data Supplement). Mice subjected to TAC and treated with scrGM exhibited pronounced decline in cardiac function, increased hypertrophy and ventricular dilatation from 14-days post-TAC (Figure 6E through 6I and Figure VIII F and VIII H in the online-only Data Supplement). In contrast, TAC mice treated with Herna1GM3 exhibited a blunted response to the induction of ventricular dilatation and hypertrophy, while maintaining cardiac function up to 42-days post-TAC surgery (Figure 6F and 6G and Figure VIII G and VIII H in the online-only Data Supplement). These findings were recapitulated by using an independent Herna1-targeting ASO gapmer, Herna1GM1 (Figure VIII I through VIII L in the online-only Data Supplement). Hence, Herna1 function is critical for the development of pathological stress–induced hypertrophic heart disease.

Next, we interrogated Herna1 function in mice exhibiting overt indications of pathological growth, dilatation, and contractile dysfunction to evaluate the therapeutic implications of Herna1 inhibition. C57Bl/6 mice were randomly assigned into 2 groups, with the groups subjected to either sham or 1K1C surgery and further subdivided for scrGM or Herna1GM3 treatment on pathology development (Figure 7A). The 1K1C protocol leads to cardiomyopathy subsequent to the development of hypertension. 28,29 1K1C surgery was performed and hypertrophy allowed to progress until overt indications of cardiac dysfunction were observed by echocardiography, at which time gapmer treatment was initiated and echocardiography was performed at regular intervals to monitor disease progression (Figure 7A). Blood pressure was assessed to confirm the elevation on 1K1C surgery (Figure 7B). In line with TAC experiments Herna1 expression was only elevated in cardiac left ventricle of mice on hypertension-induced pressure overload (Figure 7C). Herna1GM3 led to the inhibition of pathology-induced Herna1, Syt17, Smg1 expression and reduced Upf1 phosphorylation (Figure 7D and Figure IX A in the online-only Data Supplement). At 56 days post-1K1C, cardiac dysfunction was observed in the 1K1C group and mice from the respective groups were further subdivided for scrGM or Herna1GM3 therapy. After disease progression by echocardiography, 1K1C-operated mice treated with scrGM displayed progressive decline in cardiac function as evidenced by ventricular dilatation and reduced cardiac ejection fraction throughout the 91-day duration (Figure 7E through 7G and Figure IX B through IX D in the online-only Data Supplement). However, 1K1C mice treated with Herna1GM3 demonstrated resolution of disease-associated pathologies including gradual improvement of cardiac function and reversion of hypertrophic cardiac growth (Figure 7F and 7G and Figure IX B through IX D in the online-only Data Supplement). It is notable that, despite the protection conferred by Herna1GM3 in 1K1C mice, a mild increase in physical heart weight was detected at the end of the protocol (Figure 7G), although this was not reflected in 2-dimensional surface area measurements of cardiomyocytes from these hearts (Figure 7H and 7I). Consistent with the echocardiography measurements, mice from the 1K1C scrGM-treated group displayed reduced overall survival in comparison with 1K1C mice treated with Herna1GM3 (Figure 7I). These findings were confirmed by using an independent Herna1-targeting ASO gapmer, Herna1GM1 (Figure IX E through IX H in the online-only Data Supplement). Thus, pathological stress–induced Herna1 expression is critical for
maintaining key aspects of hypertrophic heart disease–associated pathologies in mouse models.

**Herna1 Function Can Be Uncoupled Via Smg1 and Syt17 In Vivo**

In vitro phenotypic analysis and gene expression profiling of Smg1 and Syt17 function revealed cooperation of both genes in normalizing cardiomyocyte hypertrophy and metabolism, but a unique capacity of Syt17 in correcting the maladaptive contractility induced by stress (Figure 5H and 5I and Figure VIIE and VIIF in the online-only Data Supplement). To assess if these Herna1-flanking genes confer similar effects in vivo, we subjected mice expressing Cre recombinase under the control of the ventricle-specific myosin light chain 2v (MLC2v) promoter (MLC2v-cre/+30) to TAC and delivered a modified adeno-associated virus 9 (AAV9) where shRNA transcription is dependent on Cre recombinase activity, thus restricting shRNA expression to the cardiac ventricle. AAV-shRNAs targeting either Smg1 (AAV9-fl/fl-shSmg1) or Syt17 (AAV9-fl/fl-shSyt17) were administered individually or simultaneously as depicted in Figure XA in the online-only Data Supplement. A scrambled nonsilencing RNA construct was used as control. As shown in Figure XB through XD in the online-only Data Supplement, efficient mRNA and protein knockdown of the respective targets was achieved by...
AAV9-mediated shRNA cardiac transduction, whereas Herna1 RNA levels were maintained (Figure XE in the online-only Data Supplement). Thereafter, mice treated with the respective shRNAs were subjected to sham or TAC surgery. As indicated in Figure XF in the online-only Data Supplement, TAC surgery significantly increased blood flow across the aorta of mice of all groups to a similar extent. At 4 weeks postsurgery, a dramatic decline in cardiac systolic function was observed in control mice injected with an AAV9 bearing a nonsilencing shRNA (AAV9-fl-fl-mRNA) and in mice inactivated for Smg1 in the myocardium. In contrast, mice treated with AAV9-fl/fl-shSy17 revealed normal cardiac function despite TAC-induced pressure overload (Figure XG).
in the online-only Data Supplement). In accord, lysates of left ventricular biopsies from these mice showed normalized Serca2 protein levels, and increased Ser16-phosphorylation of Phospholamban in comparison with sham or TAC-operated mice injected with AAV9-fl-fl-nsRNA, as well (Figure XD in the online-only Data Supplement). Left ventricular posterior wall thickness was largely normalized by simultaneous inactivation of Smg1 and Syt17 (Figure XH in the online-only Data Supplement). In line with these results, cardiac hypertrophy was reduced in TAC-operated mice inactivated for Smg1 and Syt17 in the myocardium in comparison with TAC-operated mice treated with AAV9-fl-fl-nsRNA (Figure XI and XJ in the online-only Data Supplement). Thus, simultaneous inactivation of the flanking genes led to normalization of all aspects of cardiac function, dimension, and morphology, hence recapitulating HerNA1 in vivo inactivation.

**Herna1 Correlates With Human Cardiac Hypertrophy and Is Necessary for Disease Transition**

In silico analysis indicated conservation of this gene cluster structure in various species, including humans, where HERNA1 is similarly flanked by SMG1 and SYT17 in the genome and shares 38% overall sequence similarity with the mouse homolog (Figure 1B and Figure IIIA and IIIB in the online-only Data Supplement). Hence, we interrogated HERNA1 function in human cardiomyopathic samples. HERNA1 induction occurred concomitant to elevated SMG1 and SYT17 expression in independent patient cohorts of aortic stenosis–induced cardiomyopathy and idiopathic hypertrophic obstructive cardiomyopathy31 (Figure 8A through 8G and Table I in the online-only Data Supplement). In contrast, we detected an inverse correlation of the HERNA1-SMG1-SYT17 axis in ventricular biopsies of patients with dilative cardiomyopathy and in ventricular biopsies of a Muscle LIM protein (Mlp)–/– with dilative cardiomyopathy and in ventricular biopsies of a detected an inverse correlation of the I in the online-only Data Supplement). In contrast, we assayed HERNA1 function in HIF-driven metabolic reprogramming and cardiomyocyte contractility. As shown in Figure 8M and 8N and Figure XII through XIIIF in the online-only Data Supplement, HERNA1GM2 suppressed the pathological shift to glycolysis and contractile dysfunction caused by HIF1αΔODD expression. The data obtained from left ventricular biopsies of patients who had pressure-overload heart failure recapitulates the expression profile of the mouse models of left ventricular pressure overload, indicating a potential role for the HERNA1-SMG1-SYT17 axis in driving human heart disease. It is more important that the clearly beneficial effects of HERNA1 depletion in iPSC-hCM protecting from structural, metabolic, and functional remodeling suggests HERNA1 as a novel RNA target for the treatment of heart failure.

**DISCUSSION**

Collectively, this work reports a novel mode of hypoxia-dependent gene regulation in pressure overload–induced heart disease initiated by HIF1α activation of the HERNA1 eRNA and its binding to and stimulation of mRNA synthesis of its neighboring gene promoters SYT17 and SMG1. This mode of gene regulation (as opposed to direct transcriptional activation of SYT17 and SMG1 by HIF1α) provides an effective means of engineering cell-specific hypoxia transcriptional responses and offers a potential mechanistic explanation of at least some of the contextual effects that HIF1α mediates in different tissues and pathological settings.34–37 A schematic of this model is shown in Figure XIII in the online-only Data Supplement.

Hence, the requirement for HIFα in development and disease may reflect the need for extensive remodeling of the RNA landscape in cardiac pathology that is known to be coupled to the appearance of transcripts and splice variants of metabolic and sarcomeric proteins not typically expressed in the normal heart.38–41 In this regard, we have recently uncovered SF3B1-mediated alternative pre-mRNA splicing as a previously unknown step between HIF-driven transcription and metabolism, cell growth, and the development of hypertrophic heart disease.31 It is conceivable that the activation of both the HIF1α-SF3B1 and HIF1α-HERNA1 axes in response to hypoxia provides a means to coordinately control central posttranscriptional gene regulatory processes to bring about key changes in the RNA landscape essential for implementing adaptive and maladaptive changes in
cell phenotype. Of note, the presented specificity for HIF1α as a driver of the characterized pathological stress–induced hypoxic response was ensured by the chosen screening strategy, focusing on in vitro and in vivo models where transcript expression was studied as a function of HIF1α (Figure 1G and 1H and Figures IA, IIB, IIC, and IVA in the online-only Data Supplement).

Given the correlation between HERN1, SMG1, and SYT17 coexpression in independent human cohorts of hypertrophic cardiomyopathy and aortic stenosis, it is conceivable to suggest a role of this axis in driving cardiac pathology. Indeed, suppression of stress-induced Herna1 production in vivo in mice resolved established cardiomyopathy through repression of Syt17 and Smg1 transcription, indicating that a tight coupling of enhancer transcription and successive induction of promoters in their vicinity is disease relevant. Both aortic stenosis (as the most prevalent valvular heart disease) and hypertrophic cardiomyopathy (as the primary cause of sudden cardiac death) represent a large fraction of cardiac disease whose therapy today is inefficient in preventing heart failure. In contrast to the general rather poor sequence conservation of long-noncoding RNAs among mammals, HERN1 sequence shares a stretch of 330 bp that displays high sequence conservation among mammals (Figure 1B and Figure IIIA and IIB in the online-only Data Supplement), thus offering a high translational potential through the definition of target sequences that work across species for therapeutic development. In contrast to the nontargeted nature of current treatment regimens, the contextual nature of HERN1 expression facilitates specific targeting of the diseased ventricular myocardium. Hence, targeting of context-specific disease-induced eRNAs, such as HERN1, represents an attractive avenue for developing targeted therapeutic modalities for the treatment of a variety of pathologies including heart disease, cancer, diabetes mellitus, and neuropathies.

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Disclosures
None.

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